

# 2<sup>nd</sup> Biomonitoring research on persistent organic pollutants (POPs) in the surrounding environment of the cement plant - Cementáreň Turňa nad Bodvou

Slovakia, May 2024



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December 9<sup>th,</sup> 2024





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All photographs are made by ToxicoWatch during biomonitoring sampling May 8-11<sup>th</sup> 2024., Graphs and tables are designed by ToxicoWatch, except mentioned else as in references and links. The map pictured at the front: alamy.com. For the map location pictures in this study was used Google-maps.

Front page, Surrounding environment of the cement plant Cementáreň Turňa nad Bodvou, embedded between the mountains of the Slovak Karst National Park (NP Slovenský kras), May 8th, 2024

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2<sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024





# 1. Executive summary

# 2<sup>nd</sup> TW-Biomonitoring cement plant - Cementáreň Turňa nad Bodvou, Slovakia - May 2024

In 2023, the civil organisation Zelený živel o.z. representing environmentally concerned residents of Turnianska kotlina, took the initiative to contact Zero Waste Europe in Belgium and ToxicoWatch (TW) in the Netherlands. The first TW research, started with initial biomonitoring on October 30-31, 2023, in the region of Turňa nad Bodvou, near the cement kiln. This second biomonitoring research, started on May 8-11<sup>th</sup> 2024, is a follow-up. The TW research aims to assess the deposition of persistent organic pollutants (POPs), such as dioxins (PCDD/F/dI-PCB), Polycyclic Aromatic Hydrocarbons (PAH), PFAS, and Heavy Metals (HM), in the surrounding area of the cement kiln. For this multi-year study (2023-2025), the area around the cement plant Cementáreň Turňa nad Bodvou located in the Košice Region of Slovakia is the central location for biomonitoring research. Reference samples were from the Slovak Karst National Park (NP Slovenský kras), with the guidance of official park rangers. All analysis results were performed by accredited labs in the Netherlands.

From May 8-11, 2024, 63 samples were collected by the TW team, including eggs/eggshells of backyard chicken, wildlife meat of deer, Carp fish (*Cyprinus carpio*) wildlife bird eggshells of Heron (*Ardea*), mosses (*Bryophyta*), pine needles (*Picea abies*), water from natural water stream and wells, sediment from natural water streams and wells, and soil.

Key Findings of the 2<sup>nd</sup> TW biomonitoring:

- 1. Exceeding values for dioxins of the EU limit of 3.3.pg BEQ/g/fat (Dr CALUX) and 5.0 TEQ/g fat (GC-MS) were found in the eggs of backyard chickens.
- 2. The results of dioxins in mosses (*Bryophyta*) and pine needles (*Picea abies*) were highly elevated compared to reference sites.
- 3. Dioxin patterns (congeners) indicate a source of co-incineration of alternative industrial fuel.
- 4. Analysis of 14 Heavy Metals (HM) Silver (Ag), Aluminium (Al), Arsenic (As), Barium (Ba), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Mercury (Hg), Manganese (Mn), Nickel (Ni), Lead (Pb), Tin (Sn) and Zinc (Zn) showed elevated levels of all heavy metals in mosses (*Bryophyta*) compared to the references from the Slovak Karst National Park (NP Slovenský kras), EU-limits and average levels of heavy metals in vegetables.
- 5. Comparative studies by Danucem Slovensko a.s., (conducted by Ekotoxikologické centrum Bratislava, ECB) and the Košice Regional Government (conducted by Ekolive) confirm the findings of TW regarding increased dioxins in backyard chicken eggs but underestimate the elevated presence of Heavy Metals (HM) in vegetation and soil this research area.
- 6. The analysis results in sediment at the reference sites in the Slovak Karst National Park > 20 km west of the cement kiln show significantly lower values than the results of fourteen (14) heavy metals in the soil at seven (7) sites within a radius of 3.5 km around the cement plant.
- 7. A seriously contaminated children's playground in Dvorníky-Včeláre, located just 630 meters north of the cement kiln, was found to contain high levels of lead (Pb) and Arsenic (As).
- 8. High concentrations of Heavy Metals (HM14) were found in Mosses (*Bryophyta*), near the cement kiln, indicating a serious contamination of the soil in private vegetable gardens.

- 9. PFAS contamination, with chemical analysis LC-MS/MS on ∑ 24 PFAS substances, was found at all backyard chicken egg locations.
- 10. High values of PFAS with the PFAS CALUX assay were found in Brook Turňa (potok Turňa) water stream and sediment near the cement kiln, compared with the wells of Brook Turňa (potok Turňa) water wells at reference locations in the Slovak Karst National Park, Hrhov, Jablonov and Zádiel.
- 11. PFOS contamination was detected in the liver of Carp fish (*Cyprinus carpio*) from **Lake Hrhov** (**Hrhovské rybníky**), based on chemical PFAS analysis (LC-MS/MS ≥ 24).
- 12. Specific combustion-related PAH congeners (4- and 5-ring), such as Benzo[a]pyrene, were found in mosses (*Bryophyta*) and pine needles (*Picea abies*) in the surrounding area of the cement kiln, notably also in pine needles (*Picea abies*) at one location within the protected area of Slovak Karst National Park.
- 13. The specific congener patterns of dioxins (PCDD/F/dl-PCB) and polycyclic aromatic hydrocarbons (PAH  $\sum$  16) in the eggs of backyard chickens and vegetation showed elevated levels of combustion-related contamination.

These findings are a clear call for action for the responsible authorities to ensure that people living in the surrounding area of the cement kiln are provided with an environment free from industrial POP emissions. The precautionary principle should be applied as a guideline in this regard.

ToxicoWatch strongly recommends:

1. A structural, yearly biomonitoring research programme on POP emissions, to monitor increases or decreases in POP contamination in the surrounding environment of the cement kiln in Turňa nad Bodvou.

2. The establishment of a Technical Working Group (TWG) involving all parties (independent research, representatives of concerned citizens, regional government, and the cement kiln industry), to work on technical improvements, such as filter systems, and a transparent monitoring system of for flue gas emissions of POPs and dust emissions from mining, production, transport, and waste. This initiative aims to stop the contamination of the area where people live and the surrounding natural environment of the Slovak Karst National Park.

Biomonitoring results highlight the underestimation of emissions of dioxins, PFAS, PAH and heavy metals from the cement industry.



Figure 1: Results heavy metals As, Ba, Pb on children's playground in Dvorniky, May 11th 2024

# 2. Acronyms

B[a]P	Benzo[a]pyrene
BAT	Best Available Techniques
BEQ	Bioanalytical EQuivalents
BREF	Best Available Techniques (BAT) Reference Document for Waste Incineration
BBT	Best Available Techniques (BAT)
dI-PCB	Dioxin-Like Polychlorinated Biphenyls
dm	Dm=Dry matter or ds=dry substance NL: droge stof
DR CALUX®	Dioxin Responsive Chemical-Activated LUciferase gene eXpression
ECB	Ekotoxikologické centrum Bratislava s.r.o.
EFSA	European Food and Safety Authority
GC-MS	Gas Chromatography Mass Spectrometry GC-MS
НМ	Heavy metals
LB	Lower Bound
LOD	Limit of Detection
LOQ	Limit of Quantification
MB	Medium Bound
MSWI	Municipal Solid Waste Incineration
ndl-PCB	Non-Dioxin-Like Polychlorinated Biphenyl (Non-Dioxin-Like PCB)
mg	Milligram: 0.001 gram
ng	Nanogram: 0.00000001 gram
μg	Microgram 10 <sup>-3</sup> gram
OTNOC	Other Than Normal Operating Conditions
РАН	Polycyclic Aromatic Hydrocarbons
РСВ	Polychlorinated Biphenyl
PCDD	Polychlorinated Dibenzodioxins
PCDF	Polychlorinated Dibenzofurans
PBDD/F	Polybrominated-dibenzodioxins and furans
pg	Picogram; 10 <sup>-12</sup> gram
РОР	Persistent Organic Pollutants
SKNP	Slovak Karst National Park
SVHC	Substances of Very High Concern
TCDD	2,3,7,8-tetrachloordibenzo-p-dioxine
TDI	Tolerable Daily Intake
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalents
TW	ToxicoWatch
TWI	Tolerable Weekly Intake
UB	Upper Bound (UB)
	Unintentional POP (Persistent Organic Pollutants)
μ <u></u>	Wetweight or fresh weight
WW MARE	wet weight of fresh weight
VVTE	waste to Energy (waste incinerator)

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## 3. Introduction

This second ToxicoWatch (TW) biomonitoring study focuses on the sampling conducted from 8-11, 2024, in the surrounding environment of the cement plant - Cementáreň Turňa nad Bodvou. It is a follow-up to the first published biomonitoring report based on the sampling from October 30-31, 2023.

Thirteen (13) different (bio)matrices were sampled: eggs and eggshells from backyard chickens, eggshells of wildlife birds Heron (*Ardea*), mosses (*Bryophyta*), pine needles (*Picea abies*), meat of a domestic cow, wildlife meat of deer and Carp fish (*Cyprinus carpio*), soil, sediment, water from natural water streams and sheep wool. In total, sixty-three (63) samples were collected and analysed for 1 to 4 different kinds of POPs, including dioxins, PFAS, PAH, and 14 heavy metals (HM): Silver (Ag), Aluminium (Al), Arsenic (As), Barium (Ba), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Mercury (Hg), Manganese (Mn), Nickel (Ni), Lead (Pb), Tin (Sn), and Zinc (Zn). (Bio)assays were used to monitor non-targeted dioxins (by DR CALUX), PFAS (by PFAS CALUX), and PAH (by PAH CALUX). Besides these (bio)assays, chemical analyses were performed on dioxins (PCDD/F/dI-PCB with GC-MS), PFAS (LC-MS/MS), and PAH (GC-MS/MS).

Ecologically significant areas adjacent to the second TW sampling site include the Protected Bird Area Slovak Karst (SKCHVÚ 027) and the National Nature Reserve - Zádiel Gorge (Zádielska dolina), part of the Slovak Karst National Park.

To interpret the analytical results for persistent organic pollutants (POPs), such as dioxins, PFAS, PAHs and heavy metals that were detected in the May 2024 collected samples, the geological environments of the hills surrounding the cement kiln must be considered, especially concerning wind fumigation and the subsequent deposition of these substances of very high concern (SVHC).



Figure 2: Area of TW biomonitoring research, the surrounding environment of the cement kiln near the villages of Dvorníky-Včeláre, with the Slovak Karst National Park in the distance, May 11, 2024.

# 4. Cement kiln industry

### 4.1. General production

The cement industry, in general, has two distinct faces. On the one hand, it forms the backbone of society and is closely linked to a country's economic development. It provides jobs and serves as a significant financial source of financial income for many countries. Concrete and cement are therefore essential, with current global production reaching as much as 1 tonne per person per year.<sup>1</sup>

The ambitions of the cement industry are notably high, as reflected in numerous industry publications.<sup>2</sup> However, it is striking that hardly a word is devoted to the emissions of substances of very high concern (SVHC). Is the cement industry ready to eliminate toxic emissions? Investigating environmental releases of persistent organic pollutants (POPs) would be a worthwhile initiative.

Currently, the heavily promoted 'green card' of reducing  $CO_2$  emissions - since cement kilns are significant contributors to  $CO_2$  -, seems to overshadow the critical need to address the elimination of hazardous toxic emissions, like dioxins, PFAS, PAH, and heavy metals. These pollutants, emitted by the cement industry, take a heavy toll on the surrounding environment.

The health of the population living near these facilities is under constant pressure due to inadequate monitoring of dioxins, PFAS, PAHs, and heavy metals. Substances like dioxins, which are extremely toxic even at very low concentrations, remain minimally monitored. Yet, due to their persistence, bioaccumulation, and toxicity, they are carcinogenic, mutagenic, reprotoxic, and neurotoxic – posing significant risks to human health and the environment.



Figure 3: PBT – Persistent-Bioaccumulation-Toxic features of POPs

<sup>&</sup>lt;sup>1</sup> J. Res. Technol. Eng. 5 (2), 2024, 12-27

<sup>&</sup>lt;sup>2</sup> Cementing the European Green Deal, REACHING CLIMATE NEUTRALITY ALONG THE CEMENT AND CONCRETE VALUE CHAIN BY 2050. CEMBUREAU The European Cement Association, 2024 https://www.cembureau.eu/library/

#### 4.2. Cement plant - Cementáreň Turňa nad Bodvou

It is to be welcomed that both Danucem Slovensko a.s and the Košice Regional Government have started biomonitoring studies in 2024 via Ekotoxikologické centrum Bratislava (ECB) and Ekolive, respectively. Biomonitoring on POPs in the environment should be conducted structurally by independent parties with no financial interests or political ties to the cement kiln industry. Monitoring the environment on behalf of public communities provides insight into the level of POP contamination over time, which could lead to technical improvements in the cement kiln production line to eliminate toxic contamination in the environment.

The upcoming permit to increase capacity and switch to alternative fuels, such as used car tyres and waste oil, could exacerbate the POP contamination problem in the environment. Many researchers warn about the negative impact of the combustion of alternative fuels, such as used car tyres, plastic waste, and PCB oils. If a permit is granted based on a monitor commitment that only covers 0.1% of production time for highly toxic substances like dioxins, this is not a genuine effort to reduce hazardous emissions. Not to mention the extremely short time frame for monitoring the conglomeration of heavy metals. In addition to the absence in the mandatory measurements of Aluminium (Al), Barium (Ba), Mercury (Hg), Cadmium (Cd), Zinc (Zn), Tin (Sn), and Silver (Ag), hardly any heavy metals are monitored in the end products.<sup>3</sup> PFAS also does not seem to be controlled at all, even though these substances are abundantly present in the waste stream and are detected near the cement kiln (see Chapter 7).

Several studies have highlighted complaints from residents about air pollution when cement kiln plants are located nearby. People living close to the plant often experience confusion and public irritation due to the thick layer of dust that collects on house roofs, in (vegetable) gardens, and on parked vehicles. Unfortunately, many of these residents are still unaware of the potential risks of cement dust in their vicinity, leaving them constantly exposed to a variety of toxic pollutants. Cement production is inextricably linked to air and environmental pollution. Switching to alternative fuels can cause more environmental damage than fossil fuels, despite the green labelling. This is especially true for the burning of used car tyres, plastics, and waste oils.<sup>4</sup> Combustion of used tyres produces atmospheric pollutants such as dioxins, dibenzofurans, NO<sub>x</sub>, SO<sub>x</sub>, and heavy metals such as Ti, Al, Pb, Ni, Mn, Fe, Cr, and Zn.<sup>5</sup> However, data from Danucem Slovensko a.s. cement plant - Cementáreň Turňa nad Bodvou reported 0.0 kg of use of end-of-life tyres use (Code 16 01 03, European Waste Catalogue) for 2023.<sup>6</sup> This data conflicts with the information TW received from concerned residents and contrasts with the upcoming permit to incinerate used car tyres as an alternative fuel. Most significantly, the results of this study indicate serious contamination of dioxins, PAH, PFAS and heavy metals in the environment of Turňa nad Bodvou, which can also be explained by the burning of alternative fuels.



Figure 4: The surrounding area of cement plant Cementáreň Turňa nad Bodvou, village of Dvorníky in front, May 11th, 2024

<sup>4</sup> P.M. Mayer et al. (2024). Science of the Total Environment 927 (2024) 171153

<sup>5</sup> Chen et al., (2022). Disposal methods for used passenger car tires: one of the fastest growing solid wastes in China. Green Energy and Environment 7, 1298–1309. https://doi.org/10.1016/j.gee.2021.02.003.

<sup>&</sup>lt;sup>3</sup> Li, C.; Nie, Z.; Cui, S.; Gong, X.; Wang, Z.; Meng, X. The life cycle inventory study of cement manufacture in China. J. Clean. Prod. 2014, 72, 204–211.

<sup>&</sup>lt;sup>6</sup> Report on the operation and inspection of the combustion plant for the year 2019, 2020, 2021 and 2023 Danucem Slovensko a.s., Cement plant Turňa nad Bodvou

#### 4.2 Cement kiln Dust emissions

There is a lack of thorough biomonitoring research on the deposition of hazardous substances such as dioxins and heavy metals in the environment surrounding cement kilns, open mining lime pits, and coincinerator plants. The mining industry has significantly contributed to national economic development by supplying raw materials, offering employment opportunities, and spurring regional economic growth. However, it has caused varying degrees of ecological damage in the vicinity of mining sites, including vegetation destruction, soil erosion, atmospheric and water pollution, and waste accumulation, all of which threaten the sustainability of both the economy and the environment.<sup>7</sup> The main environmental issues associated with lime production are air pollution and energy use. The lime-burning process is the primary source of emissions and the principal energy consumer. Secondary processes such as lime slaking and grinding can also contribute to hazardous dust pollution, while subsidiary operations (namely crushing, screening, conveying, storage, discharge and shipment) are minor in terms of both dust emissions and energy usage.<sup>8</sup>

Three types of dust emissions must be considered: first, limestone dust (from the production of raw limestone material extracted from the mountains); second, dust from the cement kiln industry during production; and third, cement dust emissions from manufacturing of products, storage, bagging, and shipment.



Figure 5: Infographic of pathways of cement kiln dust

Lime is produced by burning calcium and/or magnesium carbonates at temperatures between 900 and 1200/1400°C. The chemical reaction of calcium carbonate thermal decomposition is often referred to as 'calcination' and can be expressed as follows:

 $\begin{array}{cc} CaCO_3 \text{ heat} \longleftrightarrow CaO + CO_2 \uparrow \\ 100 \text{ g} & 56 \text{ g} \text{ 44 g} \end{array}$ 

Calcination in the cement kiln production process accounts for 44% of CO<sub>2</sub> emissions, which is a substantial contribution from this industry sector. Dust emissions of persistent organic pollutants (POPs) should not be overshadowed by efforts to reduce CO<sub>2</sub> emissions (since the cement kiln industry is a large contributor to CO<sub>2</sub>) by utilising high-risk alternative fuels (such as contaminated plastic, rubber, tyres, PCB oils etc.) under a 'green' label promotion. In addition, more biomonitoring should be applied to the mining of limestone and the dust emissions during production, transport, use of products (cement), and disposal of end-of-life products (cement).

