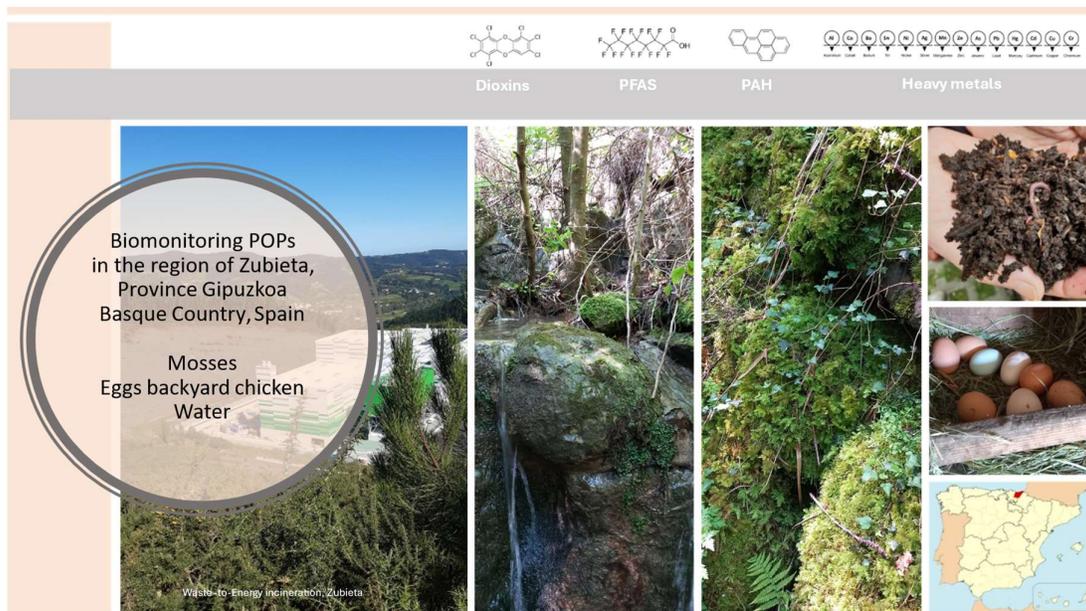


Biomonitoring research on persistent organic pollutants (POPs)
in the surrounding environment of
the WtE waste incinerator in Zubieta 2024

Interim report



Dioxins

PFAS

PAH

Heavy metals

Biomonitoring POPs
in the region of Zubieta,
Province Gipuzkoa
Basque Country, Spain

Mosses
Eggs backyard chicken
Water

Waste-to-Energy Incineration, Zubieta

March - 2025



Biomonitoring research on persistent organic pollutants, (POPs)
in the surrounding environment of
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Interim report

Thanks to Zero Waste Europe for support of this research on persistent organic pollutants (POPs).



Thanks to the private participants of this research for making this study possible by allowing the TW team collecting samples of their backyard chicken eggs, soil, vegetation, and water streams

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Acronyms

APCD	Air Pollution Control Devices
BAT	Best Available Techniques
BEQ	Bioanalytical EQUIvalents
dl-PCB	Dioxin-Like Polychlorinated Biphenyls
DR CALUX®	Dioxin Responsive Chemical-Activated LUCiferase gene eXpression
EFSA	European Food and Safety Authority
GC-MS	Gas Chromatography Mass Spectrometry GC-MS
LB	Lower Bound
LOD	Limit of Detection
MB	Medium Bound
MWI	Medical Waste Incineration
MSWI	Municipal Solid Waste Incineration
ng	Nanogram; 10 ⁻⁹ gram
OTNOC	Other Than Normal Operating Conditions
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Dibenzodioxins
PCDF	Polychlorinated Dibenzofurans
PBDD/F	Polybrominated-dibenzodioxins and furans
pg	Picogram; 10 ⁻¹² gram
POP	Persistent Organic Pollutants
PFOS	Perfluorooctanesulfonic acid
PFOA	Perfluorooctanoic acid
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnDA	Perfluoroundecanoic acid
PFDoA	Perfluoroundecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFBS	Perfluorobutanesulfonic acid
SVHC	Substances of Very High Concern
TCDD	2,3,7,8-tetrachloordibenzo-p-dioxine
TDI	Tolerable Daily Intake = Aanvaardbare Dagelijkse Inname
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalents
TW	ToxicoWatch
TWI	Tolerable Weekly Intake
UB	Upper Bound (UB)
UPOP	Unintentional POP (Persistent Organic Pollutants)
µg	Microgram 10 ⁻³ gram
WtE	Waste to Energy (waste incinerator)

Table of contents

ACRONYMS.....	3
TABLE OF CONTENTS.....	4
1. INTRODUCTION	5
2. SAMPLING.....	6
3. ANALYSIS METHODS	7
3.1. DIOXINS.....	7
3.2. PFAS.....	7
3.3. HEAVY METALS [14].....	7
4. RESULTS.....	8
4.1. DIOXINS.....	8
4.1.1. Dioxins in Eggs.....	8
4.1.2. Dioxins in mosses (Bryophytes).....	11
4.2. PFAS.....	13
4.2.1. PFAS in eggs of backyard chicken.....	13
4.2.2. PFAS in mosses.....	14
4.2.3. PFAS in water	14
4.3. HEAVY METALS [14]	15
4.3.1. Heavy Metals in water.....	15
4.3.2. HEAVY METALS IN SOIL.....	16
4.3. HEAVY METALS IN MOSSES (BRYOPHYTES)	17
5. CONCLUSIONS	18

1. Introduction

In 2019, the ToxicoWatch (TW) foundation, at the request of the local group Zubieta-Lantzen, initiated, a multi-year biomonitoring research on persistent organic pollutants (POPs) in the environment surrounding the Waste-to-Energy (WtE) incinerator in Zubieta. The waste incinerator located in the province of Gipuzkoa, within the municipal district of Donostia/San Sebastián, in the vicinity of the village of Zubieta, in the Basque Country, northern Spain.

The biomonitoring started in 2019 with a **baseline ('zero-measurement') study**, conducted before the incinerator commenced operations in 2020. This biomonitoring research utilised a comprehensive range of matrices, including backyard chicken eggs, pine needles (*Pinus Radiata*), vegetation/leaves, like Holly (*Ilex aquifolium*), mosses (*Bryophytes*), soil, sediment, water and even breast milk.

This interim report, based on the sampling of 2024, focuses is on the environmental impact of persistent organic pollutants, including polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (dl-PCBs), heavy metals (HM), and perfluoroalkyl substances (PFAS). To address the analytical challenges associated with such an extensive group of POPs, a range of methodologies. **Innovative analytical tools**, such as the PFAS CALUX bioassay, have been employed to measure the impact of these highly toxic fluorinated substances. Further technical details can be found in previous TW biomonitoring reports from 2019 and 2023.

In May 2024, the TW team presented the results of five-year biomonitoring study to the public and scientific community in Hernani, and Usurbil, as well at the Gipuzkoa regional government's headquarters. The necessity of regular biomonitoring was underscored to enhance understanding of the ecological impact of environmental pollution. To safeguard environmental health, it is imperative to eliminate or at least reduce emissions of substances of very high concern (SVHC) into the environment.



Figure 1: Waste-to-Energy Incinerator Zubieta, Province Gipuzkoa, Basque Country

2. Sampling

In 2024, the following sampling matrices were collected: eggs from backyard chickens, moss, soil, water from natural streams, and tap drinking water from Usurbil (used as reference). At seven (7) locations within a 5 km radius of the Zubieta WtE waste incinerator, samples of backyard chicken eggs were collected (see Figure 2). The TW team visited each location to conduct a site inspection and collect 6-10 fresh eggs.

Each site visit included an inspection of the chicken enclosure and the completion of a TW questionnaire by the chicken coop owner. Photographs were taken during sampling to ensure traceability and reduce ambiguity regarding the origin of the eggs and sampling location. The total contents of the eggs (yolk and white) were stored in HDPE laboratory containers and frozen until analyses.

Additionally, seven (7) locations within 1 km of the incinerator – covering all wind directions - were selected for the sampling of moss, vegetation, and soil.

- Vegetation: From mosses (*Bryophyta*), 200-300-gram of fresh pine needles were collected for analyses. Samples were stored in HDPE laboratory bags, kept cool, dark, and dry.
- Soil and sediment: 250 grams of material were sampled from the topsoil layer (0-5 cm depth).

Water samples (500 -1000 ml) were collected at four (4) locations along a freshwater stream near the incinerator (designated W1-W4).