<sup>8</sup> Schorcht F. et al (2013). Best Available Techniques (BAT) Reference Document for the production of cement, lime and Magnesium Oxide. Industrial Emissions Directive 2010/75/EU Integrated Pollution Prevention and control

<sup>&</sup>lt;sup>7</sup> N. Li et al. (2024). Ecological Indicators 158 (2024) 111371.

# 5. Sampling & Analyse methods

This second biomonitoring report should be read as a follow-up to the first biomonitoring report, which was published and presented at the local village hall in the village of Dvorníky-Včeláre on May 7, 2024.

In this second biomonitoring report, based of TW sampling from May 8-11, 2024, the focus is on eggs from backyard chickens owned by private chicken coop owners, mosses (*Bryophyta*), pine needles (*Picea abies*), soil, sediment, water, meat from a domestic cow, and wildlife meet from deer and Carp fish (*Cyprinus carpio*), as well as eggshells of backyard chickens and wildlife birds Heron (*Ardea*).

The persistent organic pollutants (POPs) targeted by lab analysis are dioxins, PFAS, PAH, and 14 heavy metals: Silver (Ag), Aluminium (Al), Arsenic (As), Barium (Ba), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Mercury (Hg), Manganese (Mn), Nickel (Ni), Lead (Pb), Tin (Sn), and Zinc (Zn). By applying bioassays (DR CALUX), a much broader spectrum of dioxin toxicity is detected than with the very limited 29 chlorinated dioxins identified by chemical GC-MS analysis. PFAS and PAH are also monitored in this research using screening assays like PFAS CALUX and PAH CALUX, for the same reason as for dioxins, allowing a much broader scan of their toxicity, beyond the limited chemical analysis of 24 PFAS substances and 16 PAH compounds.

Ecologically significant areas are adjacent to the cement kiln, including the Protected Bird Area Slovak Karst (SKCHVÚ 027) and National Nature Reserve - Zádiel Gorge (Zádielska dolina),, which forms part of the Slovak Karst National Park. The sample objectives and locations are summarised in the sample overview map below. An overview of locations, analysis methods and results for each (bio)matrix can be found in the attached Annex 1.

A total of 63 samples were collected by the TW team from May 8-11, 2024. All analysis results were performed by accredited labs in the Netherlands: BioDetection Systems (BDS), Amsterdam and Normec, Groen Agro Control in Delfgauw. The official lab analysis reports of this 2<sup>nd</sup> research are attached in Annex 2.



Figure 6: TW 2<sup>nd</sup> sampling overview map, May 8-11<sup>th</sup> 2024

#### 5.1. Soil

From May 8-11 of this year, TW took fourteen (14) soil samples from nine (9) locations in all wind directions within a 3,5 km radius surrounding the cement plant - Cementáreň Turňa nad Bodvou. Soil samples were taken from private property and a public children's playground and open-air sports facility located 630 meters north of the cement kiln. See Annex 1: Samples & Analysis Overview

Soil samples of more than 100 grams were taken from the top layer of the soil (< 5 cm) and collected in plastic LDPE lab bags. The collected soil samples were stored dry, cool and dark until lab analysis.



Figure 7: Soil sample locations & analysis, May 8-11th 2024

#### 5.2 Water

Water samples were collected from six (6) natural water streams. Two samples from the Slovak Karst National Park were taken as reference samples. Locations one (1) and two (2) were collected with the permission and guidance of official park rangers of the Slovak Karst National Park, located 25-30 km west of the cement plant. Location five (5) is from the Hrhov natural water well stream, 7 km away, and can therefore be considered as a reference sample. Location four (4) is from the natural water stream running through the centre of the village of Zádiel. At Location Six (6), water was sampled from a stream passing by the Jablonov Gas plant, located 12 km west of the cement kiln. Location one (1), Brook Turňa (potok Turňa), is situated 100 m southeast of the cement kiln. The natural brook is used for both water intake and discharge for production processes by the cement plant.

For each location, TW collected 2x 500 ml water samples, which were directly stored in plastic HDPE lab containers. The samples were stored dry, cool and in the dark until lab analysis.

At three (3) locations, water samples were analysed for PFAS using a chemical analysis method (LC-MS/MS) for 24 PFAS substances. At these same three (3) locations, a bioassay (PFAS CALUX) was performed to measure a broader range of PFAS toxicity. Additionally, at three (3) water sample locations, heavy metal analyses (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn and Zn) were performed.



Figure 8: Water sample locations & analysis, May 8-11th 2024

#### 5.3 Sediment

Sediment samples were taken at five (5) locations. At each location, 2x 500 ml plastic HDPE lab containers were filled with sediment sample material. Using a lab spoon, the top sediment layer (< 5 cm) was taken from the water streams. All the collected sediment samples were stored dry, cool and in the dark until lab analysis.

The sediment samples were analysed using four different methods: 2 samples for dioxins (PCDD/F/dl-PCBs) with the innovative bioassay DR CALUX, 3 samples for 14 heavy metals (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn and Zn), 2 samples for PFAS using the bioassay PFAS CALUX, and 3 samples for PFAS with chemical analysis (LC-MS/MS) for 24 PFAS substances.



Figure 9: Sediment sample locations & analysis, May 8-11th 2024

KRAS-01

1

Ck-01

KRAS-BR

HRH-01

# 5.4. Vegetation

There is a fundamental lack of thorough biomonitoring research on vegetation regarding the deposition of substances of very high concern (SVHC), such as dioxins, PFAS, PAH, and heavy metals, in the environment of the cement kiln industry. TW has monitored fourteen (14) heavy metals, dioxins, and PAH in mosses (*Bryophyta*), pine needles (*Picea abies*), as well as in fruit, and correlated the analysis results with the EU limits for feed (animal and pet food) and food.

### 5.4.1. Pine needles (*Picea abies*)

At eight (8) locations, pine needles were collected from pine trees (*Picea abies*) at private locations in all wind directions within a 3,5 km radius of the cement kiln. These locations are three (3), four (4), six (6), seven (7), and eight (8). The reference location in the Slovak Karst National Park included two (2) pine needle samples (1A and 1B) at 25 km to the west. The reference samples were taken at this location with the permission and guidance of the official rangers of the Slovak Karst National Park (SKNP).

At each location, the pine needles were taken from different branches of the pine *tree (Picea abies)* at a height of 1.50-2 meters in various wind directions. The sample material (> 100 grams) was stored in plastic LDPE lab bags and kept dry, cool and in the dark until lab analysis. The pine needles were analysed for different substances using three analytical methods: six (6) samples were analysed for dioxins (PCDD/F/dl-PCBs) with the innovative bioassay DR CALUX, all eight (8) samples were analysed for polycyclic aromatic hydrocarbons (PAH) using chemical analysis PAH/HP-LC-FLD/ GC-MS/MS) on 16 PAH substances; and all eight pine needle samples were analysed for 14 Heavy Metals (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Zn).



Figure 10: Pine needles (Picea abies) sample locations & analysis, May 8-11th 2024

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

## 5.4.2. Mosses (*Bryophyta*)

In the second biomonitoring, moss samples from seven (7) locations (4-10), both private and public, within the 3,5 km radius surrounding the cement kiln were collected. The mosses were collected from stone, wood, or metal roofs of sheds, as well as from soil/ground. For reference, moss samples were collected from three (3) locations (1-3) in the Slovak Karst National Park, from natural decaying tree trunks and forest floors. This was done with the permission and guidance of the official park rangers, at 25-30 km to the west.

The collected moss samples (> 100 grams) were stored in plastic LDPE lab bags, and kept dry, cool and in the dark until lab analysis. The moss samples were analysed for different substances using four analytical methods: 8 samples were analysed for dioxins (PCDD/F/dl-PCBs) using the innovative bioassay DR CALUX.



	Date Sample	Total	Species samples	Location Village	Biomarker	TW-RF-NR 2024	Dioxin DR CALUX	Dioxin GC-MS	Heavy Metals HM-14	PAH CALUX	PAH LC-MS-MS Σ 16	PFAS LC-MS/MS Σ 24	PFAS CALUX
34	9-5-2024	1	Mosses ground	Kras NP- 01-Bron	REF	24TWS-Mos-KS-Bron-01	v		v			v	
35	9-5-2024	2	Mosses ground	Kras NP- 02	REF 🐠	24TWS-Mos-KS-02			v			v	
36	9-5-2024	3	Mosses ground	Kras NP- 03	REF	24TWS-Mos-KS-03			v		v		
37	11-5-2024	4	Mosses Roof	Dvornike / Loc. 1	306	24TWS-Mos-Dv01	v		v			v	
38	11-5-2024	5	Mosses ground	Dvornike / hill	-002-	24TWS-Mos-Dv03	v		v		v		
39	11-5-2024	6	Mosses ground	Dvornike / Loc. 4 / Playground	-02	24TWS-Mos-Dv-04	v		v			v	
40	9-5-2024	7	Mosses Roof	Včeláre - Loc. 1	-102	24TWS-Mos-Vc01	v		v		v	v	
41	11-5-2024	8	Mosses Roof	Zádiel - Loc. 1	-500-	24TWS-Mos-Za01	v		v		v		
42	8-5-2024	9	Mosses Roof	Zádiel - Loc. 2	102	24TWS-Mos-Za02	v		v		v	v	
43	8.5.2024	10	Mosses Roof	Hostowce - Loc 1	.004	24TWS-Mos-He01	V		V		v	V	



Figure 11: Mosses (Bryophyta) sample locations & analysis, May 8-11th 2024

2<sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

All ten (10) moss samples underwent heavy metal analysis for 14 elements (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn and Zn). For polycyclic aromatic hydrocarbons (PAH), six (6) samples were analysed using the chemical analysis method PAH/HP-LC-FLD/ GC-MS/MS) for PAH  $\geq$  16 substances, and 7 samples were analysed for PFAS using chemical analysis (LC-MS/MS) for PFAS  $\geq$  24 substances.

TW has monitored heavy metals in moss, pine needles, and fruit. The findings are compared with the EU limits of feed (animal and pet food) and food.<sup>9</sup> When the limits set for leafy vegetables are applied to the mosses (Bryophyta) as bioindicators, excess levels can be observed for most, if not all metals.



Figure 12: Mosses (Bryophyta) Reference sample locations & analysis, May-11th 2024

<sup>&</sup>lt;sup>9</sup> https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023R0915

### 5.5 Eggs/eggshells of backyard chicken

In this second biomonitoring study, eggs from backyard chickens were again sampled at the same locations as in the October 30-31, 2023, sampling. In addition to collecting eggs from backyard chickens, chicken feed was sampled at all seven (7) locations for additional analysis of possible confounding substances.

At each egg location, TW collected 9-10 fresh chicken eggs, mixed the egg content (egg white and egg yolk), and stored them in 500 ml plastic HDPE lab containers, kept dry, cool and in the dark until lab analysis.

From May 8-11 of this year, dioxin analysis was performed at seven (7) egg locations using the bioassay DR CALUX. The results exceeding DR CALUX EU limit (1.7 for PCDD/F and 5.0 for the sum of dioxins PCDD/F/dl-PCBs) were additionally analysed by a chemical analysis (GC-MS) for 29 chlorinated dioxins, as required by EU regulation. At five (5) locations, eggshells were analysed for 14 heavy metals (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Zn). This analysis was also performed on eggshells from Herons (*Ardea*) taken from the reference location near near Lake Hrhov (Hrhovské rybníky),, with permission and guidance from the official Slovak Karst National Park rangers. At six (6) egg locations of backyard chickens, eggs were analysed for PFAS using the chemical (LC-MS/MS) method for 24 PFAS substances.



Figure 13: Eggs and Eggshells of backyard chicken sample locations and analysis, May 8-11th 2024

### 5.6. Eggshells of wildlife bird Heron (Ardea)

At five (5) locations, eggshells were analysed for 14 heavy metals (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Zn). This analysis was also performed on eggshells from Herons (*Ardea*) taken from the reference location near near Lake Hrhov (Hrhovské rybníky),, with permission and guidance of the official rangers of the Slovak Karst National Park.



Figure 14:Eggshells of wildlife bird Heron (Ardea) sample location and analysis, May 8-11th, 2024

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

## 5.7. Meat of wildlife deer, Carp fish (Cyprinus carpio) and domestic cow

In this second biomonitoring research (May 2024), TW was provided with meat from a young deer (<1 year) by a local farmer. The deer had been living nearby forests of his village, Včeláre. Additionally, meat from the farmer's domestic young calf was provided for analysis on dioxins (PCDD/F/dl-PCB) during the sampling week in May. Both fresh pieces of meat (each 350-500 grams) were handed over in a plastic bag, in a deep-frozen state, as they had been stored for human consumption. The meat was delivered the lab in this way for analysis.

At the same location where TW collected the wildlife Heron (*Ardea*) eggshells, near **Lake Hrhov** (**Hrhovské rybníky**), wild carp fish (*Cyprinus carpio*) was caught for human consumption. A portion of this fresh fish meat (350-500 grams) and the liver were given to TW for analyses on dioxins (PCDD/F/dl-PCB) using the bioassay DR CALUX and for PFAS analysis using the chemical analysis PFAS LC-MS/MS on 24 PFAS substances. The fresh meat was directly stored in a deep freezer in an LDPE lab bag until analyses. The fisherman later mentioned that this fish had been in the freshwater basin near **Lake Hrhov** (**Hrhovské rybníky**) for only a few weeks after being brought there from another lake.



Figure 15: Meat of wildlife deer, Carp fish (Cyprinus carpio), domestic cow location samples & analysis May 8-11, 2024

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

### 5.8. Wool of domestic sheep

During the first biomonitoring sampling visit in the region surrounding the cement plant - Cementáreň Turňa nad Bodvou in October 2023, TW collected sheep wool samples from local sheep in the village Včeláre. TW cut the wool directly from the sheep and collected two plastic LDPE lab bags (each > 50 grams) for analysis on dioxins (PCDD/F/dl-PCB) using the bioassay DR CALUX, and polycyclic aromatic hydrocarbons (PAH) using the bioassay PAH CALUX. The sheep wool collection procedure was repeated on the same sheep in Včeláre, although the sheep were now located on the other side of the village, where the sheep shed is also located. In Hosťovce. TW was able to sample wool from a small group of sheep. The sheep wool collected in 2024 has not yet been analysed for dioxins and PAH. The results will be implemented in the 3rd biomonitoring report in 2025.



Figure 16: Wool of domestic sheep sample locations and analysis, May 8-11th, 2024

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

# 6. Analysis methods

In this second TW Biomonitoring research, DR CALUX and GS-MS were used for dioxin analysis. Polycyclic Aromatic Hydrocarbons (PAH) were analysed by chemical analysis of  $\sum$  16 PAH substances. PFAS were analysed using the PFAS CALUX assay and chemical analysis LC\_MS/MS for  $\sum$  24 PFAS substances. Heavy metals (HM) were analysed for 14 elements, with results expressed in mg/kg dry matter (dm, or ds for dry substance). The graph of heavy metals also uses the expression in wet weight, recalculated from the dry matter results.

TW uses a colour-indicative scale to provide better visualisation of the results. The values in these TW colour indicative scales are 2x, 3x, etc. the EU-limits (for chicken eggs, feed, drinking water), or are compared with the background level or occurrence values in vegetables. For the 14 heavy metals, the indicative grey colours are based on 2x, 3x, 4x and 5x the lowest value of the TW results for each specific heavy metal.

TW Biomonitoring - Lab analysis methods

						1				
	Dioxins DR C	ALUX (mb)		Dioxins GC-MS (mb)						
PCDD/F	dl-PCB	PCDD/F/dl-	PCB PCDD/F	dl-PCB	PCDD/F/dl-PCB		PA	н	4 PAH	16 PAH
			GC-MS-ub	GC-MS	GC-MS	ERaCALUX	PAH C	ALUX	GC-MS/MS	GC-MS/MS
Vegetati	on: DR CALUX	(MB,DW, 88 % dw)	2.5		5.0	( 1 11)			88% DW	88% DW
Medi	umbound (mb)	dry weight (dw)	1.75	1./5	**	pg/1/b	B[a]p equ	uvalent	<u>24 PAH</u>	<u>&gt; 16 PAH</u>
P5 0	2019: Unner	hound (ub)		2019: Upperbound (ub	1 1	Estración eq./1	ng bar eq./	gproduct	11676	יינג / נג
					,					
	Dioxins			Biassay analysis <u>Dioxin</u> :	5		Bioassay an	alysis <u>PAH</u>	Chemical a	nalysis <u>PAH</u>
т	TW Indicative s	cale for Eggs		TW Indicative scale for Eg	gs	TW indicative scal	e TW	Indicative scale	Results	TW ind. scale
	DR CA	LUX		GC-MS		ERaCALUX	PAH C	ALUX	PAH GC-MS/MS	PAH GC-MS/MS
PCDD/F	dl-PCB	(PCDD/F/dl-	PCB) PCDD/F	dl-PCB	(PCDD/F/dI-PCB)	pg / 17b	B[a]p	eq.	Σ 4 PAH	Σ4 PAH
	pg BEQ /	/ g fat		pg TEQ /g fat		Estradiol eq./l	ng BaP eq./	g product	ng/g product	ng/g product
> 6.6	> 2.5	> 10	> 7.5		> 15.0		> 500	) ng	> 500 ng	> 500 ng
> 3.3	> 1.0	> 6.6	> 5.0		> 10.0		> 250	ng	> 250 ng	> 250 ng
>1.7	> 0.5	> 3.3	> 2.5	> 1.75	> 5.0		> 100	ng	> 100 ng	> 100 ng
< 1.7	< 0.5	< 3.3	< 2.5	< 1.75	< 5.0		> 10	ng	> 10 ng	> 10 ng
							< 10	ng	< 10 ng	< 10 ng
TW Inc	dicative scale V	egetation / (Feed)								
DR CALUX										
PCDD/F	dl-PCB	(PCDD/F/dl-	PCB)							
ng	TCDD eq./g dr	v weight (dw)								
> 2.5	×25	> 2 22								
>10	>10	>1.66								
21.0	> 0.5	> 0.92								
20.5	20.5	20.83								
× 0.5	×0.5	10.05								
						-				
PFAS		PFAS	PFAS	PFAS	PF/	AS	Harris Martala			
			HIC-14	PIIC-14			Heavy wietais			
wet weight (	(ww) w	ng/g et weight (ww)	(wet weight / ww)	(wet weight / ww)	Mediumbo	und (mb)	14			
μg PFOA eq.	./g. r	ng PFOA eq./g	μg PFOA eq./g	μg PFOA eq./g	μg / kg	μg / kg				
μg		ng	μg	ng	2019: Lower	bound (LB)	mg/kg			
Bio	Bioassay analysis <u>PFAS</u>			nalysis <u>PFAS</u>	Chemical an	alysis <u>PFAS</u>	<u>Heavy Metals</u>			
T	W Indicative s	cale	TW Indic	ative scale	TW Indica	tive scale	TW Ind. Scale			
PFAS CALL	UX	PFAS CALLUX	PFAS FITC-T4	PFAS FITC-T4	PFAS LC-	MS/MS	Heavy Metals			
wet weight (	(ww) w	et weight (ww)	wet weight (ww)	wet weight (ww)	Σ4 PFAS	Σ 24 PFAS	mos/veg			
μg PFOA eq	./g. r	ng PFOA eq./g	μg PFOA eq./g	ng PFOA eq./g	μg / kg	- ng / g	mg/kg			
			<u>&gt;</u> 0,0768 ng		<u>≥</u> 5,1	<u>≥</u> 5,1				
			≥ 0,0384 ng		≥ 3,4	≥ 3,4				
			> 0.0192 ng		> 1.7	> 1.7				
			> 0.0096 ng		> 1.45	> 1.45				
			< 0.0048 ng		< 1.45	< 1.45				
			< 0,0040 lig		~ 1,75	\$ 1,75				

Figure 17: TW Biomonitoring lab analysis methods

#### 6.1. Dioxin analysis

The analysis of dioxins (PCDD/F/dl-PCB) starts with the screening bioassay method DR CALUX. This method measures a broader range of dioxin activity compared to the limited chemical analysis (GC-MS), which tests only 29 chlorinated dioxin compounds (see figures below). The chemical GS-MS analysis provides more data on the specific congener patterns of the chlorinated dioxins, which helps to determine the source of the dioxin contamination. See the figures below for EU limits for the bioassay DR CALUX and chemical GC-MS analysis.