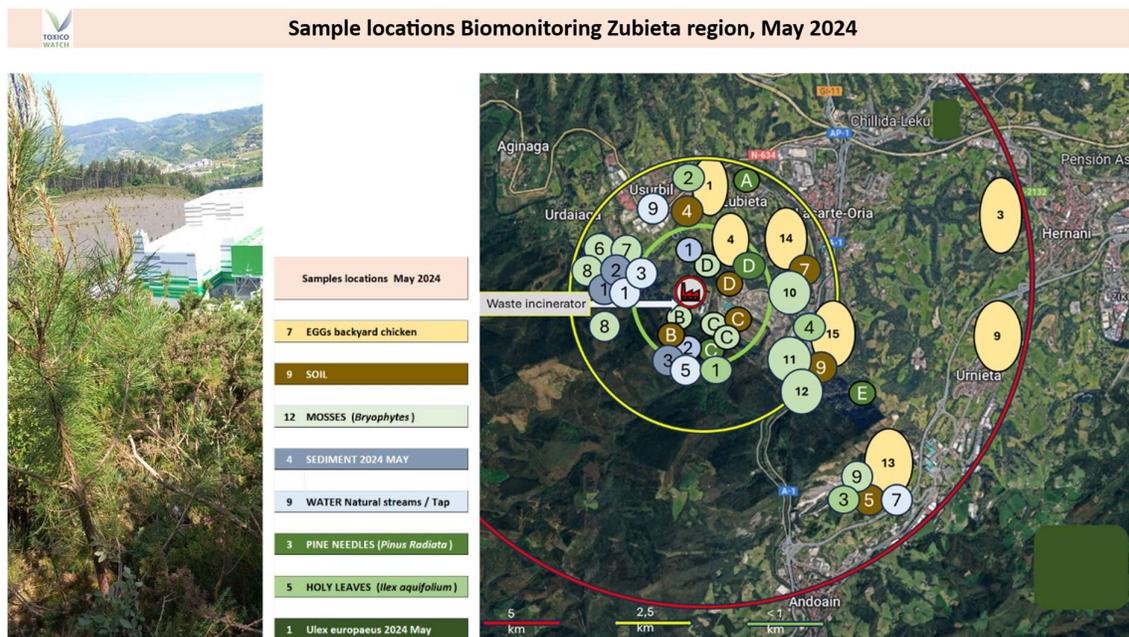


Figure 2: (Bio)matrices locations in the surrounding environment of WtE incinerator Zubieta, May 2024

3. Analysis Methods

3.1. Dioxins

For the 2024 research, the DR CALUX® bioassay was used to analyse dioxins/furans (PCDD/F) and dioxin-like PCBs (dL-PCBs). When DR CALUX results for backyard chicken eggs exceeded the EU regulatory limits - 1.7 pg BEQ/g fat for PCDD/F and 3.3. pg BEQ/g fat for the sum of dioxins (PCDD/F/dl-PCB - GC-MS analyses is applied for confirmation. The analyses are performed by BioDetection Systems (BDS) in Amsterdam, the Netherlands (NL), an accredited laboratory under RvA L401. Chemical analysis for PFAS and heavy metals are conducted by the accredited laboratory Normec, Groen Agro Control, located in Delft, the Netherlands (NL). PFAS chemical analyses is carried out using LC-MS/MS to detect a total of 24 PFAS compounds, while ICP-MS is used for heavy metals analysis.

3.2. PFAS

The biomonitoring research uses the bioassay PFAS CALUX® and the chemical analyse (LC-MS/MS). In previous TW biomonitoring studies, the assay FITC-T4 was also used. These (bio-)assays can detect a broader spectrum of fluorinated substances than chemical LC-MS/MS analysis, limited to \sum 24 PFAS compounds. Current EU regulation only addresses \sum 4 PFAS compounds (EFSA-4). Which is a very limited subset of the wide range of toxic PFAS substances that are now commonly found in contaminated environments.

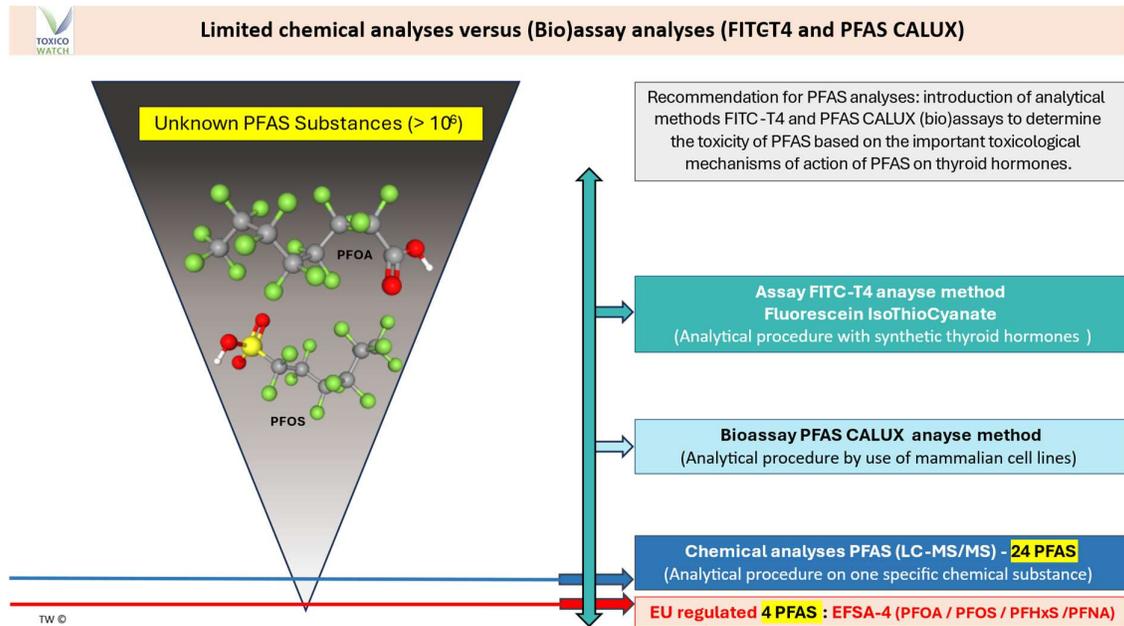
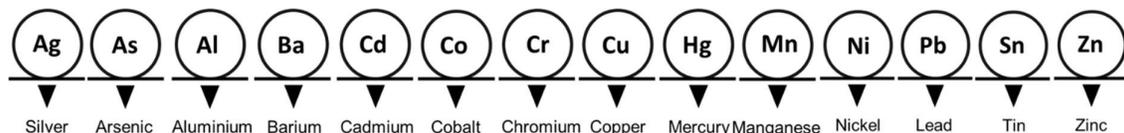


Figure 3: Chemical PFAS analysis vs. different (bio)assay PFAS analysis

3.3. Heavy Metals [14]

In this biomonitoring research, TW analysed samples of backyard chicken eggshells, soil and mosses (*Bryophytes*) for 5-14 heavy metals (see figure below). The analysis was carried out by the accredited laboratory Normec Groen Agro Control, using ICP-MS. The method follows Normec protocols A068 and A095, in accordance with NEN-EN 13805, and NEN-EN-ISO 17294-2 standards.



4. Results

4.1. Dioxins

4.1.1. Dioxins in Eggs

In 2024, a total of seven (7) egg locations were sampled for dioxins analysis. Of these, four (4) locations did not comply with the EU limit for DR CALUX. The eggs from these four sites were also analysed using GCMS for confirmation. The result for the sum of dioxins (PCDD/F/dl-PCB) ranged from **2.90 to 38.00 pg TEQ/g fat**. The chemical results ranged from **1.45 – 20.63 pg TEQ/g fat**.

The two graphs below provide an overview of dioxin levels detected using DR CALUX between 2019 and 2024 in the vicinity of the Zubieta waste incinerator. Notably, the Hernani location recorded a dioxin level of **38 pg TEQ/g fat**, the highest concentration measured by TW in 13 years across Europe in backyard chicken eggs.

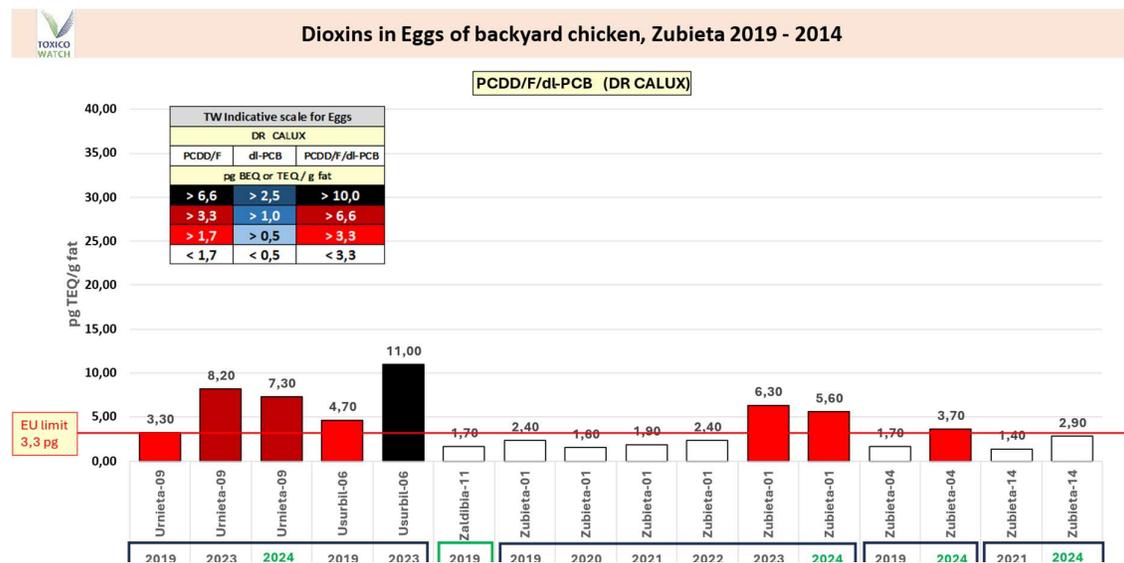
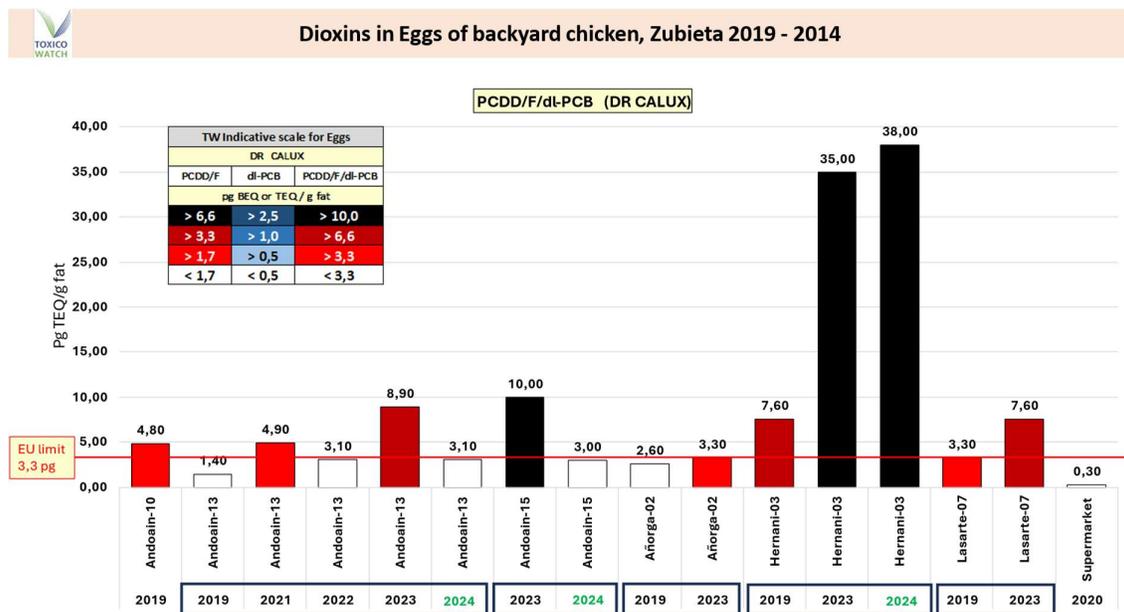


Figure 4: Dioxin (PCDD/F/dl-PCB) by DR CALUX analysis in Eggs of backyard chicken 2019-2024

The following figures compare PCDD/F results in 2019 and 2024. They show a marked increase in dioxins (PCDD/F) and total dioxins (PCDD/F/dl-PCB), illustrated using an indicative colour scale based on EU-limits. Of particular concern is the substantial increase in dioxin contamination within the 3 - 5 km radius around the Zubieta waste incinerator. This area is also home to other industrial activities, including a cement kiln, aluminium smelting and wood treatment facilities.

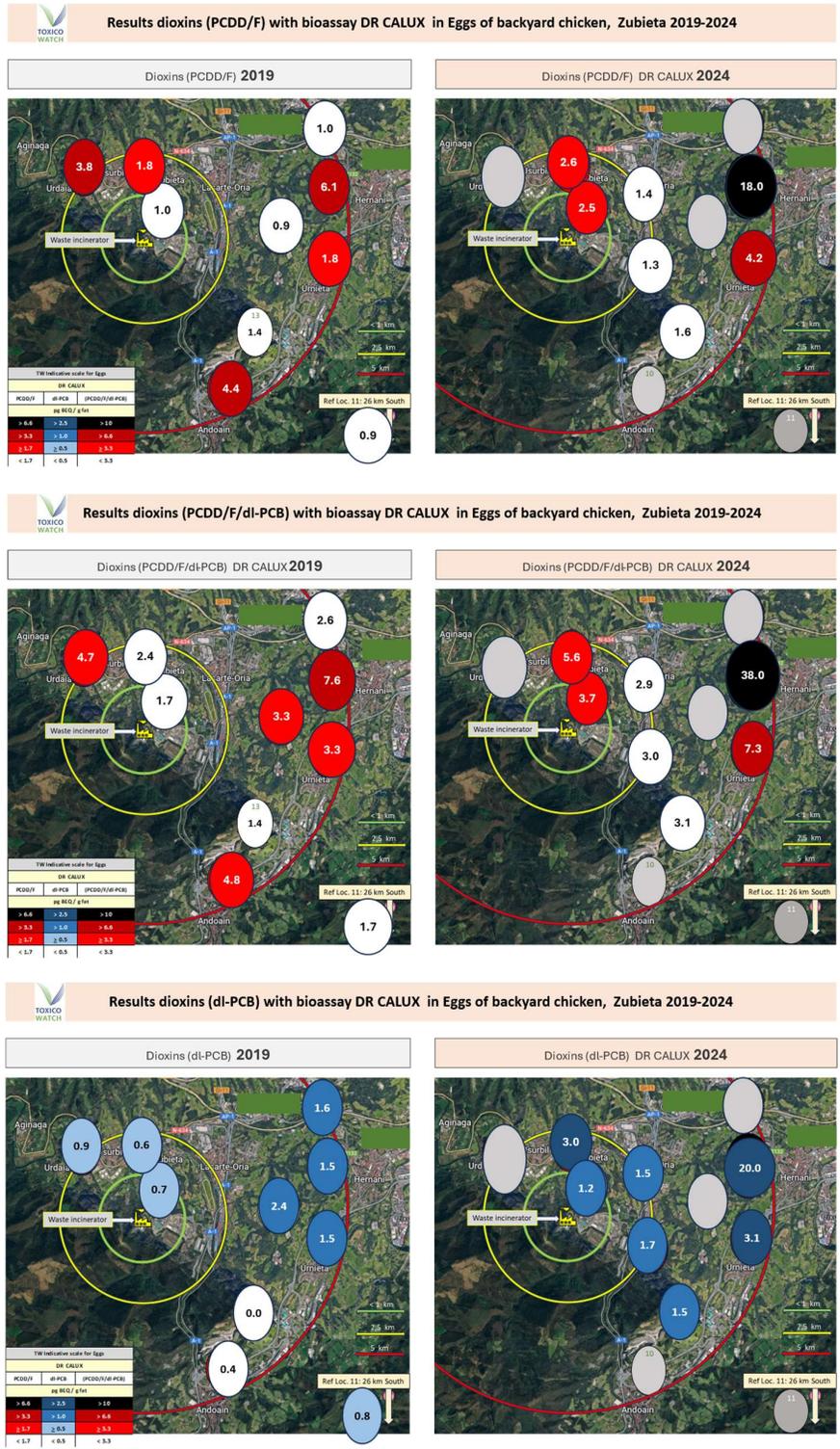


Figure 5: Comparison Overview locations of dioxin Results in backyard chicken eggs, 2019 - 2024

At egg site 1, also known as the 'Press Site', located 1,690 metres from the incinerator, an increase in dioxins levels has been observed over the years using the DR CALUX bioassay. As outlined in the analysis methodology (page 3), there is a fundamental difference between bioassay and chemical analysis approaches. Chemical analysis is limited to detecting only 29 chlorinated congeners of dioxins (PCDD/F) and dioxin-like PCBs. In contrast, the bioassay also responds to other dioxin-like compounds, such as brominated dioxins and mixed halogenated dioxin-like substances.