Figure 19: Dioxin 29 congeners of chemical analysis (GC-MS) analyses for chicken eggs



Figure 18: Dioxins by bioassay DR CALUX vs chemical (GC-MS)

2<sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

### 6.2. Polycyclic Aromatic Hydrocarbons (PAH)

In various national and EU regulations regarding PAHs, the summation of concentrations of 4 or 10 PAH substances is typically adopted. The table below shows the different relative toxicity values, expressed as relative potency (REP) factors. For example, Benzo[b]fluoranthene, the most toxic PAH, is 10,000 times more toxic than anthracene. This demonstrates that simply adding up the concentrations of PAH congeners is insufficient to show the actual toxic load. The regulations need to be updated.

An additional problem, which also affects other highly hazardous substances, is the extremely limited analytical capabilities. Out of the approximately 400 different PAH substances 700, only 16 can be analysed using the chemical analysis method GC-MS/MS, while brominated, chlorinated or other substituted PAHs are not examined.

abutical	Nr of rings	<b>ΡΑΗ Σ16</b>	REP
lalytical			
nt PAH	2	Naphthalene*	0*
hemical	3	Acenaphthylene	0,0001
rinated	3	Acenaphthene	0,0001
mateu	3	Fluoren	0,0001
	3	Phenanthrene	0,0001
	3	Anthracene	0,0001
Г	4	Fluoranthene	0,0001
54	4	Pyren	0,0001
<u>24</u> 7	4	Benzo[a]anthracene	0,3
	4	Chrysene	0,8
	5	Benzo[b]fluoranthene	5
	5	Benzo[a]pyrene	1
	5	Benzo[k]fluoranthene	3,7
	6	Dibenz[a,h]anthracene	1,3
	6	Benzo[g,h,i]perylene	0,0001
	6	Indeno[1,2,3,c,d]pyrene	1,3

In the figure below, the 16 different PAH substances are shown and ordered by the number of benzene rings in their PAH molecular structure.

PAH



Figure 20: Polycyclic aromatic hydrocarbons (PAH), 16 different congeners

#### 6.3. PFAS

PFAS are widely used in industrial applications and consumer products because of their physical/chemical characteristics such as low grade of degradation, surfactant properties, and thermic flame resistance. Chemical target analyses (LC/MS) accurately quantify individual compounds; however, it may underestimate the total PFAS presence. Many PFAS are possible thyroid hormone (TH) system-disrupting compounds because they can -amongst others- inhibit the TH thyroxine (T4) from binding to its transport protein transthyretin (TTR). An alternative approach to chemical analysis of limited target PFAS substances is to monitor the total contribution of PFAS in the environment by applying the effect-based PFAS reporter gene bioassay.

The Chemically Activated LUciferase gene eXpression (CALUX) method is a bioassay technique to detect organic compounds like PFAS based on their ability to induce or inhibit the expression of specific reporter genes. The PFAS-CALUX is based on thyroid hormone transport disruption potential, revealing the presence of unknown PFAS beyond the targeted ones. Effect-based PFAS bioassay data correlate well with LC/MS-derived converted data showing that in vitro toxicity analysis of total PFAS content in water samples using the PFAS reporter gene bioassay is a promising and suitable strategy to cover complex mixtures of PFAS and to assess PFAS in water and the environment in general.<sup>10,11,12</sup>



Figure 21:PFAS Lab analysis methods

<sup>11</sup> Behnisch, P.A. et al (2021). Developing potency factors for thyroid hormone disruption by PFASs using TTR-TR6 CALUX bioassay and assessment of PFASs mixtures in technical products. Environm. Int. 157, 106791-106798.

<sup>&</sup>lt;sup>10</sup> J.K.H. de Schepper et al (2023). The contribution of PFAS to thyroid hormone-displacing activity in Dutch waters: A comparison between two in vitro bioassays with

chemical analysis, Environment International 181 (2023) 108256

<sup>&</sup>lt;sup>12</sup> Behnisch P.A. et al. (2022). Evaluation of Thyroid Hormone Disruption by PFAS in WWTP Influent/Effluent and Surface Waters in the Netherlands, Organohalogen Compd. 83 (2022)

#### 6.4. Heavy Metals (HM)

In TW Biomonitoring research, analysis was performed on 14 elements heavy metals: Silver (Ag), Aluminium (Al), Arsenic (As), Barium (Ba), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Mercury (Hg), Manganese (Mn), Nickel (Ni), Lead (Pb), Tin (Sn) and Zinc (Zn), In some cases the lab could analyse 6, 12 or 13 of these elements in specific matrices.



# 7. Analysis Results of 2<sup>nd</sup> TW Biomonitoring, May 8-11<sup>th</sup> 2024

#### 7.1. Results in Soil

The following results have not been received yet: 3 results on dioxins (PCDD/F/dl-PCB) by bioassay DR CALUX, 3x results on polycyclic aromatic hydrocarbons (PAH) by bioassay PAH CALUX, 1x PFAS analysis with the PFAS CALUX assay, and 5 results on heavy metals (14 elements).

	Date Sample	Total	Species samples	Location Village	Bion	narke	TW-RF-NR 2024	Dioxin DR CALUX	Dioxin GC-MS	Heavy Metals HM-14	PAH CALUX	PAH LC-MS-MS Σ 16	PFAS LC-MS/MS Σ 24	PFAS CALUX
1	11-5-2024		Soil / Vegetable garden	Dvorniky - Loc. 1	1	d.	24TWS-Soil-Dv01						v	
2	11-5-2024		Soil / Chicken enclosure	Dvorniky - Loc. 2 KH	2	<u>S</u> ,	24TWS-Soil-Dv-02							
3	11-5-2024	2b	Soil / vegetable garden	Dvorniky - Loc. 2 KH			24TWS-Soil-Veg-G-Dv02							
4	11-5-2024	2c	Soil -ASH	Dvorniky - Loc. 2			24TWS-Soil-Ash-Dv02							
5	11-5-2024	3	Soil / Vegetable garden	Dvorniky - Loc. 3 (roundabout)	3	ġ,	24TWS-Soil-Veg-G-Dv03							
6	11-5-2024	4	Soil / Playground	Dvorniky - Loc. 4/ Playground	4	S.	24TWS-Soil-Dv-04			v			v	
7	9-5-2024		soil / Vegetable garden	Zádiel - Loc. 1/veg garden	5	Ś.	24TWS-Soil-Za-01			v				
8	9-5-2024	6a	Road Soil stone	Zádiel - Loc. 2 Slakken Road	6	Ś.	24TWSK-Road-Za-02							
9	9-5-2024	6b	Soil Vegetable garden	Zádiel - Loc. 2 Moestuin			24TWSK-Soil-Za-02			v				
10	9-5-2024		Soil - Ash	Zádiel - Loc. Z			24TWSK-ash-Za-02							
11	9-5-2024		Soil / Chicken enclosure	Včeláre - Loc. 1 / chicken	7	S.	24TWS-Soil-VC-1A			v				
12	9-5-2024		Soil / Vegetable garden	Včeláre - Loc. 1 / veg. garden			24TWS-Soil-VC-1B			v				
13	9-5-2024		Soil / Vegetable garden	Turňa nad Bodvou - Loc. 1	8	S.	24TWS-Soil-TnB-01			v				
14	8-5-2024	9	Soil / Chicken enclosure	Hosťovce - Loc. 1	9	ő.	24TWSK-Soil-Hs-01							



### 7.1.1. Heavy metals (HM) in Soil in general

Heavy metals are ubiquitous in the environment due to both natural processes and human activities, and they pose a significant threat to ecosystems and human health. Sources of heavy metals emissions include mining, (including open lime mining), industrial production (such as foundries, smelters, oil refineries, petrochemical plants, pesticide production, and chemical industry), untreated sewage sludge, and diffuse sources such as metal pipes, traffic, and combustion products from waste incineration and cement kilns. <sup>13,14</sup> Although several metals are essential for metabolic processes, there is a critical concentration at which toxic effects occur.

<sup>&</sup>lt;sup>13</sup> Ogunbileje, J. O. et al. (2013). Lead, mercury, cadmium, chromium, nickel, copper, zinc, calcium, iron, manganese and chromium (VI) levels in Nigeria and United States of America cement dust. Chemosphere, 90(11), 2743–2749. doi:10.1016/j.chemosphere.2012.11.058

<sup>&</sup>lt;sup>14</sup> https://www-unep-org.

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

The research conducted by TW is aimed at protecting the environment, animals, and people, and applies stricter standards than those set by the (Regional) Government of Slovakia (see table). This implies that the standards applied by the Slovak government for soil may pose serious risks to the physical and mental health of the human population, especially children. For instance, in the case of lead (Pb), it means that a child can experience serious cognitive/ learning problems by playing on a playground where lead (Pb) concentrations in the soil have been found, as indicated by the results shown in TW's 2<sup>nd</sup> biomonitoring study at Dv-04/playground.

The applicable limits for heavy metals in this report by TW are related to levels at which the least damage to humans and nature is expected. For soil, a certain exposure to children is assumed when they play on contaminated soil and, for example, unintentionally ingest soil particles while eating. The RIVM calculations assume 100 mg of soil per day. Additionally, exposure for people working in vegetable gardens should be considered. Consuming products from the vegetable garden, as well as eggs from your backyard chickens, is discussed in the vegetation chapter. The limits for heavy metals in Slovak are shown in the table below.

Emissions of heavy metals from industrial establishments are one of the major sources of environmental pollution. Heavy metals like lead (Pb) and mercury (Hg) in contaminated soils can be transported by water, wind, and other human activities, resulting in health impacts and environmental effects. Cement industries have been reported as a major source of heavy metal emission into the environment, with several studies showing higher concentrations of heavy metals around cement kiln industries.<sup>15</sup>

TW performs biomonitoring of hazardous substances of very high concern (SVHC), such as dioxins and heavy metals in the surrounding environment of the combustion-related industries. The limits for heavy metals in this report are related to safe levels of vegetable consumption from private vegetable gardens and safe food limits for eggs from backyard chickens. The differences between the Netherlands/EU and Slovakia are shown in the table below.

	Limit levels soil												
		Safe level NL/EU NL											
		vegetable garden/children playground	Industry	Intervention	Ekolive								
As	Arsenic	20	76	76	65								
Ва	Barium	22 (190)	920	920	900								
Cd	Cadmium	0.6	4.3	13	10								
Cr	Chromium	55	180	180	450								
Со	Cobalt	15	190	190	180								
Cu	Copper	40	190	190	500								
Pb	Lead	50	530	530	250								
Hg	Mercury	0.15	4.8	36	2.5								
Ni	Nickel	35	100	100	180								
Sn	Tin	6.5	900	900									
Zn	Zinc	140	720	720	1500								

<sup>&</sup>lt;sup>15</sup> Ogunbileje, J. O. et al. (2013). Lead, mercury, cadmium, chromium, nickel, copper, zinc, calcium, iron, manganese and chromium (VI) levels in Nigeria and United States of America cement dust. Chemosphere, 90(11), 2743–2749. doi:10.1016/j.chemosphere.2012.11.058

### 7.1.1.1Lead (Pb) in Soil



A level of lead (Pb) of 110 mg/kg ds was found at location 4, in the soil of the playground of Dvorníky, 630 m north of the cement plant. The European Food & Safety Authority (EFSA) and the Dutch health services (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) have warned of a decline in children's IQ if lead (Pb) levels are found in soil above the background levels. Exposure to food alone can lead to an increase of lead (Pb) in blood lead levels, EFSA is considered a cause for concern and is associated with an IQ loss of 1 point. A loss of 1 IQ point is significant, affects socio-economic status and labour productivity. <sup>16</sup>. Children are mainly exposed to soil lead (Pb) through the ingestion of soil particles.

According to EFSA model calculations, at a soil content of 100 mg/kg lead (Pb), a comparable amount of lead (Pb) (0.53  $\mu$ g/kg body weight per day) will be ingested via the diet through the ingestion of soil particles during children's activities on the playground.<sup>17</sup>

Children are mainly exposed to soil lead (Pb) through the ingestion of soil particles. In the case of vegetable gardens, there is also exposure through the consumption of crops grown on contaminated soil.



Figure 22: Heavy metal, Lead (Pb) in soil, location Dv-04, 2024

For children's playgrounds (both formal and informal), and in (private) gardens, measures should mainly

<sup>&</sup>lt;sup>16</sup> https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2010.1570

<sup>&</sup>lt;sup>17</sup> Otte P.F. (2015). Diffuse loodverontreiniging in de bodem, Advies voor een gemeenschappelijk beleidskader RIVM Rapport 2015-0204

focus on reducing the ingestion of soil particles. In addition, the cultivation of consumer crops should be discouraged or adapted (e.g. grown only in containers) to ensure that exposure through the consumption of the cultivated crops is reduced to an acceptable level. <sup>18</sup>

For interpretation, EFSA has found a relationship between blood lead levels and neurotoxic effects (such as the loss of IQ points). The calculated relationship is an average value. The effect for an individual child may vary due to differences in factors such as behaviour (exposure), kinetics and sensitivity to lead.

### 7.1.1.2 Arsenic (As) in Soil

Besides the finding of high levels of lead (Pb) at the playground in Dvorníky, elevated levels of Silver (Ag), Barium (Ba) and Arsenic (As) were found. Arsenic is one of the oldest historical agents of targeted poisoning. The heavy metal arsenic (As) was found at a level of 48 mg/kg ds, which exceeds the Dutch safety limit for Arsenic in soil.<sup>19</sup>

Once arsenic is in the environment, it cannot be broken down, so the amounts added can spread globally and harm the health of people and animals. Plants absorb arsenic quite readily, so high concentrations can be present in food. In addition, arsenic is bioaccumulative, which is one reason birds die if they eat fish already containing significant amounts of arsenic.



Figure 23: Heavy metal Arsenic (As) in soil, Location Dv-04, 2024

The graph below shows Arsenic (As) levels in soil across different European countries, with samples

<sup>19</sup> Swartjes F.A. et al (2017). Gezondheidsrisico's ten gevolge van arseen in bodem en grondwater in Apeldoorn , RIVM Rapport 2017-0198

<sup>&</sup>lt;sup>18</sup> Otte P.F. et al (2015). Diffuse loodverontreiniging in de bodem, Advies voor een gemeenschappelijk beleidskader, RIVM Rapport 2015-0204

from locations in the surrounding area (< 1-3 km) of a waste incineration plant, except for the asterisk-marked locations, where no incinerator is present in the environment.



Figure 24: TW data (Arsenic (As) in Soil in Europe 2007-2024

## 7.1.1.3. Results Heavy Metals (HM) in Soil

The graphs below show the results for each of the 14 specific heavy metal elements in ten (10) locations. To interpret these results TW made a TW colour indicative sale based on the lowest result value of each specific heavy metal. The up-grey colouring is based on 2x, 3x, 4x and 5x of this lowest value. For some metals, the Slovak soil limits for a specific heavy metal are implemented at the bottom of the table in Figure 26.

In the map of this figure, the soil sample locations are marked with a red and/or black triangle concerning the high value of the results at these locations according to TW indicative colour scale for the results.

In this table for reference, Sediment from locations of the Slovak Karst National Park (HRH-O1 and KARS-BR) are implemented. The results for the fourteen (14) heavy metals in the reference locations show lower values than the results of heavy metals in the seven (7) locations within a short distance of 3,5 km from the cement kiln.