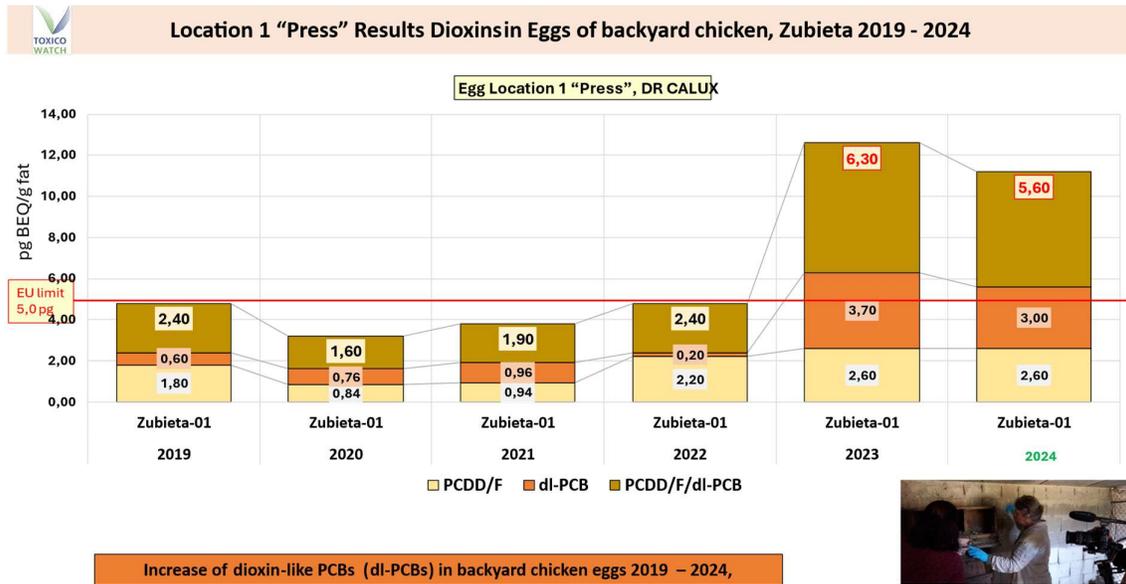


Figure 6: Location 1 "Press", dioxin pattern (DR CALUX) 2019-2024

The Hernani location, which exhibited the highest DR CALUX response, recorded an increase of 1130% in dl-PCB levels compared to 2019. Despite the ban of the production and trade of dl-PCBs for over 50 years, these substances continue to be detected- not only in eggs, but also in moss samples collected in 2022. The cause of this sharp rise in dl-PCBs must be investigated, in line with EU regulations mandating the elimination of emissions of persistent organics pollutants. A further noteworthy increase in dioxin-like PCBs has also been observed at Egg Location Urnieta, situated 3500 metres from the incinerator.

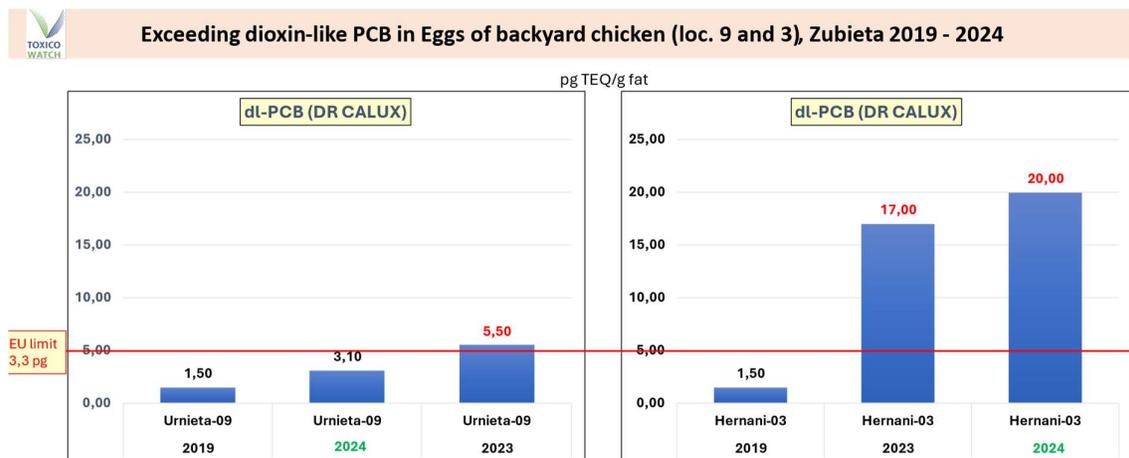


Figure 7: Exceeding dioxin-like PCB in Eggs of backyard chickens (Loc. 9 and 3), Zubieta 2019-2024

4.1.2. Dioxins in Mosses (*Bryophytes*)

At the start of this biomonitoring research in 2019, no dioxins were detected above the limit of detection (LOD) in moss samples near the incinerator, based on DR CALUX bioassay analysis. In subsequent years, increased dioxin levels were measured. In 2022, an exceptionally high dioxin level of **29.8 pg TEQ (TCDD)/g product** was found at location C, situated 500 m from the waste incinerator. This spike maybe linked to frequent shutdowns and start-ups of the incinerator that year. However, this hypothesis could be ruled out s by further analysis of uncorrected minute data from semi-continuous flue gas measurements. **By 2024, the dioxin levels in moss had decreased to a range of 0.6 – 1.8 pg TEQ(TCDD)/g product dry weight 88%.** However, when compared to the EU limits for animal feed, the upper end of this range exceeds the EU regulatory threshold of 0.83 pg TEQ/g 88%. It is important to note that the EU dioxin legislation is based on chemical analysis using GC-MS, with a reference limit of 1,25 pg TEQ/g. In line with the methodology used for dioxin determination in eggs, a conversion factor of 66.6% is applied to DR CALUX results for comparability.

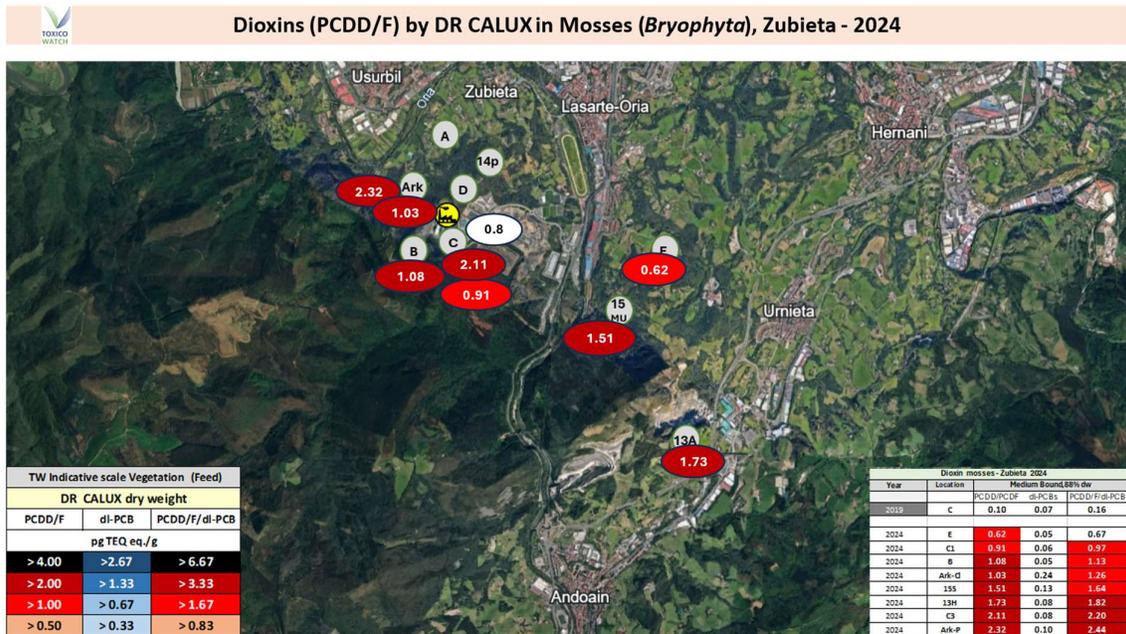


Figure 8: Results dioxins (PCDD/F) in Mosses (*Bryophytes*), 2024

In 2022, elevated levels of dioxins in mosses were found, likely linked to emissions from operational disruptions at the waste incinerator. A heatmap of dioxin concentrations in mosses, using Eurofins' 2019 baseline measurements as a reference, indicates a persistent increase in dioxin levels. Although a decrease in levels was observed in 2024, dioxin concentrations were still found to be 8 to 28 times higher than in 2019. The recorded values, ranging from 0.97 to 2.44 pg TEQ/g 88% dw, reflect a significant and ongoing presence of dioxins in the environment.

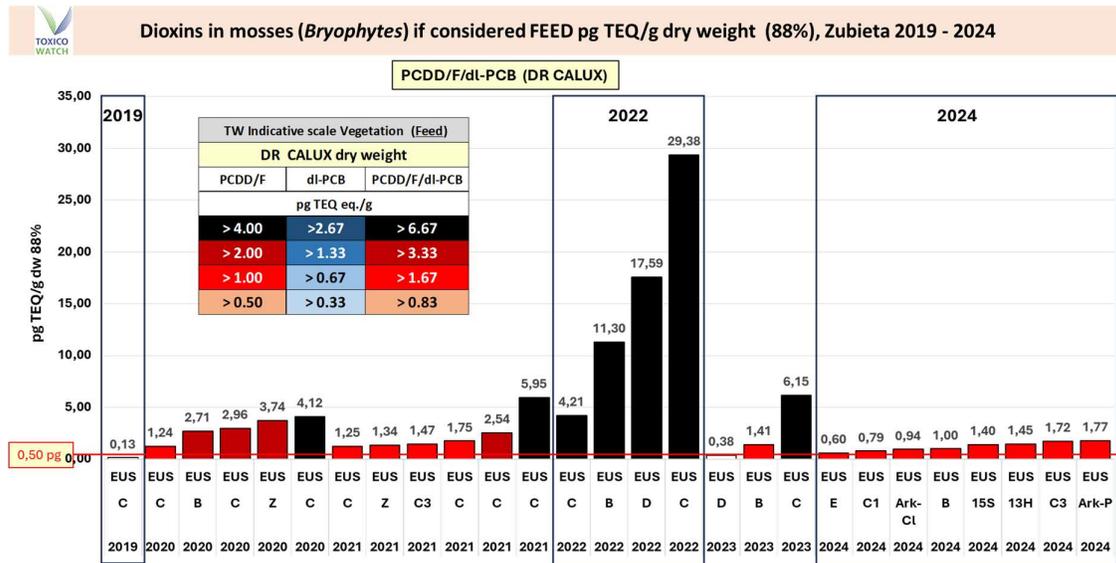


Figure 9: Dioxin in mosses (Bryophytes) marked by TW color indicative scale (88% dry weight)

The spike in dioxin levels (PCDD/F/dl-PCB) observed in 2022, as shown in the graph above, is particularly noteworthy. A comprehensive investigation would be necessary to determine the underlying causes of these emissions. The use of mosses (Bryophytes) as biological indicators of pollution has proven to be a highly sensitive method and effective method, as further evidenced by the heavy metal analysis on moss samples collected from the same location.

Dioxin analysis on mosses - Zubieta 2019 - 2024							
Year	Location	Medium Bound, 88% dw			Heatmap		
		PCDD/PCDF	dl-PCBs	PCDD/F/dl-PCB	PCDD/PCDF	dl-PCBs	PCDD/F/dl-PCB
2019	C	0.10	0.07	0.16	0.08	0.1	0.13
2020	B	3.28	0.10	3.45	41	1.2	43
2020	C	0.00	0.00	5.25	0	0.0	66
2020	C	1.35	0.23	1.58	17	2.9	20
2020	C	3.61	0.10	3.78	45	1.2	47
2020	Z	4.60	0.10	4.76	57	1.2	60
2021	C	1.35	0.24	1.59	17	3.0	20
2021	C	1.94	0.30	2.23	24	3.7	28
2021	C	3.15	0.08	3.23	39	1.0	40
2021	C	6.57	1.02	7.59	82	12.7	95
2021	C3	1.48	0.39	1.87	18	4.9	23
2021	Z	1.51	0.10	1.71	19	1.2	21
2022	B	9.17	9.17	18.34	115	114.6	229
2022	C	3.56	1.38	4.94	45	17.2	62
2022	C	39.03	8.99	48.02	488	112.4	600
2022	D	19.55	13.79	33.34	244	172.4	417
2023	B	1.39	0.16	1.55	17	2.1	19
2023	C	5.15	1.49	6.64	64	18.7	83
2023	D	0.26	0.12	0.38	3	1.5	5
2024	13H	1.73	0.08	1.82	22	0.9	23
2024	15S	1.51	0.13	1.64	19	1.6	21
2024	Ark-Cl	1.03	0.24	1.26	13	3.0	16
2024	Ark-P	2.32	0.10	2.44	29	1.3	31
2024	B	1.08	0.05	1.13	13	0.6	14
2024	C1	0.91	0.06	0.97	11	0.7	12
2024	C3	2.11	0.08	2.20	26	1.0	28
2024	E	0.62	0.05	0.67	8	0.6	8

Exceeding Factors	
1.5 - 2.0	
2.0 - 5.0	
5.0 - 10.0	
10.0 - 50.0	
50.0 - 100.0	
> 100.0	

Table 1: Results Dioxins (PCDD/F/dl-PCB) results in mosses concentrations and heatmap by DR CALUX

4.2. PFAS

4.2.1. PFAS in Backyard Chicken Eggs

In 2021, eggs from backyard chicken in Zubieta and Andoain were analysed for PFAS using the FITC-T4 bioassay screening method. Both locations showed results of **1900 µg PFOA equivalent/kg (1.9 ng/g)**. In 2022, TW began conducting PFAS chemical analyses using LC-MS/MS on various biomatrices. In 2023, the PFAS levels increased substantially at three egg sampling locations, with LC-MS/MS results ranging from: **3.1 – 10.54 µg/kg ∑ 24 PFAS (mb)**. The highest concentration—**10.54 µg/kg ∑ 24 PFAS (MB)**—was found at Andoain, located 2 km from the incinerator.

In 2024, PFAS levels in backyard eggs from Andoain decreased to **0.31 µg/kg**. However, at Hernani concentrations increased from **5.92 µg/kg in 2023 to 10.91 µg/kg in 2024**. In Urnieta, a town 4 km from the incinerator, PFAS were also detected, showing the same congener profile as in Hernani (4 km) and Andoain (3 km), indicating that this region is under significant PFAS pressure. At the press location (Egg location 1), only PFOS was identified. PFOS was also found to be the dominant C8 compound in all the egg samples, with highest concentration - **7.3 µg/kg** – detected at Hernani. This value exceeds the EU limit for PFOS by a factor 7.3.

Chemical PFAS analyses eggs of backyard chicken, 2022 -2024														
Year	Sample		EFSA-4											
			∑ 4 PFAS	∑ 24 PFAS	C8 PFOS	C8 PFOA	C9 PFNA	C6 PFDA	C11 PFUnDA	C11 PFDoA	C13 PFTriDA	C14 PFTeDA	C4 PFBS	
			Lowerbound (LB) µg/kg											
2022	Egg	Zubieta-01	0.35	0.35	0.35									
2022	Egg	Andoain-13	0.11	0.11	0.11									
2023	Egg	Zubieta-01	1.70	1.70	1.70									
2023	Egg	Andoain-15	5.50	9.49	4.40	0.29	0.81	0.82	0.83	1.00	0.74	0.60		
2023	Egg	Hernani-03	5.02	5.92	4.40	0.16	0.46	0.28	0.25		0.19	0.18		
2023	Egg	Lasarte-07	0.11	0.11			0.11							
2024	Egg	Zubieta-01	1.30	1.30	1.3									
2024	Egg	Hernani-03	8.35	10.91	7.3	0.16	0.52	0.37	0.49	0.52	0.88	0.67		
2024	Egg	Urnieta-09	2.99	3.99	2.6		0.23	0.16	0.18	0.24	0.33	0.25		
2024	Egg	Andoain-15	0.31	0.31	0.31									

Table 2: Chemical PFAS analyses Eggs of backyard chicken 2022-2024

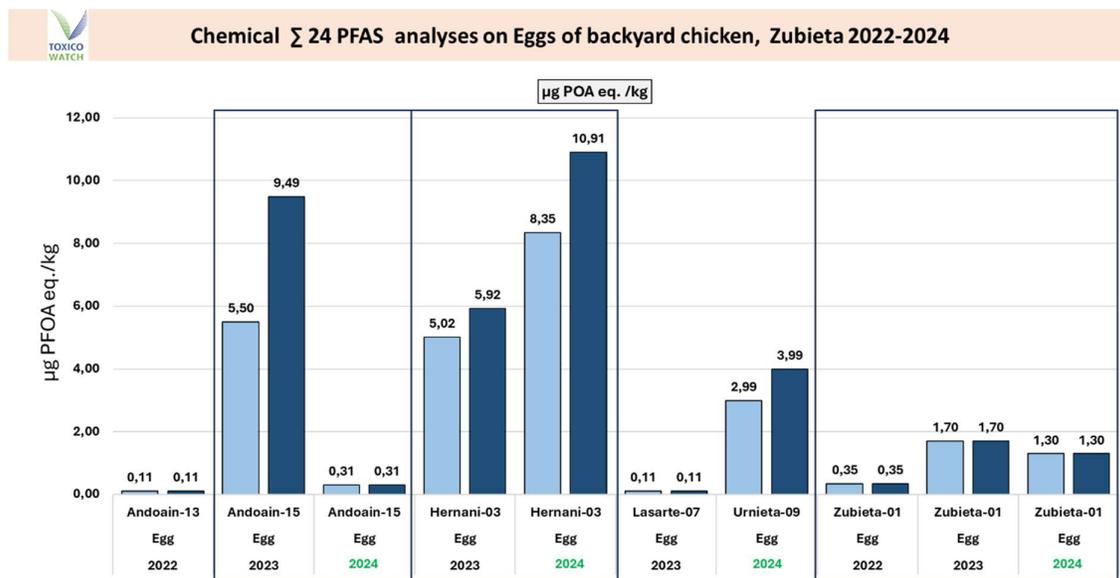


Figure 10: Chemical analysis PFAS on Eggs of backyard chicken 2022-2024

4.2.2. PFAS in Mosses

In 2020, moss samples from locations B and C were tested using the PFAS CALUX bioassay, with the results ranging from 1500 to 5000 ng PFOA eq./gram wet weight (ww). In 2021, the FITC-T4 assay detected a PFAS level of 17000 ng PFOA eq./g in moss.

In 2022, chemical analysis of $\Sigma 24$ PFAS congeners showed values of 1.7 – 2.7 ng/g at locations D and C, respectively. The primary PFAS compound detected was perfluorobutanesulfonic acid (PFBS), a chemical substitute for the banned substance perfluorooctanesulfonic acid PFOS, known for its persistence, toxicity, and bioaccumulation. In 2023, no PFBS was detected. Notably, while the C4 compound PFBS was found in 2023 in moss near the incinerator, it was not detected in eggs, which is a significant observation.