					Soil (+ Sediment as REFERENCE ) and Road material, May 8-11, 2024 : mg/kg (ds)													
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
					Al	Ag							Hg	Mn				
Lab date	Lab Nr	TW-REF-NR	Loc.		Aluminium	Silver	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
29-11-2024	C6758102	24TWS-SED-HRH-01	HrH-01	Ob	2469,00	0,037	3,80	34,00	0,660	2,80	5,60	6,60	0,022	348,00	7,50	9,50	0,210	35,00
29-11-2024	C6758097	24TWS-SED-KARS-BR	Karst-BR	0a	8236,00	0,030	4,80	123,00	0,310	5,40	13,00	7,50	0,033	500,00	9,80	14,00	0,520	30,00
		24TWS-Soil-Dv-01	Dv-01	1														
3-12-2024	C6759160	24TWS-Soil-Dv-02	Dv-02	2	12035,00	0,150	27,00	188,00	0,610	9,70	31,00	40,00	0,160	835,00	32,00	67,00	4,500	216,00
29-11-2024	C6758183	24TWS-Soil-Veg-G-Dv03	Dv-03	з	10264,00	0,120	18,00	155,00	0,520	8,10	24,00	41,00	0,150	995,00	26,00	31,00	3,000	151,00
23-7-2024	C6700949	24TWS-Soil-Dv-04	Dv-04 play	4	1626,00	0,140	48,00	583,00	0,370	9,60	24,00	18,00	0,086	11229,00	25,00	110,00	0,780	85,00
23-7-2024	C6700950	24TWS-Soil-Za-01	Za-01	5	1452,00	0,130	16,50	150,00	0,610	8,50	24,30	22,70	0,130	10650,00	27,50	39,20	1,600	182,00
3-12-2024	C6759162	24TWS-Soil-Za-02	Za-02	6a	19092,00	0,150	19,00	209,00	0,750	13,00	40,00	36,00	0,150	1191,00	44,00	47,00	5,400	142,00
3-12-2024	C6759158	24TWK-ROAD-ZA-02	Za-02-RD	6b	34059,00	0,025	0,83	516,00	0,024	0,10	13,00	0,72	0,170	5442,00	< 1,5	0,37	0,020	8,30
23-7-2024	C6700952	24TWS-Soil-VC-1A	Vc-01a	7a	1613,00	0,052	9,80	158,00	0,510	9,60	30,00	21,00	0,056	8508,00	24,00	46,00	1,200	196,00
23-7-2024	C6700953	24TWS-Soil-VC-1B	Vc-01b	7b	2519,00	0,079	13,00	187,00	0,700	10,00	39,00	28,00	0,100	11763,00	33,00	46,00	1,800	290,00
23-7-2024	C6700955	24TWS-Soil-TnB-01	TnB-01	8	2800,00	0,120	17,00	170,00	0,470	14,00	35,00	34,00	0,120	26739,00	32,00	50,00	1,700	114,00
29-11-2024	C6758107	24TWS-Soil-Hs-01	Hs-01	9	13950,00	0,120	22,00	184,00	0,520	15,00	32,00	29,00	0,130	1080,00	30,00	39,00	1,600	115,00
		SK-Hygienic Limit							0,800		130,00	36,00	0,300			85,000		140,00
										T	V Indicative s	cale mg/kg	5					
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
					AI	Ag								Mn				
					Aluminium	Silver	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
					> 24000,00	> 0,480	> 40,00	> 560,00	> 0,800	> 24,00	> 80,00	> 35,00	> 0,240	> 8000	> 30,00	> 100,0	> 4,500	> 200,00
					> 12000,00	> 0,240	> 20,00	> 280,00	> 0,400	> 12,00	> 40,00	> 17,50	> 0,120	> 4000	> 15,00	> 50,00	> 2,250	> 100,00
					> 6000,00	> 0,120	> 10,00	> 140,00	> 0,200	> 6,00	> 20,00	> 8,75	> 0,060	> 2000	> 7,50	> 25,00	> 1,125	> 50,00
					> 3000,00	> 0,060	> 5,00	> 70,00	> 0,100	> 3,00	> 10,00	> 4,36	> 0,030	> 1000	> 3,25	> 12,50	> 0,563	> 25,00
					> 1500,00	> 0,030	> 2,50	> 35,00	> 0,050	> 1,50	> 5,00	> 2,19	> 0,015	> 500	> 1,63	> 6,25	> 0,282	> 12,50

Figure 25: Location map of soil samples and table of heavy metal results with a TW indicative colours of result values



Figure 26: Results 14 heavy metals, Ag, Al, As, Cd, Co, Cr, Cu, in Soil, May 8-11th, 2024

2<sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024



Figure 27: Results heavy metals Hg, Mn, Ni, Pb, Sn. Zn in soil, May 8-11th, 2024

#### 7.1.2 PFAS in Soil

Results will be expected in December and will be included in the third biomonitoring report.

#### 7.1.3 PAH in Soil

Results will be expected in December and will be included in the third biomonitoring report.

#### 7.1.4 Dioxins in Soil

Heavy metals, dioxins, and furans with high migration capacity are generated during the incineration process and can cause long-term harm to the environment and human health. This secondary pollution has attracted widespread attention, and as a result, monitoring and controlling hazardous emissions has become an important part of the waste incineration system. TW performs biomonitoring of hazardous substances of very high concern (SVHC), such as dioxins and heavy metals in the surrounding environment of the combustion-related industry.

Results will be expected in December and will be included in the third biomonitoring report.

### 7.2. Results in Water streams

All six (6) water samples from natural water streams, as shown on the map below, were analysed for PFAS: 3 samples using a chemical analysis (LC-MS/MS) and four water samples using a screening assay PFAS CALUX. The water stream Brook Turňa (Turniansky potok) was sampled at four locations (1, 2, 3, and 6). Sample point 1 of the Brook Turňa, is near the cement plant - Cementáreň Turňa nad Bodvou. Samples 2 and 3 are at the beginning of the well in the Slovak Karst National Park. The analyses were performed chemically with LC-MS/MS and with the bioassay of the PFAS CALUX.

Heavy metal analyses will be performed on three waste samples, with results expected by the end of November or December.



Figure 28: Results heavy metals Hg, Mn, Ni, Pb, Sn. Zn in soil, May 8-11th, 2024
#### 7.2.1 PFAS in natural water streams

Per- and poly-fluoroalkyl substances (PFAS) are pollutants found everywhere in the environment and are known to contaminate drinking water and food sources. Recent research shows that despite high temperatures, PFAS and other fluorinated substances may form and/or persist during incineration, risking release into the environment through air emissions and incinerator residues. Spoilage of technical treatments or fire extinguishers spill-off into water streams can also be a source.

In the first TW Biomonitoring research in Slovakia (2023), the screening method using the FITC-T4 assay was applied to indicate PFAS activity in the **Brook Turňa (Turniansky potok)** waste stream near the cement kiln (Location: CK / 1). TW found elevated activity of 21  $\mu$ g PFOA/I, which calls for further research. In 2024, TW expanded its PFAS research to natural water samples from the Slovak Karst National Park (Location: Karst/2 and Karst water well/3), Zádiel water stream (Location: Za-c/ 4), Hrhov water well stream (Location: HrHv/5), and the sample point of the **Brook Turňa** near Jablonov (Location: Jab / 6).

PFAS measurement through chemical analyses (LC-MS/MS) is minimal, as explained in TW's previous report (1<sup>st</sup> Biomonitoring 2023) on PFAS. In this research, TW applied an innovative PFAS CALUX method to these matrices. The promising PFAS CALUX method is also used by (semi) governmental water protection organisations in the Netherlands and the US to provide a more reliable overview of all possible PFAS. Based on the European Drinking Water Directive (EU) 2020/2184 <sup>20</sup>, **the Dutch government implemented a drinking water limit value of 100 ng per litre for 20 PFAS compounds, which will come** into force by 12 January 2026. The Dutch drinking water already complies with this limit value (van der Aa et al., 2022). In addition, the Dutch government expressed the intent to lower the legal drinking water **limit value for PFAS to 4.4 ng PFOA eq./ litre (PEQ) in the future, based on EFSA guidelines**.<sup>21</sup> The results from the PFAS CALUX bioassay are expressed as  $\mu g$  PFOA equivalent/ litre ( $\mu g$  PEQ/I) and have been corrected for the environmental background level of these samples, which is 0.14  $\mu g$  PFOA eq./I for water and  $\mu g$  PEQ/g for other matrices, such as food, sediment, and vegetation.



Figure 29:PFAS different analysis methods (Chemical (LC-MS/MS) vs assay PFAS CALUX and FITC-T4

<sup>&</sup>lt;sup>20</sup> https://eur-lex.europa.eu/eli/dir/2020/2184/oj

<sup>&</sup>lt;sup>21</sup> National Institute for Public Health and the Environment (2023) Risk assessment of exposure to PFAS through food and drinking water in the Netherlands RIVM report 2023-0011



Figure 30: PFAS results in natural water streams, May 8-11th, 2024

### 7.2.2 Heavy Metals in natural water streams

On three (3) water locations, heavy metals (14 elements) were analysed, as shown on pages 13 and 31. The results are expected in December and will be included in the 3<sup>rd</sup> TW Biomonitoring report.

# 7.3. Analysis results in Sediment

For the sediment samples taken on the five (5) locations on the map below, four (4) different analyses are performed. All these four (4) analysis methods for dioxins (DR CALUX), HM, chemical and assay analysis on PFAS (LC-MS/MS  $\geq$  24 and PFAS CALUX) are implemented on the sediment sample taken in the **Brook Turňa (Turniansky potok)**, near the cement plant on 1100 m distance.



Figure 31: Results sediment analysis, May 2024

### 7.3.1. PFAS in Sediment

Sediment samples were taken from the **Brook Turňa (Turniansky potok)**, in the Slovak Karst National Park, near the cement plant. See all five (5) locations on the map in the figure on page 14. The result of the PFAS CALUX assay shows **1600 µg PFOA eq./g dm** in the **Turniansky potok** near the cement plant. The result is corrected with the environmental background level of 830 µg PFOA eq./g dm, measured in the Slovak Karst National Park. The lab results of the assay PFAS CALUX are given in micrograms per gram (µg PFOA eq./g), and for comparison reasons in Figure below expressed in µg/kg. The figure shows the gap between the "known" congeners by chemical analyses and the "unknown" PFAS congeners detected by the assay (PFAS CALUX). The chemical PFAS analysis shows the presence of **0.2 µg PFOS/kg dm** in the sediment near the cement kiln. This value is expressed as a lower bound (LB), meaning only results above the Limit of Quantification (LOQs) are considered.



Figure 32:PFAS results in sediment in natural water streams, Slovakia May 8-11th 2024

### 7.3.2. Dioxins in Sediment

Analyses for this study are pending and will be included in the third biomonitoring report.

### 7.3.3. Heavy Metals in Sediment

At two reference locations in the Slovak Karst National Park, sediments are analysed for heavy metals. The results show much lower values than the analysis on soil in locations at a shorter distance of 3,5 km of the cement kiln. See also chapter and heavy metals 7.5.2. and in heavy metals on soil 7.1.1.3.

TOXIC	0		He	avy	/ Metals	s(14) ii	n Sed	imen	t & Sc	oil, M	lay 8-1	1 <sup>th</sup> 2	024					
								Soil	(+ Sediment a	s REFERENCI	E ) and Road ma	iterial, May 8	l-11, 2024 : m	ng/kg (ds)				
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
					AI	Ag		Ba					Hg	Mn		Pb		
Lab date	Lab Nr	IW-REF-NR	Loc.		Aluminium	Silver	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
			_															
29-11-2024	C6758102	24TWS-SED-HRH-01	HrH-01	Ob	2469,00	0,037	3,80	34,00	0,660	2,80	5,60	6,60	0,022	348,00	7,50	9,50	0,210	35,00
29-11-2024	C6758097	24TWS-SED-KARS-BR	Karst-BR	0a	8236,00	0,030	4,80	123,00	0,310	5,40	13,00	7,50	0,033	500,00	9,80	14,00	0,520	30,00
		24TWS-Soil-Dv-01	Dv-01	1														
3-12-2024	C6759160	24TWS-Soil-Dv-02	Dv-02	2	12035,00	0,150	27,00	188,00	0,610	9,70	31,00	40,00	0,160	835,00	32,00	67,00	4,500	216,00
29-11-2024	C6758183	24TWS-Soil-Veg-G-Dv03	Dv-03	3	10264,00	0,120	18,00	155,00	0,520	8,10	24,00	41,00	0,150	995,00	26,00	31,00	3,000	151,00
23-7-2024	C6700949	24TWS-Soil-Dv-04	Dv-04 play	4	1626,00	0,140	48,00	583,00	0,370	9,60	24,00	18,00	0,086	11229,00	25,00	110,00	0,780	85,00
23-7-2024	C6700950	24TWS-Soil-Za-01	Za-01	5	1452,00	0,130	16,50	150,00	0,610	8,50	24,30	22,70	0,130	10650,00	27,50	39,20	1,600	182,00
3-12-2024	C6759162	24TWS-Soil-Za-02	Za-02	6a	19092,00	0,150	19,00	209,00	0,750	13,00	40,00	36,00	0,150	1191,00	44,00	47,00	5,400	142,00
3-12-2024	C6759158	24TWK-ROAD-ZA-02	Za-02-RD	6b	34059,00	0,025	0,83	516,00	0,024	0,10	13,00	0,72	0,170	5442,00	< 1,5	0,37	0,020	8,30
23-7-2024	C6700952	24TWS-Soil-VC-1A	Vc-01a	7a	1613,00	0,052	9,80	158,00	0,510	9,60	30,00	21,00	0,056	8508,00	24,00	46,00	1,200	196,00
23-7-2024	C6700953	24TWS-Soil-VC-1B	Vc-01b	7b	2519,00	0,079	13,00	187,00	0,700	10,00	39,00	28,00	0,100	11763,00	33,00	46,00	1,800	290,00
23-7-2024	C6700955	24TWS-Soil-TnB-01	TnB-01	8	2800,00	0,120	17,00	170,00	0,470	14,00	35,00	34,00	0,120	26739,00	32,00	50,00	1,700	114,00
29-11-2024	C6758107	24TWS-Soil-Hs-01	Hs-01	9	13950,00	0,120	22,00	184,00	0,520	15,00	32,00	29,00	0,130	1080,00	30,00	39,00	1,600	115,00
		SK-Hygienic Limit							0,800		130,00	36,00	0,300			85,000		140,00
										Т	N Indicative s	cale mg/k	5					
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
					AI	Ag							Hg					
					Aluminium	Silver	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
					> 24000,00	> 0,480	> 40,00	> 560,00	> 0,800	> 24,00	> 80,00	> 35,00	> 0,240	> 8000	> 30,00	> 100,0	> 4,500	> 200,00
					> 12000,00	> 0,240	> 20,00	> 280,00	> 0,400	> 12,00	> 40,00	> 17,50	> 0,120	> 4000	> 15,00	> 50,00	> 2,250	> 100,00
					> 6000,00	> 0,120	> 10,00	> 140,00	> 0,200	> 6,00	> 20,00	> 8,75	> 0,060	> 2000	> 7,50	> 25,00	> 1,125	> 50,00
					> 3000,00	> 0,060	> 5,00	> 70,00	> 0,100	> 3,00	> 10,00	> 4,36	> 0,030	> 1000	> 3,25	> 12,50	> 0,563	> 25,00
					> 1500,00	> 0,030	> 2,50	> 35,00	> 0,050	> 1,50	> 5,00	> 2,19	> 0,015	> 500	> 1,63	> 6,25	> 0,282	> 12,50

Figure 33: Table of Sediment results for 14 heavy metals comparing with heavy metals at soil locations, May 8-11th 2024

# 7.4. Results in Pine needles (Picea abies)

### 7.4.1. Dioxins in Pine needles

TOXICO		Dioxins (P	CDD/F/dl-PCB) in pi	ne need	dles ( <i>Picea abi</i>	<i>es</i> ), Slovakia	a May - 20	24			
-								Dioxins DR CAL	UX (mb)		
							PCDD/F	dl-PCB	PCDD/F/dl-PCB		
Date Sample	Total	Species samples	Location Village	Biomarker	TW-RF-NR	Analyse Method		DR CALUX (dw	ı), 88 %		
							pg BEQ (TCDD)/g fat (veg: product)				
9-5-2024		Pine needles - Picea abies	Kras NP- 1A	REF	24TWS-PN-KS-1A	DR CALUX					
11-5-2024		Pine needles - Picea abies	Dvornìky - Loc. 1	<b>▲</b>	24TWS-PN-Dv01	DR CALUX					
9-5-2024		Pine needles - Picea abies	Včeláre - Loc. 2 (near CK)	4	24TWS-PN-Vc02	DR CALUX					
11-5-2024		Pine needles - Picea abies	Zádiel - Loc. 1	<b>A</b>	24TWS-PN-Za01	DR CALUX					
8-5-2024		Pine needles - Picea abies	Turňa nad Bodvou - Loc. 1	4	24TWS-PN-TnB-01	DR CALUX					
8-5-2024	8	Pine needles - Picea abies	Hosťovce - Loc. 2	4	24TWS-PN-Hs02	DR CALUX					
CARGE IN ADVISED OF ADVISED	Charles in an				-		-		//=		
States 1		SARA-NO			and the second second		I W Indic	ative scale veg	etation / (Feed)		
Chi an Sh		COL A COM	Company Income and		and the second			DR CALU	IX		
Mr. W.S.	A 41	The share		1			PCDD/F	dl-PCB	(PCDD/F/dI-PCB)		
. Ala				S MULLES			pg T	CDD eq./g dry	weight (dw)		
				7.20			≥ 2.5	<u>≥</u> 2.5	<u>≥</u> 3.32		
	. AT			122	A DECEMBER OF		≥ 1.0	<u>≥</u> 1.0	<u>≥</u> 1.66		
			ALL AND ALL AND ALL	Zenas Billi	A CONTRACTOR		≥ 0.5	<u>≥ 0.5</u>	<u>≥</u> 0.83		
KS-1A/1B 1	2	Dv-01 3	/c-02 4 Za-01 5	Hs-01	6 TnB-01 7	Hs-02 8	< 0.5	< 0.5	< 0.83		

Figure 34: Results dioxin (PCDD/F/dl-PCB in Pine needles (Picea abies)

On six (6) locations, as shown on page 16, samples of pine needles (*Picea abies*) were analysed for dioxins using the bioassay DR CALUX, as shown in the graphs below. The levels of dioxins in pine needles ranged from 0.48 – 3.03 pg TCDD eq./g (dm) with the bioassay DR CALUX in Dvorníky. In Zádiel, there was a 100% increase to 3.03 pg TEQ, while Hosťovce showed an 83% decrease, from 2.86 to 0.48 pg TEQ/g dm. This represents the opposite trend of dioxins found in the eggs of backyard chickens at locations in Zádiel and Hosťovce in 2023 and 2024 (see chapter 7.6. Results in Eggs of backyard chicken).



Figure 35: Dioxins in pine needles (Picea abies), May 8-11th 2024

The graphs below show that on 6ix (6) out of seven (7) locations show exceeding results for the sum of dioxins (PCDD/F/dl-PCBs) in pine needles (*Picea abies*) when the EU limit of 0,75 pg/g TEQ in feed would be applied in case the pine needles are consumed as food. In the graphs, TW indicative code colours are used for vegetation, based on the EU limit of 0,75 pg TEQ/g for food.



*c*<sub>0.5</sub> *c*<sub>0.75</sub> *c*<sub>0.7</sub>

Dv-01

2024

0,91

Hs

2023

2,86

Hs

2024

0,48

Ks-01

1,79

2024

TnB-01

2024

1,23

Vc-02

2023

2,85

Vc-02

2024

1,26

Za-01

2023

1,52

Za-01

2024

3,03

0,00

PCDD/F/dl-PCB

Dv-01

2023

0,77

pg TCDD eq./g dry weight (dw)

> 2.5

> 1.0

<u>></u> 0.5

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

### 7.4.2. Heavy Metals in pine needles

In 2024, concentrations of heavy metals were analysed at 7 locations in pine needles of the common native gymnosperm tree species, Picea abies. In Annex 2, comparisons are shown for heavy metals in soil, mosses (Bryophyta) and pine needles (Picea abies). Only barium (Ba), mercury (Hg) and zinc (Zn) in pine needles showed comparable results to those found in the mosses (Bryophyta), without exceeding those levels. TW analysed 14 heavy metals (HM) in the collected pine needles, and the results are shown in the next figure with 14 graphs, one for each heavy metal element. In the first TW biomonitoring research (October 2023), 14 heavy metals were analysed at two (2) locations, which are highlighted in square markings.

TOXICO		Heavy Metals	(HM) sampling locations pi	ne needle	es (Picea abies) -	2024	
Date Sample	Total	Species samples	Location Village	Biomarker	TW-RF-NR	Analyse Method	Heavy Metals 14 mg/kg dm
9-5-2024		Pine needles - Picea abies	Kras NP- 1A	REF	24TWS-PN-KS-1A	Heavy Metals	v
9-5-2024		Pine needles - Picea abies	Kras NP- 1B	REF	24TWS-PN-KS-1B	Heavy Metals	v
11-5-2024	2	Pine needles - Picea abies	Dvornìky - Loc. 1		24TWS-PN-Dv01	Heavy Metals	v
9-5-2024	33	Pine needles - Picea abies	Včeláre - Loc. 2 (near CK)		24TWS-PN-Vc02	Heavy Metals	v
11-5-2024	4	Pine needles - Picea abies	Zádiel - Loc. 1		24TWS-PN-Za01	Heavy Metals	v
8-5-2024	5	Pine needles - Picea abies	Hosťovce - Loc. 1 (sheep/garden)	<b>▲</b>	24TWS-PN-Hs01	Heavy Metals	v
8-5-2024	6	Pine needles - Picea abies	Turňa nad Bodvou - Loc. 1	▲	24TWS-PN-TnB-01	Heavy Metals	v
8-5-2024	7	Pine needles - Picea abies	Hosťovce - Loc. 2		24TWS-PN-Hs02	Heavy Metals	v
K5-14/18	1 2			a-01 5			H5-02 8
	Jovice	Krásnohorská Dihá Luká 1 2	Draaa Drenove Kováčova Drenove Luči I dra Jabi onov nači Turnov	) Husitský ko ka	stol Bôrka Zádielska tiest Zádielsk Sv Bôrka Zádielsk	have a tiest Zadiel 5 vorniky-Vcelar 0 0 0 0 0 0 0 0 0 0 0 0 0	a) P Turna nac Bodvou Rova Ves over Chorváty

*Figure 37: Locations heavy metal (HM-14) results in pine needle (Picea abies), May 8-11th 2024* 

Pine needles from location 1A/B in the Slovak Karst National Park (SKNP) was provided by the official park rangers of the SKNP. Remarkably, high levels of cadmium (Cd), lead (Pb) and Tin (Sn) were found in this sample.



2<sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

## 7.4.3. Polycyclic Aromatic Hydrocarbons (PAH) in Pine needles

Chemical analyses (GC-MS/MS) of PAH were performed on pine needles of *Picea abies* at seven (7) locations in the surrounding environment of the cement kiln, as shown in the map on page...

TOXICO			Results PAH in pine	e needl	es ( <i>Picea ab</i>	<i>ies</i> ), Slova	kia May 2024		
							medium bound (mb) 2023	medium bou	nd (mb) 2023
							РАН	4 PAH	16 PAH
Date	Total	Species samples	Location Village	Biomarker	TW-RE-NR	Analyse	PAH CALUX	GC-MS/MS	GC-MS/MS
Sample						Method	Benzo[a]pyrene equivalent	Σ4 PAH	<u>Σ</u> 16 PAH
							ng BaP eq./g product	ng/g	ng / g
9-5-2024	1	Pine needles - Picea abies	Kras NP- 1A 🏯	REF	24TWS-PN-KS-1A	PAH GC-MS/MS			
9-5-2024		Pine needles - Picea abies	Kras NP- 1B	REF	24TWS-PN-KS-1B	PAH GC-M5/M5			
11-5-2024		Pine needles - <i>Picea abies</i>	Dvornìky - Loc. 1		24TWS-PN-Dv01	PAH GC-M5/M5			
9-5-2024	3	Pine needles - <i>Picea abies</i>	Včeláre - Loc. 2 (near CK)	<b>A</b>	24TWS-PN-Vc02	PAH GC-MS/MS			
11-5-2024	4	Pine needles - Picea abies	Zádiel - Loc. 1	<b>A</b>	24TWS-PN-Za01	PAH GC-MS/MS			
8-5-2024	5	Pine needles - Picea abies	Hosťovce - Loc. 1 (sheep/garden)	<b></b>	24TWS-PN-Hs01	PAH GC-MS/MS			
8-5-2024	6	Pine needles - <i>Picea abies</i>	Turňa nad Bodvou - Loc. 1	<b></b>	24TWS-PN-TnB-01	PAH GC-MS/MS			
8-5-2024	7	Pine needles - <i>Picea abies</i>	Hosťovce - Loc. 2		24TWS-PN-Hs02	PAH GC-MS/MS			
							TW Indicative sc	ale Results	TW Indicative scale
							PAH CALUX	PAH GC-MS/MS	PAH GC-MS/MS
							Benzo[a]pyrene equivalent	Σ4 PAH	<b>Σ16 PAH</b>
							ng BaP eq./g product	ng/g product	ng/g product
							> 500 ng	> 500 ng	> 500 ng
							> 250 ng	> 250 ng	> 250 ng
							<u>≥</u> 100 ng	<u>&gt;</u> 100 ng	<u>≥</u> 100 ng
							≥ 10 ng	≥ 10 ng	<u>≥</u> 10 ng
							< 10 ng	< 10 ng	< 10 ng

Figure 38: Results PAH in pine needles (Picea abies), May 8-11th 2024

The PAH results from the samples collected in May 2024 ranged from  $48.1 - 207.5 \ \mu g \sum 16 \ PAH/g \ dm$ . Location seven (7), at Turňa nad Bodvou, showed **elevated levels of Phenanthrene and Fluorene, at.**  $43.0 \ and \ 86.0 \ \mu g/kg \ dm$ , respectively. The high concentration of fluoranthene is also confirmed by the Ekolive survey 2024 of soil samples in the eastern part of their study area.



Figure 39: PAH in Pine needles (Picea abies), May 8-11th 2024



Figure 40:  $PAH \sum 16$  results in pine needles (Picea abies), May 8-11<sup>th</sup> 2024



Figure 41: PAH  $\sum$  16 % in pine needles (Picea abies), May 8-11<sup>th</sup> 2024

As a reference, TW used pine needles (*Picea abies*) from the Slovak Karst National Park (SKNP), provided by the official (SKNP) rangers research/management team. **Remarkably, a high value of 6.7 \mug Benzo(a)Pyrene/g was found at this reference location** in the Slovak Karst National Park. The reason for this elevation level of PAH in pine needles is unknown. It is possible that the physical landforms of the TW sample locations, which can be described as a bowl surrounded by mountains on three sides, play a role in the deposition of industrial emissions of substances of very high concern (SVHC). See the map in the figure below. The cement kiln is in the flat area of this bowl, bordered by mountains to the north, south and west, at a relatively short distance (18km) from the Slovak Karst National Park. It is unknown whether the steel industry near Košice, in the east, plays a role in the PAH and heavy metal contamination in the environment of this nature reserve, or whether it is related to it recent forest fires. The Ekolive study found concentrations of B[a]P of 740 µg/kg in the east and north of their study area. Also notable are the high concentrations of B[a]P found in TW's 2<sup>nd</sup> biomonitoring study in mosses (Bryophyta) in SKNP (see the next chapter 8).



Figure 42: Physical landforms Slovak Karst National Park, area of 2<sup>nd</sup> TW Biomonitoring, May 2024

# 7.5. Results in Mosses

### 7.5.0. What are mosses (Bryophytes)?

*Bryophytes* are three groups of non-vascular land plants: mosses, hornworts, and liverworts. Each of these groups encompasses several hundred different species. Mosses are, in addition, the most important group within the non-vascular plants. **Non-vascular plants** are plants that **do not have a**:

- 1. **Xylem** transport system, for water/nutrient distribution from the roots upwards to the green leaves via cohesion and adhesion actions.
- 2. **Phloem** transport system, for energy/sugar-rich sap distribution, collected by chlorophyll cells from direct sunlight (photosynthesis) into the leaves, and then downwards to the root system and to all plant parts where energy or storage is needed.

Although Mosses lack these vascular transport systems, some mosses have more basic and simple vascular tissues that allow them to internally transport the water they collect by capillary action. When mosses first dry out, they do not die directly. First, the mosses turn brown and go dormant. Mosses can stay in this dormant state for various lengths of time before rehydration is necessary, depending on the species. Mosses are characterised by leaves that are only one cell wide, attached to a stem used for water and nutrient transportation. Mosses can absorb a substantial amount of water and have historically been used for insulation, water absorption, and as a source of peat.

*Bryophytes* are a group of plant species that reproduce via spores rather than flowers or seeds and are mostly found in damp environments. Like most green plants, mosses are mostly green in colour due to the chlorophyll pigments in the chloroplasts of plant cells, which are needed for photosynthesis, when they are not dehydrated.

In TW biomonitoring research, mosses from the *Bryophyta* group were sampled to examine by diverse whether the environment is contaminated with persistent organic pollutants, and POPs, like dioxins, PFAS, PAH and Heavy Metals.



Figure 43: Mosses (Bryophyta)

## 7.5.1. Dioxins in Mosses (Bryophytes)

For this 2<sup>nd</sup> biomonitoring research, TW analysed moss samples (*Bryophyta*) from eight (8) locations for dioxins using the bioassay DR CALUX in the surrounding environment of the cement plant - Cementáreň Turňa nad Bodvou. For reference, moss samples (*Bryophyta*) were taken from more than 20 km waste of from the cement plant, near natural water springs in the mountains of the Slovak Karst National Park. These ecologically protected areas were visited for sampling with the guidance of official SKNP rangers.

The figure below shows the dioxin results for each location. The location numbers correspond to those numbers on the overview location map for mosses (*Bryophyta*) sampled in May 2024, on page 17.

TOXICO			Results Dioxir	ns in mo	osses ( <i>Bryophyta</i> ), Sl	ovakia May 2	024						
							1	Dioxins DR CAL	UX (mb)				
							PCDD/F	dl-PCB	PCDD/F/dl-PCB				
Date Sample	Total	Species samples	Location Village	Biomarker	· TW-RF-NR	Analyse Method	DR CALUX (dw), 88 %						
							pg BEC	Q (TCDD)/g fat	(veg: product)				
	Vege	tation / Mosses	/ Bryophyta	F	EED / Vegetation /Mosses - N	Aedium bound (mb),	88% Dry Weig	ht/ (dw) or Dry	Matter (dm)				
9-5-2024	1	Mosses ground	Kras NP- 01-Bron	REF	24TWS-Mos-KS-Bron-01	DR CALUX							
11-5-2024	4	Mosses Roof	Dvornìke - Loc. 1	5002	24TWS-Mos-Dv01	DR CALUX							
11-5-2024	5	Mosses ground	Dvornìky / Hill	-002-	24TWS-Mos-Dv03	DR CALUX							
11-5-2024	6	Mosses ground	Dvorniky / Playground	-002-	24TWS-Mos-Dv-04	DR CALUX							
9-5-2024	7	Mosses Roof	Včeláre - Loc. 1	-002-	24TWS-Mos-Vc01	DR CALUX							
11-5-2024	8	Mosses Roof	Zádiel - Loc. 1	-002	24TWS-Mos-Za01	DR CALUX							
8-5-2024	9	Mosses Roof	Zádiel - Loc. 2	-002	24TWS-Mos-Za02	DR CALUX							
8-5-2024	10	Mosses Roof	Hosťovce - Loc. 1	5002	24TWS-Mos-Hs01	DR CALUX							
							TW India	ative scale Veg	etation / (Feed)				
								DR CALU	x				
							PCDD/F	dl-PCB	(PCDD/F/dl-PCB)				
							pg T	CDD eq./g dry	weight (dw)				
							<u>&gt; 2.5</u>	<u>&gt;</u> 2.5	<u>&gt;</u> 3.32				
							≥1.0	≥ 1.0	≥1.66				
							<u>≥</u> 0.5	<u>≥</u> 0.5	<u>≥</u> 0.83				
							< 0.5	< 0.5	< 0.83				

Figure 44: Results of dioxins (PCDD/F/dl-PCB in mosses (Bryophyta), May 8-11th 2024

The value of mosses (*Bryophytes*) in biomonitoring environmental pollutants from industry is increasingly recognised.<sup>22</sup> *Bryophytes* are frequently overlooked in biodiversity and ecosystem function assessments, even though they contribute significantly to ecosystem processes.<sup>23</sup> Moss data serve as an indicator of pollution levels but do not carry health thresholds. That is why a comparison was made with EU feed regulation to indicate at what level other food and feed can be contaminated.

TW measured dioxins using the bioassay DR CALUX in biomatrices such as mosses (*Bryophytes*) and pine needles (*Picea abies*) in the environment of the cement kiln. In the graph below, a comparison is shown between sampling in October 2023 and May 2024. The results show higher levels of dioxins in mosses in 2023 compared to those in May 2024. The levels of dioxins (PCDD/F) in mosses were **3.32 – 23.76 pg TCDD/TEQ/g dm in 2023** and **0.68 – 7.09 pg TCDD/TEQ/g dm in 2024**.

<sup>&</sup>lt;sup>22</sup> Jovan S, et al. (2024). Putting biomonitors to work native moss as a screening tool for solid waste incineration. Environ Monit Assess. 2024 Nov 7;196(12): 1177..

<sup>&</sup>lt;sup>23</sup> Slate, M.L., et al. (2024), Impact of changing climate on bryophyte contributions to terrestrial water, carbon, and nitrogen cycles. New Phytol, 242: 2411-2429. <u>https://doi.org/10.1111/nph.19772</u>

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

The cement plant - Cementáreň Turňa nad Bodvou is closed annually from December to February for at least 6 weeks for maintenance work. Photosynthesis of vegetation stops/slows down below 10 degrees C, which reduces the metabolic activity of plants, and so *Bryophyta* mosses as well.

*Bryophytes* produced more compounds, such as fatty acids and conjugates, carbohydrates, and glycosyls, phenylpropanoids and polyketides, flavonoids and anthocyanins in spring and summer than in autumn.<sup>24</sup> The capacity of saturation of fat-related compounds in the October sampling is more pronounced and can be the reason for higher levels of findings POPs in October 2023. Besides this hypothesis, the sampling time from February to May 8-11<sup>th</sup> is much shorter, only 3 months than the time in 2023 from February to the end of October, which is 9 months.





Figure 45: Results (sum) of dioxins (PCDD/F/dl-PCB) in mosses (Bryophyta), May 8-11th 2024

<sup>&</sup>lt;sup>24</sup> Peters K, et al (2019). Chemical Diversity and Classification of Secondary Metabolites in Nine Bryophyte Species. Metabolites. 2019 Oct 11;9(10):222.

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

There is a more pronounced contribution of dI-PCB to the toxicity of dioxins in mosses in 2023. In eggs, where the chemical (GC-MS) analysis for dioxins could more specify the congeners, the dominant presence of PCB 126 is evident. This is a congener related to combustion.



Figure 46: Results dl-PCB in mosses (Bryophyta), May 8-11th 2024

In Figure 49, the results of dioxin (PCDD/F/dI-PCB) in mosses (Bryophyta) are given in the first graph for the 1<sup>st</sup> TW biomonitoring research in October 30-31<sup>st</sup> 2023 and 2<sup>nd</sup> in May on 8-11<sup>th</sup> 2024. The second graph shows the dioxin results for 2023/2024 per location. Sampling in May gives lower results but is still above the standard of 0.75 pg TEQ/g ds. As previously discussed, these lower values are explained by the fact that the sampling period after the winter stop of the cement kiln is considerably shorter.



Figure 47: Results sum of dioxins (PCDD/F/dl-PCB in mosses (Bryophyta), May 8-11th 2024

### 7.5.2. Heavy metals in Mosses (Bryophyta)

The figure below shows the results of the 14 heavy metals analysed in mosses from seven (7) locations in the surrounding environment of the cement plant - Cementáreň Turňa nad Bodvou. For reference, TW collected three (3) samples from the protected area of Slovak Karst National Park (SKNP). See the location map, 5.4.2. for all ten moss (Bryophyta) samples **on page 17**.

As a screening tool, TW interprets the elemental content of moss mainly in relative, spatial terms (i.e., comparing where concentrations are relatively high vs. low) to help guide the need for more detailed research. Especially, the presence and/or deposition of heavy metals in mosses should prompt further investigation into its source(s). Since mosses lack a root system (See information on *Bryophytes* on page 49 (7.5.0.), heavy metals in mosses are related to air deposition.