PFAS analyses mosses, LCMSMS, 2021 -2023												
Year	Sample		FITC-T4	PFAS CALUX			EFSA-4					
			PFOA eq.	PFOA eq.	PFOA eq.	$\Sigma 4$ PFAS	$\Sigma 24$ PFAS	PFOS	PFOA	PFNA	PFDA	PFBS
			$\mu\text{g/g}$	$\mu\text{g/g}$	ng/g	ng/g (lb)	C8	C8	C9	C6	C4	
2022	Mos	Location C				0.00	2.70					2.70
2022	Mos	Location D				0.00	1.70					1.70
2023	Mos	Location C				0.00	0.00					
2023	Mos	Location D				0.00	0.00					

PFAS analyses mosses, FITC-T4, PFAS CALUX, 2021 -2024												
Year	Sample		FITC-T4	PFAS CALUX			EFSA-4					
			PFOA eq.	PFOA eq.	PFOA eq.	$\Sigma 4$ PFAS	$\Sigma 24$ PFAS	PFOS	PFOA	PFNA	PFDA	PFBS
			$\mu\text{g/g}$	$\mu\text{g/g}$	ng/g	ng/g (lb)	C8	C8	C9	C6	C4	
2020	Moss	Location D		5.00	5000.00							
2020	Moss	Location C		1.50	1500.00							
2020	Moss	Location D		3.90	3900.00							
2020	Moss	Location B		2.80	2800.00							
2021	Moss	Lasarte-Oria (3300)	4.20		4200.00							
2021	Moss	Location C	17.00		17000.00							

Table 3: PFAS analyses in mosses.

4.2.3. PFAS in Water

In 2020, water samples from the *Arkaitz erreka stream* were analysed using the PFAS CALUX bioassay, with results of 63 ng PFOA eq./litre downstream and 140 ng PFOA eq./litre upstream. In 2024, at a mountain water source in Andoain, the level of perfluorooctanoic acid (PFOA), was measure at 150 ng PFOA eq. /L - a result that was unexpected by the TW team, given the seemingly pristine, a natural setting on top of a hill. The PFAS CALUX method quantifies the combined toxic effect of various PFAS congeners. It is currently employed by the Dutch government for screening surface waters and to inform policy measures aimed at reducing emission at the source. The PFAS result from the 2024 Andoain water sample exceeds the Dutch guideline limit in water, which is 0.3 nanograms per litre for PFOA, as established in 2023 (Smit, 2022).

PFAS analyses mosses, sediment and water, FITC-T4, PFAS CALUX, 2021 -2024							
Year	Sample		FITC-T4	PFAS CALUX			
			PFOA eq.	PFOA eq.	PFOA eq.	$\Sigma 4$ PFAS	$\Sigma 24$ PFAS
			$\mu\text{g/g}$	$\mu\text{g/l}$	$\mu\text{g/g}$	$\mu\text{g/l}$	ng/g
2020	SED	Sediment downstream			0.06	63.00	
2020	SED	Sediment upstream			0.14	140.00	
2022	Waterpads	Arkaitz erreka					0.00
2024	Water	Andoain mountain well			0.15	150.00	

Table 4: Chemical PFAS analysis Mosses (Bryophytes), Sediment, Water 2021-2024

PFAS in water streams 2021 and calamities May 10, 2020 & July 13, 2022 - Zubieta



Figure 11: PFAS in water stream 2021, due to calamities in same water stream of research in May 2024

4.3. Heavy Metals [14]

4.3.1. Heavy Metals in Water

The following table presents heavy metals concentration in running water and water sources near Zubieta, measured between 2019 to 2024. For reference, samples were also taken from a groundwater reservoir situated 930 metres from the incinerator, prior to the facility beginning waste combustion. Two environmental incidents occurred in the Arkaitz erreka mountain stream - on 10 May 2020 and 13 July 2022 – resulting in the mass death eels. Whether the eel population will recover remains uncertain. Analysis shows a dramatic increase in heavy metal concentrations during these calamities, which has had a severe impact on the stream's vulnerable aquatic ecosystem. Specifically, **the concentrations increased by the following factors:** arsenic (As) x 61.5, chromium (Cr) x 2.580, nickel (Ni) x 132.8, lead (Pb) x 78 and zinc (Zn) x 72.

Of particular concern is the sharp increase in chromium (Cr). In 2024, elevated levels of cobalt (Co), nickel (Ni), lead (Pb), and zinc (Zn) were also detected. A particularly elevated cobalt (Co) concentration of 2.2 µg/l was recorded at a mountain water source near Andoain. Furthermore, tap water from Usurbil, sampled as a reference, showed elevated levels of cobalt (Co), copper (Cu), lead (Pb), and zinc (Zn). While these values remain within the limits of the European Drinking Water Directive (EUR-Lex (2017), the presence of aluminium (Al) above the stipulated limit (200 µg/l) was attributed to contamination from covering the sample bottle with aluminium foil. Although **the EU limit for lead (Pb) in drinking water is 5 µg/l**, exposure should be minimised as much as possible, given that no safe level for lead could be established (EFSA 2010).

Heavy metals in water, Zubieta 2024

		Heavy metals water µg/l													
Sampling	Sample	Ag	Al	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
2024	Arkaitz erreka clean	0.500	76.30	0.100	19.70	0.100	0.250	0.100	0.41	0.050	5.50	0.100	0.110	1.000	4.20
2024	Water upstream high	0.500	8.30	0.100	26.90	0.100	0.230	0.100	0.100	0.050	5.50	1.60	0.100	1.000	0.500
2024	Water well mountain Andoain	0.500	56.90	0.100	5.90	0.100	2.200	0.100	0.45	0.050	18.10	1.40	0.210	1.000	9.20
2024	Drinkwater Usurbil	0.500	336.00	0.100	8.70	0.100	0.500	0.100	7.40	0.050	6.60	0.73	0.190	1.000	8.70
2023	Groundwater Lasarte	0.500	1.00	0.100	111.00	0.100	0.100	0.100	1.30	0.050		0.13	0.050	1.000	7.30
2023	Underground water	0.500	1.00	0.100	104.00	0.100	0.100	0.100	5.80	0.050		0.67	0.160	1.000	13.00
2019	Underground water Zubieta (REF)	0.500	52.00	0.110	19.70	0.100	0.100	0.100	0.50	0.050		0.61	0.100	1.000	0.50
2024	Arkaitz erreka tube	0.500	39.90	0.210	49.80	0.100	0.230	0.100	0.49	0.050	5.50	0.100	0.100	1.000	1.40
2022	Arkaitz erreka Calamity			6.760		0.125		129.00	5.59	0.100		81.00	3.950		36.00
		< Limit of Detection (LOD)													

		Heatmap of exceeding factors of water Zubieta (reference underground water 2019)													
Sampling	Sample	Ag	Al	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
2024	Arkaitz erreka clean	2.0	1.5	0.9	1.0	2.0	2.5	2.0	0.8	2.0		0.2	2.2	2.0	8.4
2024	Water upstream high	2.0	0.2	0.9	1.4	2.0	2.3	2.0	0.2	2.0		2.6	2.0	2.0	1.0
2024	Water well mountain Andoain	2.0	1.1	0.9	0.3	2.0	22.0	2.0	0.9	2.0		2.3	4.2	2.0	38.4
2024	Drinkwater Usurbil	2.0	6.5	0.9	0.4	2.0	5.0	2.0	14.6	2.0		1.2	3.8	2.0	37.4
2023	Groundwater Lasarte	2.0	0.0	0.9	5.6	2.0	1.0	2.0	2.6	2.0		0.2	1.0	2.0	14.6
2023	Underground water	2.0	0.0	0.9	5.3	2.0	1.0	2.0	11.6	2.0		1.1	3.3	2.0	26.0
2019	Underground water Zubieta (REF)	2.0	1.0	1.0	1.0	2.0	1.0	2.0	1.0	2.0		1.0	2.0	2.0	1.0
2024	Arkaitz erreka tube	2.0	0.8	1.0	2.5	2.0	2.5	2.0	1.0	2.0		0.2	2.0	2.0	2.0
2022	Arkaitz erreka Calamity	0.0	0.0	61.5	0.0	2.5	0.0	2580.0	11.2	4.0		132.8	79.0	0.0	72.0

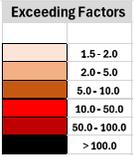


Table 5: Heavy metals in water

4.3.2. Heavy Metals in Soil

The following table presents the results of 14 analysed heavy metals in soil and sediment samples collected between 2019 to 2024. It should be noted that not all 14 metals were analysed in every instance, due to the limitations in the availability of analytical equipment and methods, particularly for aluminium (Al) and manganese (Mn).