Figure 48: Results of heavy metals-14 in mosses (Bryophyta), May 8-11th 2024

The cement kiln conducts monitoring for heavy metal emissions: As, Pb, Cr, Co, Cu, Mn, and Ni in a summarised manner and Cadmium is monitored separately. There is no monitoring for the heavy metals Al, Ba, Hg, Ag, Tn, and Zn. This is notable because the combustion of rubber and car tyres as alternative fuel involves emissions of heavy metals like zinc (Zn), which comes from the presence of zinc oxide in tyres as part of the rubber vulcanisation. Increased emissions of Ni and Pb were observed in stack emissions when tyres were used as fuel.<sup>25</sup> The combustion of waste tyres produces atmospheric pollutants such as dioxins, dibenzofurans, NOx, SOx, and heavy metals such as Zn, Mn, Cr, and Pb in remaining ash, which present disposal issues.<sup>26</sup>

TW monitors 14 heavy metals in vegetation: Ag, Al, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn and Zn. All these metals, except aluminium (Al), barium (Ba) and silver (Ag), are described in the BREF document for cement kilns.<sup>27</sup> As a screening tool, TW interprets the elemental content of moss mainly in relative, spatial terms (i.e., comparing where concentrations are relatively high vs. low) to help guide the need for more detailed research. Especially, the presence and/or deposition of heavy metals in mosses should prompt further investigation into its source(s). Since mosses lack a root system (See information on *Bryophytes* on page 49 (7.5.0.), the presence of heavy metals in mosses is related to deposition from the air.

<sup>&</sup>lt;sup>25</sup> J.A. Conesa, A. Galvez, F. Mateos, I.M. Gullon, R. Font, Organic and inorganic pollutants from cement kiln stack feeding alternative fuels, Journal of Hazardous Materials 158 (2008) 585–592.

<sup>&</sup>lt;sup>26</sup> Chen, B. et al. (2022).. Disposal methods for used passenger car tires: one of the fastest growing solid wastes in China. Green Energy and Environment 7, 1298–1309.

<sup>&</sup>lt;sup>27</sup> Schorcht F. et al (2013). Best Available Techniques (BAT) Reference Document for the production of cement, Lime and Magnesium Oxide. Industrial Emissions Directive 2010/75/EU Integrated Pollution Prevention and control

Monitoring of incinerator emissions is increasingly being conducted using mosses as bioindicators.<sup>28</sup> The ranking order of most heavy metals in mosses is as follows Zádiel> Včeláre> Hosťovce> Dvorníky> Karst. The results indicated that monitoring soils and cultivated crops in the investigated area is necessary, as it may also be advised for the old mining region 50 km to the north. This area is already impacted by emissions from operating industries and is expected to expand production in the coming years. In the Figures below, heavy metal concentrations are expressed in mg/ kg dry weight (DW) for comparison with maximum levels for contaminants in food products (according to Slovak/European regulations) the concentration values are converted to mg/kg fresh weight (FW) or wet weight (ww). The maximum limits were derived from Musilová et al. <sup>29</sup> Heavy metals (HMs can accumulate in the edible parts of plants, enter the food chain, and cause adverse toxicological effects to consumers. Their accumulation in vital organs such as the liver, kidneys, and bones can lead to many serious health disorders.

#### Heavy Metals (LB) (mg/kg dw) in moss (Bryophyta), May 8-11<sup>th</sup> 2024

				Masses (Bryophyta) May 8-11, 2024 : mg/kg (ds)														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
tob date	Lab Ma	THE OFF NO	1		Al	Ag		Ba					Hg	Mn				
Labuate	Lab IVI	IVV-REF-INR	LOC.		Aluminium	Silver	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
19-6-2024	C6693670	24TWS-Mos-KS-Bron-01	Karst-01	1	1810,00	0,026	0,750	23,00	0,200	2,80	0,560	6,90	0,110	74,20	1,80	8,80	0,450	32,00
19-6-2024	C6693672	24TWS-Mos-KS-02	Karst-02	2	497,00	0,011	0,170	23,00	0,049	0,91	0,240	8,60	0,043	18,70		1,60	0,140	21,00
19-6-2024	C6693673	24TWS-Mos-KS-03	Karst-03	3	1540,00	0,017	0,420	37,00	0,110	2,40	0,530	7,00	0,087	40,90	1,60	5,80	0,380	26,00
19-6-2024	C6693675	24TWS-Mos-Dv01	Dvorníky-01	4	6225,00	0,088	2,700	118,00	0,880	23,00	2,900	15,00	0,077	375,00	24,00	44,00	0,340	173,00
19-6-2024	C6673678	24TWS-Mos-Dv03	Dvorníky-03	5	962,00	0,024	0,540	31,00	0,110	1,90	0,300	3,90	0,036	130,00		4,00	0,270	23,00
19-6-2024	C6693578	24TWS-Mos-Dv-04	Dvorníky-04	6	4197,00	0,031	3,200	82,00	0,240	6,50	1,300	6,90	0,044	173,00	4,70	7,10	0,390	42,00
19-6-2024	C6673680	24TWS-Mos-Vc01	Včeláre-01	7	18719,00	0,230	8,500	148,00	3,000	45,00	5,900	33,00	0,120	672,00	29,00	120,00	4,100	248,00
19-6-2024	C6693682	24TWS-Mos-Za01	Zádiel-01	8	14296,00	0,180	18,000	325,00	3,900	37,00	9,700	22,00	0,100	912,00	24,00	509,00	11,000	11901,00
19-6-2024	C6693683	24TWS-Mos-Za02	Zádiel-02	9	6161,00	0,052	3,000	77,00	0,470	10,00	1,800	9,70	0,068	291,00	6,40	28,00	1,600	97,00
19-6-2024	C6693684	24TWS-Mos-Hs01	Hosťovce-01	10	3271,00	0,038	1,600	37,00	0,300	8,00	1,200	13,00	0,067	158,00	7,70	15,00	0,720	91,00
		SK-Hygienic Limit							0,800		130,00	36,00	0,300			85,000		140,00
					TW Indicative scale mg/kg (ds)													
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
					AI	Ag	As	Ba	Cd	Со	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
					Aluminium	Silver	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
								_		_		_		_	_		_	_
																	_	
													-				_	

											Mosses (Brya	iphyta) May	8-11, 2024: r	ng/kg Lower l	Bound (LB) W	et Weight (ww)				
							1	2	3	4	5	6	7	8	9	10	11	12	13	14
ab date	Lab Nr	TW-REF-NR	Loc.		ds %	wet	AI	Ag	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	NI	Pb	Sn	Zn
0101515					1000000	weight %	Aluminium	Silver	Arsenic	Barlum	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
9-6-2024	C6693670	24TWS-Mos-KS-Bron-01	Karst-01	1	32,57	67,43	589,52	0,01	0,24	7,49	0,07	0,18	0,91	2,25	0,04	24,17	0,59	2,87	0,15	10,4
9-6-2024	C6693672	24TWS-Mos-KS-02	Karst-02	2	22,97	77,03	114,16	0,00	0,04	5,28	0,01	0,06	0,21	1,98	0,01	4,30	0,00	0,37	0,03	4,8:
9-6-2024	C6693673	24TWS-Mos-KS-03	Karst-03	3	25,64	74,36	394,86	0,00	0,11	9,49	0,03	0,14	0,62	1,79	0,02	10,49	0,41	1,49	0,10	6,6
9-6-2024	C6693675	24TWS-Mos-Dv01	Dvorníky-01	4	80,41	19,59	5005,52	0,07	2,17	94,88	0,71	2,33	18,49	12,06	0,06	301,54	19,30	35,38	0,27	139,1
9-6-2024	C6673678	24TWS-Mos-Dv03	Dvorníky-03	5	88,53	11,47	851,66	0.02	0,48	27,44	0,10	0,27	1,68	3,45	0,03	115,09	0,00	3,54	0,24	20,3
9-6-2024	C6693578	24TWS-Mos-Dv-04	Dvorníky-04	6	88,16	11,84	3700,08	0,03	2,82	72,29	0,21	1,15	5,73	6,08	0,04	152,52	4,14	6,26	0,34	37,03
9-6-2024	C6673680	24TWS-Mos-Vc01	Včeláre-01	7	74,92	25,08	14024,27	0,17	6,37	110,88	2,25	4,42	33,71	24,72	0,09	503,46	21,73	89,90	3,07	185,80
9-6-2024	C6693682	24TWS-Mos-Za01	Zádiel-01	8	53,52	46,48	7651,22	0,10	9,63	173,94	2,09	5,19	19,80	11,77	0,05	488,10	12,84	272,42	5,89	6369,42
9-6-2024	C6693683	24TWS-Mos-Za02	Zádiel-02	9	43,87	56,13	2702,83	0,02	1,32	33,78	0,21	0,79	4,39	4,26	0,03	127,66	2,81	12,28	0,70	42,55
9-6-2024	C6693684	24TWS-Mos-Hs01	Hosťovce-01	10	67,82	32,18	2218,39	0,03	1,09	25,09	0,20	0,81	5,43	8,82	0,05	107,16	5,22	10,17	0,49	61,72
												TWI	ndicative sca	ale mg/k=L	b (ww)					
							1	2	3	4	5		6	8	9	10	11	12	13	14
							Al	Ag	As	Ba	Cd		Co	Cu	Hg	Mn	NI	Pb	Sn	Zn
							Aluminium	Silver	Arsenic	Barlum	Cadmium		Cobalt	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
										-		_			-	-				

Figure 49: Results heavy metals (LB) in mosses (Bryophyta), May 8-11th, 2024

<sup>&</sup>lt;sup>28</sup> Olajire, A.A., 1998. A survey of heavy metal deposition in Nigeria using the moss monitoring method. Environ. Int. 24 (8), 951–958.
<sup>29</sup> Musilová, J., et al. (2024). Impact of old environmental burden in the Spiš region (Slovakia) on soil and home-grown vegetable contamination, and health effects of heavy metals. Sci Rep 12, 16371 (2022). 8

### 7.5.2.0. Heavy metals in vegetables

To compare the results of heavy metals in the mosses with the EU safety limits for vegetation and fruit and the average limits in Slovakia. This concerns the heavy metals: Aluminium (Al, Arsenic (As), Barium (Ba), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Mercury (Hg), Nickel (Ni).

The maximum levels (ML) for heavy metals in the vegetable set have been exceeded for Cu, Pb, and Hg for the levels find in the biomatrices of mosses. Two metals Copper (Cu) and Zinc (Zn) have a bioaccumulation factor higher than one, meaning they will accumulate heavy metals from the soil into the vegetation.

#### 7.5.2.1. Silver (Ag)

Silver (Ag) is released into the environment through mining activities and the weathering of rocks. Anthropogenic sources of silver include cement manufacturing, cigarette tobacco, cloud seeding operations, coal combustion, smelting operations, steel and iron production, and urban refuse incineration. Silver concentration in mosses ranges from 0.011 mg/kg dm (reference in Slovak Karst National Park) and a maximum of 0.230 mg/kg dm measured in Včeláre.



Figure 50: Results Silver (Ag) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

#### 7.5.2.2. Aluminium (Al) in Mosses (*Bryophytes*)

The average aluminium (Al) concentration in vegetables like squash, carrots, marrow, cabbage, watercress and spinach is 27.5 mg/kg.<sup>30</sup> The Tolerable Weekly Intake (TWI) set by the European Food and Safety Authority (EFSA) is 1 mg/kg body weight/week.<sup>31</sup> The aluminium (Al) levels found in the TW biomonitoring research (2023-2024) in mosses are extremely high.

Aluminium (Al) is one of the most common metals found in the environment, and consequently, in food. However, aluminium levels have been increasing over time due to anthropogenic activities. Aluminium is a known neurotoxic agent because it tends to accumulate in the brain. Several studies have reported the correlation between aluminium levels and diseases such as Alzheimer's disease.<sup>32</sup> Aluminium also has the characteristic of interacting with the essential metals.<sup>33</sup>



Average level of aluminium (Al) in vegetables average Al concentration of 27.5 mg/kg (González-Weller et al.) Tolerable Weekly Intake (TWI) set by the EFSA at 1 mg/kg body weight/week

Figure 51: Results Aluminium (Al) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>30</sup> González-Weller D, Gutiérrez AJ, Rubio C, Revert C, Hardisson A (2010) Dietary Intake of Aluminum in a Spanish Population (Canary Islands). J Agric Food Chem 58: 10452-10457.

<sup>&</sup>lt;sup>31</sup> <u>EFSA (European Food Safety Authority) (2011) Statement on the Evaluation on a New Study Related to the bioavailability of</u> <u>aluminum in food. EFSA J 9: 2157.</u>

<sup>&</sup>lt;sup>32</sup> Shaw CA, Tomljenovic L (2013) Aluminum in the central nervous system (CNS): toxicity in humans and animals, vaccine adjuvants, and autoimmunity. Immunol Res 56: 304-316.

<sup>&</sup>lt;sup>33</sup> <u>https://www.heraldopenaccess.us/openaccess/aluminium-exposure-through-the-diet</u>

#### 7.5.2.3. Arsenic (As) in Mosses (*Bryophytes*)

The results for arsenic (As) in moss (*Bryophyta*) range from 2.7 – 18.0 mg/kg dm. The reference moss, collected in the Slovak National Park Karst, measured 0.17 mg/kg ds (dry substances) or dm (dry matter). The average arsenic level in vegetables is 0.05 mg/kg dm. There are differences in these arsenic levels between regular agriculture and private vegetable gardens. The transmission of arsenic from soil to vegetables depends on many soil quality parameters.

In the graph, arsenic levels in moss in the environment of the cement kiln are shown for 2023 and 2024. In Zádiel (Za-01), a concentration of 9.63 mg/kg wet weight (ww) is observed in this second biomonitoring research. Further research on the content of arsenic in vegetables in private gardens is needed. These TW results show a significant presence of arsenic in the environment of the cement kiln. Carcinogenic metals such as arsenic (As), cadmium (Cd), and chromium (Cr) can disrupt DNA synthesis and repair.



Figure 52: Results Arsenic (As) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

#### 7.5.2.4. Barium (Ba) in Mosses (*Bryophytes*)

Cement with barium is used as a binder with resistance to different types of radiation. Barium is widely used in manufactured materials such as tiles, automobile clutch and brake linings, rubber, brick, paint, glass, and other materials. Unusually high concentrations of this metal in soils may be a marker for anthropogenic activity. Barium is also abundantly present in waste.

Barium concentrations in the mosses of the environment of the cement kiln range from 23 – 325 mg/kg dm. The lowest value is measured in the Slovak Karst National Park, and the highest is in Zádiel. Urban garden leafy greens have a barium concentration of 45.7 mg/ kg dm, and root crops 21.5 mg/ kg dw. <sup>34</sup> There are no health-based standards for guidance values made for Ba in food crops. Therefore, the results reported here cannot be interpreted in the context of risk, however, barium levels in moss Northeast are elevated. In Zádiel, 14 times more than measured in SKNP. There is a lack of correlation between vegetable and paired soil concentrations for barium (Ba), which can be attributed to several important factors, including the strong effect of soil pH, organic matter content, and other soil properties on metal solubility and bioavailability.



Barium in leafy green vegetables : 45.7 mg/ kg dw (McBride et al. 2015).

Figure 53: Results Barium (Ba) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>34</sup> McBride et al. (2015). Environ Pollut; 194: 254–261

### 7.5.2.5. Cadmium (Cd) in Mosses (*Bryophytes*)

The limit of Cadmium in the soil of vegetable gardens in the Netherlands is 0.6 mg/kg ds (NI), which is based on the transmission into vegetables. In leafy vegetables, the safe limit is 0.2 mg/kg ds (EU-limit) and for radishes, the maximum level is 0.020 mg/kg ds. Cadmium is a neurotoxic metal, and emission sources are related to combustion processes. The use of waste as an input material has increased the levels of cadmium, zinc, lead and cobalt found in cement. <sup>35</sup> **Elevations of cadmium, lead and zinc have polluted vegetables and soil in family allotment gardens due to mining and industrial activities.**<sup>36</sup> In the figure below, concentrations of cadmium were elevated in 2024, and high levels were found 2-3 km from the plant. The levels in mosses are compared with the EU regulations for cadmium in leafy vegetables.<sup>37</sup> The numbers in the right graph are expressed in wet weight for comparison. Findings of levels 10 times over the safe limit in vegetables can cause kidney failure, bone demineralisation, and an increased risk of cancer. <sup>38</sup> The tolerable weekly intake (TWI) for cadmium is 2.5 µg kg/bw. Inhabitants in this area consume many products from their gardens. Cadmium is nephrotoxic and causes renal tubular dysfunction, renal stone formation, and osteoporosis.

This finding of high levels of cadmium in moss in this area indicates a correlation between the activities of the cement kiln and the deposits of this industry in the nearby area. The study suggests that the consumption of vegetables cultivated in the environment of the cement kiln may pose a significant health risk to consumers.





Figure 54: Results Cadmium (Cd) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>35</sup> M. Achternbosch, M. et al. (2005). Impact of the use of waste on trace element concentrations in cement and concrete, Waste Management and Research 23, 328–337.

<sup>&</sup>lt;sup>36</sup> Ćwieląg-Drabek, M., Piekut, A., Gut, K. et al. (2020). Risk of cadmium, lead and zinc exposure from consumption of vegetables produced in areas with mining and smelting past. Sci Rep 10, 3363.

<sup>&</sup>lt;sup>37</sup> (EU) 2023/915 https://eur-lex.europa.eu/legal-content/NL/TXT/PDF/?uri=CELEX:32023R0915

<sup>&</sup>lt;sup>38</sup> Dziubanek, et al (2015). Contamination of food crops grown on soils with elevated heavy metals. Ecotoxicol. Env. Saf. 118, 183–189

#### 7.5.2.6. Cobalt (Co) in Mosses (*Bryophytes*)

Levels of cobalt (Co) in the study area are 1.9 - 45.0 mg/kg dm and 1.7 - 33.7 mg/kg ww. The levels in the SNPK are 0.91 - 2.8 mg/kg dm and 0.2 - 0.9 mg/kg ww. Levels of Co are elevated in the research area. The consequences of elevated cobalt levels in vegetables are unknown. The findings highlight the need for continuous monitoring and research to prevent contamination and formulate guidelines for local food safety guidelines.



Figure 55: Results Cobalt (Co) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

#### 7.5.2.7. Chromium (Cr) in Mosses (*Bryophytes*)

Due to its wide industrial use, chromium is considered a serious environmental pollutant. The permissible value in plants is 1.3 mg/kg. The values in the graph are recalculated for wet weight. The chromium levels in the environment of the cement kiln exceed the limit for chromium in vegetables (1.3 mg/kg ww). In the literature, levels of chromium in vegetables from highly contaminated Cr soils are measured at 9.6 mg/kg. <sup>39</sup> In Včeláre and Zádiel, the permissible limit is surpassed by 34 resp. 28 times, respectively. Chromium (Cr) has been classified as a carcinogen by the International Agency for Research on Cancer. Cement dust exposure has a significant correlation with laryngeal cancer among workers exposed to cement dust in an epidemiological study. <sup>40</sup> Research found evidence that residents living near cement dust factories have an increased incidence of diseases linked to heavy metal toxicity.<sup>41</sup>



Permissible value chromium vegetables: 1.3 mg/kg ww

Figure 56: Results Chromium (Cr) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>39</sup> Cary E E and Kubota J 1990 Chromium concentration in plants: effects of soil chromium concentration and tissue contamination by soil. J. Agric. Food Chem. 38, 108–114.

 <sup>&</sup>lt;sup>40</sup> Dietz, A., Ramroth, H., Urban, T., Ahrens, W., Becher, H., 2004. Exposure to cement dust, related occupational groups and laryngeal cancer risk: results of a population based case-control study. Int. J. Cancer 108, 907–911.
 <sup>41</sup> Abimbola, A.F., Olusegun, O., Philips, K., Olatunji, A.S., 2007. The Sagamu cement factory, SW Nigeria: is the dust generated a potential health hazard? Environ. Geochem. Health 2, 163–167.

#### 7.5.2.8. Copper (Cu) in Mosses (*Bryophytes*)

Copper (Cu), a toxic metal pollution found in the soil and water of industrialised areas, causes ongoing issues for agriculture product contamination and human health hazards. However, information on copper phytotoxicity and its accumulation in vegetables is largely unknown. Copper has a high bioaccumulation factor for vegetables. Copper levels in moss in the environment of the cement kiln range from 3.9 - 33.0 mg/dm. The reference levels of copper in the Slovak Karst National Park measure 3.9 - 8.6 mg/kg dm.

The EFSA establishes an Acceptable Daily Intake (ADI) of 0.07 mg copper/kg bw, equivalent to 5 mg Cu/day for adults. It should be noted that the term 'ADI' was not fully adequate for copper, which is a micronutrient essential for life. The term 'upper limit' in the nutrient area would be more appropriate. Therefore, in the specific case of copper, the ADI is considered equivalent to a UL.'<sup>42</sup> The average concentration of copper in food samples is 1.22 mg/kg ww.<sup>43</sup> The results of copper in mosses expressed in wet weight, 3.90 - 33.00 mg/kg ww (reference samples 1.8 - 2.3 mg/kg ww), are more than ten-fold higher in the environment of the cement plant.



Maximum Limit potato 3 mg/kgww

Figure 57: Results Copper (Cu) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>42</sup> <u>https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.7728</u>

<sup>&</sup>lt;sup>43</sup> Arnika (2019). Heavy metals in soils, foodstuffs, and human hair in the mining

and metallurgical communities of Alaverdi and Akthala, Lori province of Armenia

#### 7.5.2.9. Mercury (Hg) in Mosses (*Bryophytes*)

Mercury (Hg) is recognised as a toxic, persistent, and mobile contaminant that does not degrade in the environment and is the only element in the periodic table with its environmental convention, the Minamata Convention on Mercury. Mercury exposure can also reduce photosynthesis, transpiration rate, water absorption, and chlorophyll synthesis. Mercury is toxic to humans in all its primary forms, with the most toxic being methylmercury. The WHO considers mercury to be one of the top ten chemicals or groups of chemicals of major public health concern.

In Eastern Slovakia, there is a significant environmental burden from old mining activities, causing exceedances of the limit values for Hg, Cu, Zn, As, Cd, and Pb in soils. There have been warnings about the consumption of food due to high mercury levels. This area is about 30-50 km from the area of this research. The levels of mercury found in mosses in the environment of the cement kiln range from 0.036 -0.110 mg/kg dm and recalculated to fresh weight 0.030 - 0.090 mg/kg ww. This means that the found results for mercury exceed the maximum limit (ML) for tomatoes, and carrots, which is 0.03 mg/kg ww. In Dvorníky 200% and an exceedance of 300% in Včeláre. Only the reference samples in the SKNP comply with this maximum limit for mercury (Hg). <sup>44</sup>



Maximum limit tomatoes, and carrots: 0.03 mg/kg ww, potatoes 0.02 mg/kg ww (Musilová et al. 2022)

Figure 58: Results Mercury (Hg) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>44</sup> Musilová, J., Franková, H., Lidiková, J. et al. Impact of old environmental burden in the Spiš region (Slovakia) on soil and home-grown vegetable contamination, and health effects of heavy metals. Sci Rep 12, 16371 (2022). https://doi.org/10.1038/s41598-022-20847-8

#### 7.5.2.10. Manganese (Mn) in Mosses (*Bryophytes*)

Manganese is one of the major trace metals in ordinary Portland cement, which is mainly derived from alternative fuels and secondary raw feeds during the combustion process of clinker production. No monitoring program is applied for this heavy metal by the cement kiln. However, manganese emissions and deposition are related to incinerator processes (Rovira).<sup>45</sup> Long-term exposure to high levels of manganese can result in effects on the central nervous system such as visual reaction time, hand-eye coordination and hand steadiness. Exposure to higher levels over a long period can result in a syndrome known as manganism, which leads to feelings of weakness and lethargy, tremors and psychological disturbances. The accumulation of Mn occurs mainly in the basal ganglia and leads to a syndrome called manganism, whose symptoms of cognitive dysfunction and motor impairment resemble Parkinson's disease (PD). Various neurotransmitter systems may be impaired due to Mn, especially dopaminergic, but also cholinergic and GABAergic systems.

In this study, elevated levels of manganese in mosses are found in the environment of the cement kiln. The manganese concentrations in mosses in the study area are more than 10-fold higher than the levels of manganese in SNPK 18.7 - 74.20 mg/kg dm or 4.3 - 24.2 mg/kg ww. The graph shows high levels and urges further research on vegetables grown in the garden. Consumption of high manganese (Mn) concentrations can cause neurodegenerative disorders, cardiovascular toxicity, and liver damage.



Figure 59: Results Manganese (Mn) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>45</sup> Rovira J, Mari M, Nadal M, Schumacher M, Domingo JL (2010) Environmental monitoring of metals, PCDD/Fs and PCDs as a complementary tool of biological surveillance to assess human health risks. Chemosphere 80(10):1183–1189

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

#### 7.5.2.11. Nickel (Ni) in Mosses (*Bryophytes*)

Nickel (Ni) is essential for proper plant growth and development. However, at high levels, nickel alters plant metabolic activities, and inhibits enzymatic activity, photosynthetic electron transport, and chlorophyll biosynthesis. Human exposure to nickel (Ni) mainly occurs through oral ingestion of water and food contaminated with nickel. Ni, as an immunotoxic and carcinogenic substance, can cause various health effects, including contact dermatitis, cardiovascular disease, asthma, pulmonary fibrosis, and respiratory cancer. The CONTAM Panel identified reproductive and developmental toxicity as the critical effect for the risk characterization of chronic oral exposure to Ni and established a tolerable daily intake of 2.8  $\mu$ g Ni/kg body weight (bw) per day (EFSA) 46. Due to inter- and intra-individual variability, it is not possible to back-calculate the contribution of intake from food to Ni concentration. Average occurrences in vegetables and vegetable products (including fungi) ranged from 0.065 – 0.330 mg/kg.



Figure 60: Results Nickel (Ni) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>46</sup> EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2015. Scientific Opinion on the risks to public health related to the presence of nickel in food and drinking water. EFSA Journal 2015; 13(2):4002, 202 pp.

#### 7.5.2.12. Lead (Pb) in Mosses (*Bryophytes*)

Lead is a naturally occurring element and is one of the longest-established poisons. Lead levels in mosses 2024 range from 0.4 – 272.0 mg/kg ww, expressed in dry matter 1.6 – 590.0 mg/kg dm. In Zádiel, the highest level of lead 272 mg/kg ww was found in mosses. The EU regulation for the maximum level (ML) of lead in food as vegetables is set at 0.1 mg/kg ww.<sup>47</sup> This ML implicates an exceeding of the maximum permitted limit for lead in mosses by a factor of 1000. All moss samples in the research area exceeded the maximum limit for lead in vegetables. Lead levels in moss of SNPK are: 1.6 - 8.8 mg/kg/dm and expressed in fresh weight 0.4 - 2.9 mg/kg ww. The current consensus is that no level of lead exposure should be considered "safe". New evidence regarding blood levels at which morbidities occur has prompted to reduction of screening guidelines. Measurable cognitive decline (reduced IQ, academic deficits) has been found to occur, especially in children.<sup>48</sup>Lead (Pb) is a highly toxic element, bioaccumulative, and doesn't degrade easily metabolized in the environment. Food is a significant source of Pb exposure, and the potential risk to the population may stem from the bioaccumulation of Pb in edible vegetables. Lead exposure can cause plumbism, anaemia, nephropathy, gastrointestinal colic, and central nervous system symptoms. Neurological symptoms include ataxia, encephalopathy, seizure, swelling of the optic nerve, and disorder of consciousness. In the Netherlands, the government discourages the cultivation of consumer crops or recommends growing them only in containers to reduce exposure through consumption. The findings highlight the need for more precise monitoring to ensure a safe environment for the population.



(EU) 2023/915 : 0.1 mg/kg ww. Brassica, legume, stem vegetables. Cereals 0.2 mg/kg ww.

Figure 61: Results Lead (Pb) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>47</sup> https://eur-lex.europa.eu/legal-content/NL/TXT/PDF/?uri=CELEX:32023R0915

<sup>&</sup>lt;sup>48</sup> Rădulescu, A., Lundgren, S. A pharmacokinetic model of lead absorption and calcium competitive dynamics. Sci Rep 9, 14225 (2019). https://doi.org/10.1038/

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

#### 7.5.2.13. Tin (Sn) in Mosses (*Bryophytes*)

Parameters that influence heavy metal concentration include erosion, mining, industrial processes, and incineration of commercial and domestic waste. Tin has the potential to be absorbed by plants and concentrated in green leaves due to its mobility and biological transport. The occurrence of tin in soil and vegetables has not received much study, and earlier studies were done on processed food. The concentrations of tin in vegetables are found to be several times higher than in soil or water.

The permissible limit of tin in the soil is 2 mg/kg. All soil samples around the cement kiln comply only with a factor 2-3 higher. Levels of tin in perennial and annual plants averaged 0.4 mg/kg. <sup>49</sup> The study does not clarify whether the results are expressed in fresh weight (ww) or dry matter (dm). The acceptable limit for human consumption of tin in vegetables is <1  $\mu$ g·/g. <sup>50</sup> The levels of Sn in moss in the research area are 1.6 – 11.0 mg/kg dm and 0.2 – 5.9 mg/kg ww. The reference values are 0.1 – 0.5 mg/kg ds and 0.03 – 0.15 mg/kg ww. Further research is needed if the Tin levels exceed the EU and WHO limits when vegetables are consumed in this research area of Slovakia.



Average concentration Sn Lettuce: 0.4 mg/kg, spinach: 0.5 mg/kg

Figure 62: Results Tin (Sb) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>49</sup> Bahareh Ghasemidehkordi, Ali Akbar Malekirad, Habibollah Nazem, Mohammad Fazilati, Hossein Salavati & Mohammad Rezaei (2017): Tin Levels in Perennial and Annual Green Leafy Vegetables, International Journal of Vegetable Science, DOI: 10.1080/19315260.2017.1291548

<sup>&</sup>lt;sup>50</sup> World Health Organization. 2004a. Evaluation of certain food additives and contaminants. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization, Geneva.

#### 7.5.2.14. Zinc (Zn) in Mosses (*Bryophytes*)

Zinc (Zn), an activator of enzymatic reactions, is an indispensable trace element for plants and microorganisms. However, when the environmental levels of Zn exceed those required by plants or microorganisms, toxic effects can result. Atmospheric emissions of Zn from anthropogenic sources are an important source of Zn, which can then enter the human body by dispersion, deposition, assimilation by plants and transfer through the food chain. This can result in adverse human health effects. Additionally, excessive exposure to Zn from the ambient air can cause chronic bronchitis, peritonitis, emphysema, asthma and even lung cancer. <sup>51</sup>

Zinc is a heavy metal and is not monitored by the cement kiln, although zinc is predominantly present in waste streams and especially in car tyres. When tyres are burned, zinc will be emitted as a highly volatile metal. The average level of zinc in lettuce is 2.7 mg/kg ww and in spinach 6.1 mg/kg ww. The levels found in mosses in the environment of the cement kiln are 23.0 - 11.901.0 mg/kg dm or 20.4 - 6369.4 mg/kg ww. In the SNPK the levels of moss are 21.0 - 32.0 mg/kg dm or 4.9 - 10.4 mg/kg ww.



Average concentration Zn Lettuce: 2.7 mg/kg, spinach: 6.1 mg/kg

Figure 63: Results Zinc (Zn) in moss expressed in dry (dm) and wet weight (ww) in mg/kg

<sup>&</sup>lt;sup>51</sup> Li, Z.; Huang, Y.; Li, X.; Wang, G.; Wang, Q.; Sun, G.; Feng, X. Substance Flow Analysis of Zinc in Two Preheater–Precalciner Cement Plants and the Associated Atmospheric Emissions. Atmosphere 2022,13,128. https://doi.org/ 10.3390/atmos13010128

<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024

### 7.5.2.15 Physical landforms and wind patterns

Moss location Dvorníky 03 (Dv-03) is located at 1050 m distance to the North of the cement plant. It is a small hill (around 100 m in height), covered with native deciduous and gymnosperm (coniferous) trees, mosses, shrubs and flowers. This green coverage of trees and plants, which is secluded in the graveyard as well, might protect the plants on the ground from the deposition of POPs by wind streams. The results of the heavy metals in the mosses of this location (Dv-03) are comparable with the results of the mosses of the reference locations in the Slovak Karst National Park > 20 km distance to the West. Hypotheses could be that land covered with trees, such as native forest or planted with gardens and (cemetery) parks, as well as slopes not oriented towards the main wind direction, protect from POP contamination of the surrounding area, depending on the prevailing wind direction and fumigation due to the specific physical landforms of an area.



Figure 64: Wind behavior in specific physical landforms Zádiel

Location Zádiel 01 (Za 01) shows very high values of heavy metals in the mosses (Bryophyta), like locations Vc-01 and in somewhat lower values also on Dv-01, these are all located North/West from the cement kiln, on a short distance (<3,5 km). On the moss three (3) locations further to the West (>20 km) in Slovak Karst National Park, the mosses show very low values of heavy metals and can be therefore seen as real reference samples. The reason location Zádiel 01 (Za-01) show high values of heavy metals in mosses (Bryophyta) could be the northern location. Mosses were collected near a barn, made from iron plates under the roof covering, on a long, small strip of soil covered with stone pebbles with no gutter attached. Rainwater from the roof is absorbed by mosses on the ground.

Location Zádiel 02, (Za-02) is 200 m on the other side of the road, with a long garden to the East. Mosses (Bryophyta) were collected from stone roof tiles which covered the small wooden shed on the North side of it. The garden is surrounded by high old (orchard) trees. This could be the reason for lower values of heavy metals measured at this location.



Figure 65: Moss (Bryophyta) Zádiel 01 and 02

Zádiel-02

### 7.5.3 PAH in mosses (Bryophyta)

In the various national and EU regulations of PAHs, the summation of concentrations of 4 or 10 PAH substances is adopted. Table ... on page ...shows the different relative toxicity values expressed as relative potency (REP) factors. For example, Benzo(b)fluoranthene, the most toxic PAH, is 10,000x more toxic than anthracene. This clearly shows that simply adding up the concentrations of PAH congeners is by no means enough to show the actual toxic load. The regulations need to be updated. An additional problem, which is also present with other highly hazardous substances, is that the analytical possibilities are extremely limited. Out of about 4-700 different PAH substances, only 16 can be analysed with a chemical analysis method GC-MS/MS, see Figure 21, while the brominated, chlorinated or any other substitutions are not being examined. In the figure below the 16 different PAH substances are shown and ordered by the number of benzene rings in their PAH molecular structure.

In 2024, PAH concentrations in mosses range from 109.9 - 5530.5 mg  $\geq$ 16PAH/kg dm. The increase in Hosťovce from 1065.6 mg  $\geq$ 16PAH/kg dm in 2023 to 5530.5 mg  $\geq$ 16PAH/kg dm in 2024 is striking. There has been no change in the property of this location, nor has fire or burning occurred. The graph below shows the increases of the different congeners. Especially prominent is the increase in the concentration of Benzo(a)pyrene (B[a]P).



Figure 66: Benzo(a)pyrene in mosses

The graph below shows the increases of the different congeners. Especially prominent is the increase in the concentration of Benzo(a)pyrene (B[a]P) to 1600 mg  $\geq$ 16PAH/kg. Also, Benzo(a)fluoranthene is with 2100 mg  $\geq$ 16PAH /kg ds prominent present.


Figure 67: PAH-16 in mosses (Bryophyta)

### 7.5.4. PFAS in mosses

In 2024, six moss samples were negative for PFAS. No PFAS above the LOQ could be detected. One moss sample in Včeláre measured 0.6  $\mu$ g PFTrDA/kg dm. This PFAS congener is six times found in eggs. Further screening with the chemical analyses of >4 PFAS and the application of PFAS CALUX bioassay is recommended to address the potential underestimation of PFAS contaminationin this area.



Figure 68: PFAS in mosses

2<sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024



# 7.6. Results in Eggs of backyard chicken

Figure 69: Egg sample locations

# 7.6.1. Dioxins in Eggs

In the 1<sup>st</sup> TW biomonitoring research of October 2023, high levels of dioxins were found in eggs from backyard chickens at three locations using the bioassay DR CALUX. The highest dioxin level of 9.8 pg BEQ/g fat in 2023 was found at **Zádiel (Za-O2) in 2023.** In this 2nd research (May 2024), the dioxin level at the same location Za-O2 was slightly reduced by 23% to **7.50 pg BEQ/g fat**. However, it still exceeds the EU limit of 3.3. pg BEQ/g fat, as shown in the figure below. At the **Hosťovce (Hs-O1) location**, dioxin levels have increased by 83% to **8.6 pg BEQ/g fat**. At two (2) other locations, Dvorníky and Včeláre, dioxin levels increased by 358% to 5.50 pg BEQ/g fat. At Zádiel-O1, further south, no dioxin levels above the EU limit were measured.