		Concentrations of heavy metals in soil Zubieta 2019 - 2024 mg/kg dw ub													
Sampling		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Year		Ag	Al	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
		Silver	Aluminium	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
2022	Sediment downstream	3.800	9075.00	30.100	138.00	0.420	61.300	32.20	19.70	0.120		51.80	21.500	1.400	162.00
2024	Sediment clean	0.120		10.200	98.10	0.360	23.500	17.80	3.95	0.081	7811.00	43.90	25.500	2.600	63.72
2024	Sediment tube	0.038		19.200	70.10	0.130	29.200	16.40	3.63	0.065	13383.00	39.30	26.300	0.940	18.53
2019	Zubieta A			17.00		0.20		28.00	28.00	0.05	590.00	20.00	51.00		110.00
2019	Zubieta B			8.10		0.20		15.00	16.00	0.24	230.00	10.00	77.00		87.00
2019	Zubieta C			14.00		0.20		18.00	11.00	0.05	100.00	11.00	33.00		42.00
2019	Zubieta D			12.00		0.20		18.00	16.00	0.05	210.00	17.00	28.00		57.00
2019	Zubieta E			15.00		0.20		23.00	17.00	0.05	180.00	18.00	80.00		84.00
2019	Kaparotz			15.00		0.20		22.00	13.00	0.05	300.00	24.00	23.00		65.00
2022	Zubieta C	0.04	15938.00	10.60	42.90	0.06	1.10	18.20	8.60	0.06		5.80	28.10	1.30	30.60
2023	Zubieta B	0.07	24562.00	10.00	79.50	0.08	5.60	26.00	15.00	0.08		16.00	32.00	2.00	64.00
2023	Zubieta C	0.04	23253.00	11.00	50.50	0.04	1.10	20.00	9.90	0.03		6.10	19.00	1.30	26.00
2023	Zubieta D	0.07	24487.00	11.00	78.80	0.06	5.70	24.00	18.00	0.05		22.00	29.00	1.30	85.00
2023	Zubieta-E	0.04	16197.00	11.00	32.20	0.13	7.30	16.00	16.00	0.05		20.00	43.00	1.20	95.00
2024	Press location Zubieta	0.09		12.00	93.00	0.36	7.60	15.00	44.00	0.13	8523.00	17.00	61.00	4.20	221.00
2024	Andoain-14	0.38		6.80	45.00	0.22	6.20	23.00	25.00	0.05	2899.00	18.00	14.00	1.50	92.00
2024	Andoain-15	0.06		7.50	46.00	0.22	1.70	13.00	15.00	0.07	2588.00	6.20	40.00	2.10	102.00
2024	Zubieta-4	0.08		9.40	62.00	0.12	4.70	20.00	20.00	0.05	2933.00	18.00	42.00	1.40	93.00
2024	Zubieta-B	0.04		8.00	33.00	0.17	3.30	12.00	15.00	0.13	2375.00	10.00	71.00	1.60	64.00
2024	Zubieta-C	0.04		11.00	42.00	0.09	1.60	18.00	10.00	0.05	578.00	8.30	23.00	1.00	35.00
2024	Zubieta-D	0.06		10.00	62.00	0.05	9.20	19.00	17.00	0.05	2167.00	22.00	26.00	1.20	67.00
2024	Zubieta-E	0.05		12.00	34.00	0.10	6.50	18.00	16.00	0.06	1546.00	22.00	53.00	1.50	79.00

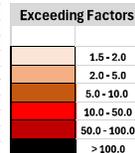
Table 6:

Concentration heavy metals in soil, Zubieta 2024

The table below is organised chronologically by locations and year, with results visualised using a TW indicative colour scale, based on the lowest measured reference value. The bottom row of the table shows the difference factors compared to the reference levels. Elevated concentrations of silver (Ag), cobalt (Co) manganese (Mn), and nickel (Ni) in sediment are attributable to the occurrence of calamities at the waste incinerator, as detailed in the subsequent chapter on water and heavy metals. Notably high levels of mercury (Hg), manganese (Mn), and zinc (Zn) were also detected at Zubieta press site (Egg location-1).

Heatmap of exceeding factors in soil 2019 - 2024 Zubieta																
	Ag	Al	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn		
Reference	0.036	9075.000	8.000	33.000	0.170	3.300	12.000	15.000	0.130	2375.000	10.000	71.000	1.600	64.000		
2024	Andoain -15	1.6	0.0	0.9	1.4	1.3	0.5	1.1	1.0	0.5	1.1	0.6	0.6	1.3	1.6	
2024	Andoain-14	10.6	0.0	0.9	1.4	1.3	1.9	1.9	1.7	0.4	1.2	1.8	0.2	0.9	1.4	
2019	Kaparotz			1.9		1.2		1.8	0.9	0.4	0.1	2.4	0.3		1.0	
2019	Press location Zubieta			2.1		1.2		2.3	1.9	0.4	0.2	2.0	0.7		1.7	
2024	Press location Zubieta	2.4		1.5	2.8	2.1	2.3	1.3	2.9	1.0	3.6	1.7	0.9	2.6	3.5	
2024	Sediment clean	3.3		1.3	3.0	2.1	7.1	1.5	0.3	0.6	3.3	4.4	0.4	1.6	1.0	
2022	Sediment downstream	105.6	1.0	3.8	4.2	2.5	18.6	2.7	1.3	0.9		5.2	0.3	0.9	2.5	
2024	Sediment tube	1.1		2.4	2.1	0.8	8.8	1.4	0.2	0.5	5.6	3.9	0.4	0.6	0.3	
2024	Zubieta 4	2.1		1.2	1.9	0.7	1.4	1.7	1.3	0.4	1.2	1.8	0.6	0.9	1.5	
2019	Zubieta B			1.0		1.2	0.0	1.3	1.1	1.8	0.1	1.0	1.1		1.4	
2023	Zubieta B	2.0	2.7	1.3	2.4	0.4	1.7	2.2	1.0	0.6	0.0	1.6	0.5	1.3	1.0	
2024	Zubieta B	1.0		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
2019	Zubieta C			1.8		1.2		1.5	0.7	0.4	1.0	1.1	0.5	0.0	0.7	
2022	Zubieta C	1.1	1.8	1.3	1.3	0.3	0.3	1.5	0.6	0.4		0.6	0.4	0.8	0.5	
2023	Zubieta C	1.0	2.6	1.4	1.5	0.3	0.3	1.7	0.7	0.3		0.6	0.3	0.8	0.4	
2024	Zubieta C	1.0		1.4	1.3	0.5	0.5	1.5	0.7	0.4	0.2	0.8	0.3	0.6	0.5	
2019	Zubieta D			1.5		1.2		1.5	1.1	0.4	0.1	1.7	0.4	0.9	0.9	
2023	Zubieta D	1.9	2.7	1.4	2.4	0.4	1.7	2.0	1.2	0.4		2.2	0.4	0.8	1.3	
2024	Zubieta D	1.6		1.3	1.9	0.3	2.8	1.6	1.1	0.4	0.9	2.2	0.4	0.8	1.0	
2019	Zubieta E			1.9		1.2		1.9	1.1	0.4	0.1	1.8	1.1		1.3	
2023	Zubieta E	1.0	1.8	1.4	1.0	0.8	2.2	1.3	1.1	0.4		2.0	0.6	0.8	1.5	
2024	Zubieta E	1.3	0.0	1.5	1.0	0.6	2.0	1.5	1.1	0.4	0.7	2.2	0.7	0.9	1.2	
Factor	Press location Zubieta			0.7	1.8		0.5	1.6	2.6	14.4	0.9	1.2		2.0		
Factor	Zubieta C 2019- 2024			0.8	0.4		1.0	0.9	1.0	5.8	0.8	0.7		1.2		
Factor	Zubieta D 2019- 2024			0.8	0.2		1.1	1.1	1.1	10.9	1.3	0.9		0.8		
Factor	Zubieta B 2019- 2024			1.0	0.9		0.8	0.9	0.5	10.3	1.0	0.9		0.7		
Factor	Zubieta E 2019- 2024			0.8	0.5		0.8	0.9	1.1	8.6	1.2	0.7		0.9		

Table 7: Heatmap of exceeding factors in soil, Zubieta 2019 - 2024



4.3. Heavy Metals in Mosses (*Bryophytes*)

In 2024, analyses of 14 heavy metals on moss, soil and water samples. In 2019, a Spanish Laboratory (Geysler) performed analyses of nine (9) heavy metals on moss samples. However, manganese (Mn) could not be analysed in these 2019 samples due to methodological limitations.