Figure 70: Dioxins in eggs of backyard chicken

An exceedance of the EU regulation for bioassay results with the DR CALUX (3.3 pg BEQ for the sum of PCDD/F/dl-PCB), mandates chemical analysis on six egg samples. The results of these measurements are shown in the graph below. The dioxin level at Zádiel exceeds the EU limit by 5.4 pg TEQ/g fat and should not be consumed. The chemical analysis shows lower TEQ levels than the bioassay of DR CALUX due to the omission of measurement of other substances with dioxin-like properties, such as brominated dioxins. See the chapter above for further details. The correlation between the DR CALUX and GC-MS results to EU regulations is explained by Hoogenboom et al. <sup>52</sup>



Figure 71: Dioxins (GC-MS\_ in eggs of backyard chicken

Remarkable is the higher value of dI-PCB measured in Hosťovce by the bioassay. The graph below clearly shows that the PCDD/F levels are always higher than the chemical analysis for dioxins. This is a strong indication that more dioxins are present in the eggs than can be measured by the limited chemical analysis.

Comparatively, the understanding and study of other unintentionally generated POPs (such as dI-PCBs and polybrominated dibenzo(p)dioxins and furans (PBDD-Fs)) from cement plants are not as well documented and may be more difficult to measure due to either their lack of presence in the flue gas or the analytical ability to detect them. Even if a plant performs to its engineered conditions to achieve stoichiometric combustion, the reality is that incomplete combustion and pollutant formations do occur. <sup>53</sup>

<sup>52</sup> Hoogenboom LAP et al. (2010). Revised EU-criteria for applying bioanalytical methods for screening of feed and food for dioxins and dioxin-like PCBs, Organohalogen Compounds Vol. 72, 1800-1905 (201)

<sup>&</sup>lt;sup>53</sup> Richards G. and Igor E. Agranovski I.E. (2017), Dioxin-like pcb emissions from cement kilns during the use of alternative fuels, Journal of Hazardous Materials, Volume 323, Part B, Pages 698-709, ISSN 0304-3894,



Figure 72: Comparison of chemical analyses and bioassay analyses on PCDD/F/dl-~PCB



Figure 73: Comparison of chemical analyses and bioassay analyses on PCDD/F and dl-~PCB separately

In the case of waste co-incineration, catalogue number 16 01 03 (worn tyres) are also recovered by the activities of R4 and R5, as the metal part of the tyre (steel cord, approximately 40% of the weight of the tyre) melts at high temperatures in the rotary kiln and becomes part of the resulting clinker product or cement. The levels of the two furans pentachlorodibenzofuran and hexachlorodibenzodifuran are prominently present at the locations of Dvorníky and Zádiel. In the study by Richards, a statistical predominance of the 1,2,3,7,8-PeCDF and 1,2,3,4,7,8-HxCDF congeners was found about combustion of alternative fuels like tyres, PCB-oil and plastic. When these two furans are placed on the maps with indicative colours a clear pattern can be observed. The chemical (GC-MS) analyses on dioxins in eggs of backyard chickens shows clearly the presence of the characteristics of these typical combustion congeners, just in the village of Dvorníky.



Figure 74: Pentachlorodibenzofuran (1,2,3,7,8-PeCDF) in eggs of backyard chicken



Figure 75: Hexachlorodibenzodifuran (1,2,3,4,7,8-HxCDF) in eggs of backyard chicken

The spatial deposition mapping of PCB 126, the most toxic dioxin-like PCB, show another pattern. The emission of PCB 126, as well as the other non-ortho PCB 169, is related to incomplete combustion. The high levels of dioxins and PCB 126 at Zádiel are likely caused by the geographical characteristics of that area, forming a bowl-like structure where emissions can be deposited more concentrated in the environment. The high levels of PCB 126 is remarkable and need to be researched further.



Figure 76: PCB 126 in eggs of backyard chicken

### 7.6.2. Heavy metals in eggshells

For this second TW biomonitoring research (May 8-11, 2024) eggshells were taken from four (4) egg locations and one (1) reference location (blue bar/green square, ref. mark) from a colony of wildlife birds of the species Heron (Ardea), living near Lake Hrhov (Hrhovské rybníky), near Slovak Karst National Park, as shown on the location map on page 19. These five (5) eggshell samples were analysed for 14 heavy metals. In the first biomonitoring research, eggshells from one location (Dv-O2) were analysed for 14 heavy metals. Heavy metals were also determined in the eggshells. Besides the eggshells of backyard chickens, TW took r reference eggshells from Herons at the Lake Hrhov (Hrhovské rybníky). The high levels of aluminium and barium in the eggshells of herons were remarkable. At the time of sampling, several herons had fallen from the trees, including young ones. Additional results from other countries have been included to provide context and help interpret these findings. Cadmium (Cd), chromium (Cr), cobalt (Co), manganese (Mn), mercury (Hg), silver (Ag) and tin (Sn) could not be measured above the limit of quantification (LOQ). High results of Zinc (Zn) and Nickel (Ni) are found in the eggshells of the wildlife Heron (Ardea).





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### 7.6.3. PFAS in Eggs backyard chicken

PFAS analyses were carried out on the eggs. PFOS was measured in all eggs. In eggs from Turňa nad Bodvou, six (6) different PFAS congeners were detected. In 2023, the highest PFAS concentration was found in Zádiel. In 2024, PFAS concentrations decreased; however, all eggs from backyard chickens were positive for PFAS. The graph below also shows the results from ECB. The difference is that TW uses PFAS analysis on 24 congeners, whereas ECB only analyses four (4) PFAS congeners.



Figure 78: PFAS in eggshells



# 7.7 Results in Meat of wildlife deer, Carp fish and domestic cow

Figure 79: Results in meat wildlife deer, carp fish and domestic cow

# 7.7.1. Dioxins in Meat

In this second TW biomonitoring, wildlife meat samples from Carp fish (Cyprinus carpio) and deer from the nearby forest in the western direction of Včeláre were provided for analysis. The Carp, approximately 2-3 months old, was caught in the waters of Lake Hrhov (Hrhovské rybníky), according to the fisherman's information. The deer, also approximately 2-3 months old, had its meat provided by a local farmer from Včeláre. The calf was approximately 2-3 months old. The bioassay DR CALUX for analysis on dioxins, showed that only dioxin-like PCB was found in the cow, as shown in the table in the figure below.



Figure 80: Dioxins in fish, deer and cow

# 7.7.2. PFAS in wildlife Carp fish

Some substances in the PFAS family are very carcinogenic at very low concentrations while spreading fast and widely throughout the environment. These substances of very high concern (SVHC) are extremely persistent and known as 'Forever Chemicals'. The consumption of 'fish and fish products is one of the most contaminated food groups with PFAS. ToxicoWatch analysed the freshly caught meat and liver of Carp fish (*Cyprinus carpio*) from the natural waters of Lake Hrhov (Hrhovské rybníky on PFAS with chemical analyses of  $\Sigma$  24 PFAS substances. The results for PFAS in the Carp liver detected 0.2 PFOS/g dm.



See also section 7.2.1. for the explanation about PFAS in water streams.



<sup>2&</sup>lt;sup>nd</sup> TW Biomonitoring in the environment of cement plant - Cementáreň Turňa nad Bodvou, May 2024



All PFAS chemical results are included in the graph below, including results on carp, fruit and moss.

Figure 82: PFAS analyses overview

# 7.8 Results in Wool of domestic sheep

The analyses of these wool samples for this study are pending and will be included in the 3<sup>rd</sup> TW biomonitoring report.



Figure 83: Sheep wool sample locations

# 8. Counter research

### 8.1 Comparison research Danucem Slovensko (ECB)

Danucem Slovakia a.s., owner of the cement plant Cementáreň Turňa nad Bodvou, ordered Ekotoxikologické centrum Bratislava s.r.o. (ECB) to undertake research on eggs of backyard chickens in the environment of the cement kiln. The eggs from 11 locations were sampled from a broader area than the research radius of 2,5 km radius applied by TW. At the site near Bratislava, Bernolákovo, a reference collected by ECB shows levels of dioxins of 0.06 pg TEQ/g fat for the sum of dioxins, which is comparable to supermarket eggs. The results of this study are compared with the results of TW's 1<sup>st</sup> and 2<sup>nd</sup> biomonitoring studies. In the graphs below, four (4) locations can be compared. The results have a similar qualitative outcome: eggs in the environment of the cement kiln are contaminated with dioxins, but quantitatively, there are remarkable differences. **In Dvorníky**, the difference factor is **2.8**, **Zádiel 5.6** and **Hosťovce**, shows a factor of **7.2** in the outcome of the chemical analyses for dioxins. Eggs sampled in Smolník , 17.2 km from the cement kiln, show extremely high dioxins at 75.9 pg TEQ/g fat and a dl-PCB content of 58.86 pg TEQ/g fat. Such values are exceptional and encountered in developing countries and warrants immediate action of this serious contamination. The result at Smolník can be considered as an outlier and highlights the difficulty of finding locations without any confounding factors, as can be found in Eastern Slovakia from old mining activities in regions relatively near Smolník.<sup>54</sup>.



Figure 84: Comparison results ECB and TW

<sup>&</sup>lt;sup>54</sup> Musilová, J., Franková, H., Lidiková, J. et al. Impact of old environmental burden in the Spiš region (Slovakia) on soil and home-grown vegetable contamination, and health effects of heavy metals. Sci Rep 12, 16371 (2022). https://doi.org/10.1038/s41598-022-20847-8

# 8.1.2. Counter research Košice Regional Government (Ekolive)

On behalf of the Kosice Regional Government, Ekolive surveyed the environmental surrounding area of the cement plant - Cementáreň Turňa nad Bodvou. Eighty-one (81) samples were analysed for soil, tree bark, mosses and two (2) eggs from backyard chickens. The analyses were performed for dioxins (chemical GC-MS), PAH (Chemical: GC-MS/MS on  $\Sigma$  16 PAH) and heavy metals.

In terms of dioxins, this research confirms the pressure of these toxic substances in the environment of cement plant - Cementáreň Turňa nad Bodvou. In soil, Ekolive did not find exceeding of dioxins, however, high levels of dioxins were found in biomass as mosses and tree bark. The Košice Regional Government (by Ekolive) confirm the findings of TW of increased dioxins in backyard chicken eggs.

Concerning the heavy metals, TW cannot agree with the conclusion that the soil complies with the Slovak rules for heavy metals, as the limits applied are intended for industrial purposes. No safety rules have been implemented to protect the health of inhabitants living in the surrounding villages and areas. Many people grow vegetables in their gardens, and they should be able to rely on the safety of their food. Likewise, children should have access to playgrounds with soil which does not pose health risks. In the Netherlands, when it comes to the healthy growth of vegetables in a vegetable garden, any increase in the background value of a heavy metal is considered an alarm signal. Based on this principle, the levels of 14 metals detected by TW indicate a problem for the environment. The mosses biomatrices exceed in this study exceed limits EU limits for food many times over. The use of mosses (*Bryophyta*) as a biomarker is becoming more prevalent and would be appropriate for the Kosice Regional Government and/or the cement industry to apply it on a structural basis. It shows that analysis of soil alone gives limited insight into the toxic load of an environment. Multiple parameters are most certainly needed such as biomonitoring in biomass

TW Recommendations for cement kiln emissions in Turňa nad Bodvou:

- (a) Semi-continuous measurements are necessary to monitor actual emissions, as the current short term of measurement represents only 0.2% per year.
- (b) Independent organisations should conduct these cement kiln industry emission measurements.
- (c) Transparency in the publication of emission data from the cement kiln, i.e. not corrected averages, just real-time data of production and incidents.

TW endorses these recommendations of Ekolive in the interest of the health of people living near industries like the cement kiln. In addition, biomonitoring should also be applied to the monitoring of mining of limestone and dust emissions during, production, transport, and use of products (cement) and end-of-life product disposal.



Figure 85: EU regulation dioxins for waste incinerators

# 9. Conclusions 2<sup>nd</sup> TW biomonitoring May 2024

From May 8-11, 2024, sixty-three (63) samples of eggs/eggshells of backyard chickens, wildlife meat from deer, Carp fish (Cyprinus carpio), wildlife bird eggshells from Heron (*Ardea*), mosses (*Bryophyta*), pine needles (*Picea abies*), water from natural water stream and wells, sediment from natural water streams and wells, and soil were collected by the TW team.

Key Findings of this 2nd TW biomonitoring:

### Soil:

1. Heavy Metals (14): Seriously contaminated soil with lead (Pb), 110 mg/kg and Arsenic (As) 48 mg/kg at the children's playground in Dvorníky-Včeláre at a short distance from the cement kiln.

### Water, natural streams:

**2.** PFAS: High value of PFAS with assay PFAS CALUX is found **1600 μg PFOA eq./g dm** measured in the Brook Turňa (potok Turňa) water stream.

### Sediment:

- **3.** Heavy Metals (14): The results of heavy metals in sediment on the reference locations in the Slovak Karst National Park at > 20 km West of the cement plant show lower values than the results of heavy metals in soil on the seven (7) locations within a short distance of 3,5 km from the cement kiln.
- **4. PFAS:** The chemical PFAS analysis shows the presence of **0.2 μg PFOS/kg** dm and with PFAS CALUX **no PFAS is found on the reference locations** in Hrhov, Jablonov and Blatný potok brook in Zádiel.

### Vegetation / Pine needles (*Picea abies*):

- PAH: The PAH results of the pine needle samples collected in May 2024 are between 48.1 207.5 μg ∑ 16 PAH/g dm. Location seven (7), at Turňa nad Bodvou, shows elevated levels of Phenanthrene and Fluorene, resp. 43.0 and 86.0 μg/kg dm.
- 6. PAH: Specific combustion-related (4 and 5 rings) PAH congeners, such as Benzo[a]pyrene, are found in mosses (*Bryophyta*) and pine needles (*Picea abies*) in the surrounding area of the cement kiln. Remarkably also in pine needles (*Picea abies*) at one location in the protected area of Slovak Karst National Park.
- 7. Heavy Metals (14): high results of lead (Pb), Cadmium (Cd) and Tin (Sn) are found in the pines of the Slovak Karst National Park.
- Dioxins: Six (6) out of seven (7) pine needle locations show results exceeding the EU-limit of 0,75 pg/g TEQ for feed for the sum of dioxins (PCDD/F/dl-PCB), in case pine needles are consumed like vegetables/feed.

### Vegetation / Mosses (Bryophyta):

- 9. Dioxins: The results show higher values for dioxins in mosses in 2023 than the samples in May 2024. The levels of dioxins (PCDD/F) in mosses were 3.32 23.76 pg TCDD/TEQ/g dm in 2023 and 0.68 7.09 pg TCDD/ TEQ/g dm in 2024. The cement kiln is closed annually from December to February for at least 6 weeks for maintenance work. In 2024 the sampling time from February to May 8-11th was much shorter, than the nine months in 2023. Besides that, photosynthesis of vegetation stops/slows down below 10 degrees C, which reduces the metabolic activity of plants, and so Bryophyta mosses as well.
- **10.** Dioxins: The results of dioxins in mosses (*Bryophyta*) and pine needles (*Picea abies*) are elevated compared to reference data.

- **11. Heavy Metals (14):** all mosses (*Bryophyta*) locations show elevated levels of heavy metals compared to the levels in the reference location in Slovak Karst National Park.
- **12.** High concentrations of heavy metals are found on mosses (*Bryophyta*), at North/West locations near the cement kiln, indicating serious contamination of the soil of private vegetable gardens. The specific physical landforms of the valley, surrounded by steep mountains on both sides with the gap in it of the Zádiel Gorge (Zádielska dolina) could cause specific wind fumigation.

### Eggs of backyard chicken:

- **13.** Dioxins; Exceeding values for dioxins of the EU limit of 3.3.pg BEQ/g/fat (Dr CALUX) and 5.0 TEQ/g fat (GC-MS) in the eggs of backyard chickens are found.
- **14.** Specific dioxin patterns (congeners) in eggs indicate a source of co-incineration of alternative industrial fuel.
- **15.** PAH: The specific **congener patterns** of dioxins (PCDD/F/dl-PCB) and polycyclic aromatic hydrocarbons (PAH ∑ 16) in the eggs of backyard chickens and vegetation show **elevated levels** of combustion-related contamination.
- **16.** PFAS: with chemical analysis, LC-MS/MS on  $\sum$  24 PFAS substances show contamination at all eggs of backyard chicken locations.

### Eggshells backyard chicken and wildlife bird Heron (Ardea)

1. Heavy Metals: Remarkable are the highest levels of aluminium (Al), zinc (Zn) and nickel (Ni) in the eggshells of a wildlife bird Heron (*Ardea*) population near the Lake Hrhov (Hrhovské rybníky)

#### Meat of wildlife deer, Carp fish, domestic cow:

**2.** PFAS: Contamination of PFOS by chemical PFAS analysis (LC-MS/MS ≥ 24) in the liver of Carp fish (*Cyprinus carpio*), from Lake Hrhov (Hrhovské rybníky).

#### Counter research:

- **3.** Danucem Slovensko a.s., (by Ekotoxikologické centrum Bratislava, ECB) confirms the findings of TW of increased dioxins in backyard chicken eggs, although differences in results and interpretation were apparent.
- **4.** The Košice Regional Government (by Ekolive) confirm the findings of TW of increased dioxins in backyard chicken eggs. It shows that analysis of soil alone gives limited insight into the toxic load of an environment. Multiple parameters are most certainly needed such as biomonitoring in biomass.

These findings are a clear call for action for the responsible authorities to take immediate action to ensure the safety of people living in the surrounding area of the cement plant. Authorities must prioritise the elimination of harmful POP industrial emissions. The precautionary principle should guide their efforts to address these significant environmental and public health concerns.

# 10. Infographics



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Annex 1. Overview of Figures report, Samples & Analysis methods & Result tables

Annex 2. Official Lab Analysis Reports



TW 2<sup>nd</sup> Biomonitoring in the surrounding environment of the cement plant - Cementáreň Turňa nad Bodvou, Slovakia, May 8-11<sup>th</sup> 2024

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