Concentrations heavy metals in moss 2024 mg/kg dw ub															
Sampling Year	Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14
		Ag	Al	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
		Silver	Aluminium	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Mercury	Manganese	Nickel	Lead	Tin	Zinc
2019	Zubieta C			0.60		0.040		5.20	1.70	0.018	83.00	1.30	2.20		14.00
2023	Zubieta B	0.023	2018.00	0.43	18.00	0.150	1.20	3.30	7.00	0.063		3.20	7.80	0.55	50.00
2024	Well mountain Andoain 15	0.210	4393.00	0.59	107.00	1.200	11.00	2.70	42.00	0.180		23.00	8.60	0.35	229.00
2024	Andoain 14 mountain	0.012	2393.00	1.80	28.00	0.070	1.30	3.40	7.00	0.023		2.60	3.00	0.37	47.00
2024	Zubieta mountain	0.028	1007.00	0.24	47.00	0.180	0.18	1.90	7.70	0.100		1.50	3.80	0.36	23.00
2024	Zubieta C1	0.064	4029.00	1.20	77.00	0.240	1.10	8.00	12.00	0.010		4.60	11.00	1.00	73.00
2024	Zubieta C3	0.053	7961.00	2.90	40.00	0.310	1.70	10.00	11.00	0.078		6.70	11.00	4.30	96.00
2024	Zubieta B	0.010	2039.00	0.02	16.00	0.010	0.71	0.02	6.10	0.010		0.05	0.010	0.01	40.00
2024	Zubieta D	0.033	7063.00	2.00	59.00	0.110	1.20	8.20	7.60	0.035		4.70	10.00	0.59	41.00
2024	Zubieta E	0.045	13633.00	3.10	67.00	0.130	3.10	15.00	9.90	0.037		4.90	36.00	0.75	60.00
2024	Zubieta Arkaitz erreka 01	0.010	3296.00	0.053	27.00	0.010	1.30	0.16	10.00	0.01		0.23	0.27	0.12	76.00
2024	Zubieta Arkaitz erreka clean	0.018	1289.00	0.57	13.00	0.098	0.72	2.60	8.30	0.058		2.20	2.20	0.35	40.00
2024	Zubieta Arkaitz erreka tube	0.020	2019.00	1.80	31.00	0.120	1.60	4.50	7.70	0.064		5.30	4.20	1.50	59.00

Table 8: Concentrations heavy metals in moss, 2019 - 2024 Zubieta

A heatmap of heavy metal concentration in moss is based on reference values from location B, which recorded the lowest overall levels. Particularly high concentrations of heavy metal levels were observed at location Andoain, near a water well used for drinking water by residents. Notably, this location also showed elevated levels of PFAS in the water raising further concern. **In moss samples collected 300 metres from the incinerator, heavy metal concentrations were found to be elevated by factors ranging from 1.9 to 7.9.** Additional moss samples collected near the water stream adjacent to the incinerator's water outlet also exhibited elevated concentrations in heavy metals, including:

- Arsenic (As): × 3.2
- Barium (Ba): × 2.4
- Nickel (Ni): × 2.4
- Tin (Sn): × 4.3

Of particular concern is the elevated level of lead (Pb) detected at location E, situated 2,680 metres south-east of the incinerator.

Heatmap heavy metals in mosses with reference location Zubieta B															
		Ag	Al	As	Ba	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
	Reference moss Zubieta	0.023	1007.00	0.43	16.00	0.040	0.71	5.20	1.70	0.018	83.00	1.30	2.200	0.55	14.00
2019	Zubieta C	0.0	0.0	1.4	0.0	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0
2023	Zubieta B	1.0	2.0	1.0	1.1	3.8	1.7	0.6	4.1	3.5	0.0	2.5	3.5	1.0	3.6
2024	Well mountain Andoain 15	9.1	4.4	1.4	6.7	30.0	15.5	0.5	24.7	10.0	0.0	17.7	3.9	0.6	16.4
2024	Andoain 14 mountain	0.5	2.4	4.2	1.8	1.8	1.8	0.7	4.1	1.3	0.0	2.0	1.4	0.7	3.4
2024	Zubieta mountain	1.2	1.0	0.6	2.9	4.5	0.3	0.4	4.5	5.6	0.0	1.2	1.7	0.7	1.6
2024	Zubieta C1	2.8	4.0	2.8	4.8	6.0	1.5	1.5	7.1	0.6	0.0	3.5	5.0	1.8	5.2
2024	Zubieta C3	2.3	7.9	6.7	2.5	7.8	2.4	1.9	6.5	4.3	0.0	5.2	5.0	7.8	6.9
2024	Zubieta B	0.4	2.0	0.0	1.0	0.3	1.0	0.0	3.6	0.6	0.0	0.0	0.0	0.0	2.9
2024	Zubieta D	1.4	7.0	4.7	3.7	2.8	1.7	1.6	4.5	1.9	0.0	3.6	4.5	1.1	2.9
2024	Zubieta E	2.0	13.5	7.2	4.2	3.3	4.4	2.9	5.8	2.1	0.0	3.8	16.4	1.4	4.3
2024	Zubieta Arkaitz erreka 01	0.4	3.3	0.1	1.7	0.3	1.8	0.0	5.9	0.3	0.0	0.2	0.1	0.2	5.4
2024	Zubieta Arkaitz erreka clean	0.8	1.3	1.3	0.8	2.5	1.0	0.5	4.9	3.2	0.0	1.7	1.0	0.6	2.9
2024	Zubieta Arkaitz erreka tube	0.9	2.0	4.2	1.9	3.0	2.3	0.9	4.5	3.6	0.0	4.1	1.9	2.7	4.2
2024	Ratio Arkaitz tube/clean	1.1	1.6	3.2	2.4	1.2	2.2	1.7	0.9	1.1		2.4	1.9	4.3	1.5

Table 9: Heatmap heavy metals in mosses, 2019 - 2024 Zubieta

5. Conclusions

The findings of this interim biomonitoring study (2024), conducted in the region surrounding the Zubieta incinerator, reveal exceedances of dioxins, perfluoroalkyl substances (PFAS), and heavy metals in the environment.

The results from backyard chicken eggs analysed using the DR CALUX bioassay show a significant increase in dioxins, including PCDD/Fs and dioxin-like PCBs (dl-PCBs). At Hernani, located 3,500 metres east of the waste incinerator, the highest dioxin concentration ever recorded in backyard chicken eggs during 13 years of TW biomonitoring across Europe was measured.

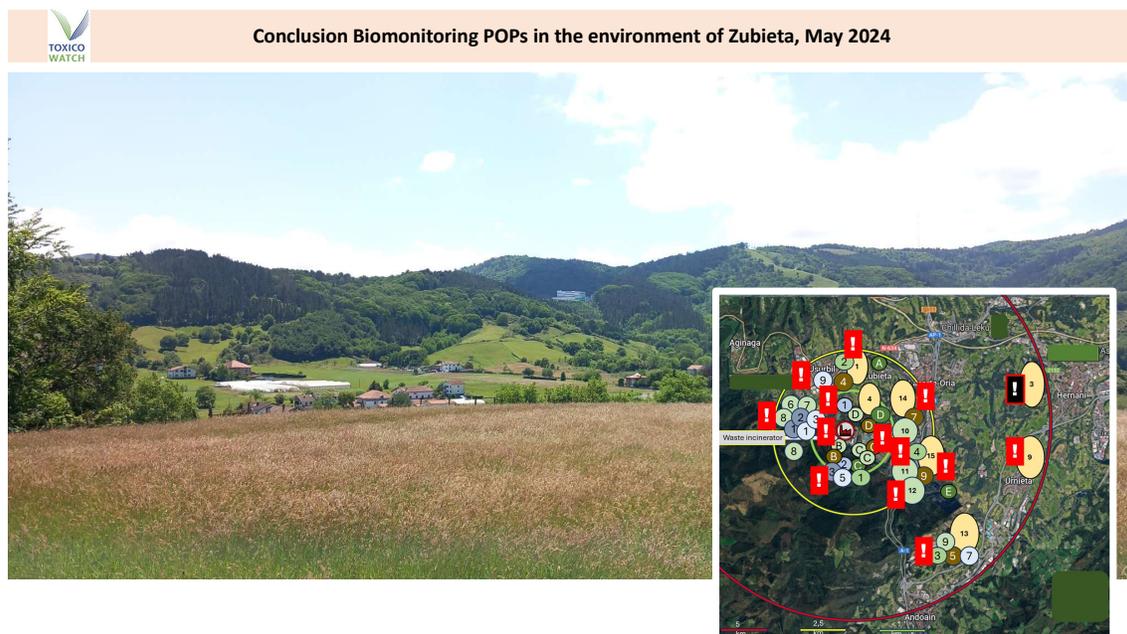
At Zubieta Press Site - where this five-year study began in 2019 with zero-measurements - a clear upward trend in dioxin levels is evident since the start of the waste incinerator's operations in 2020.

PFAS were detected in all examined backyard chicken eggs. In Hernani and Andoain, PFAS concentrations exceed the EU's maximum permissible limits. **In 2024, PFAS were also detected in a drinking water source located on a hill near Andoain, an area previously considered pristine.**

In 2019, no dioxins were found in mosses (*Bryophyta*) or pine needles (*Pinus radiata*). In the years that followed, dioxin levels in the moss increased by up to a factor of 300. Although levels have since decreased, PCDD/F concentrations in mosses remain 30 times higher than in 2019, prior to the incinerator's operation.

Rising trends in heavy metals were also observed in water, soil, mosses, and sediment. In water samples, elevated concentrations of zinc (Zn) and lead (Pb) were detected. In soil, increases in cadmium (Cd), silver (Ag), manganese (Mn) and mercury (Hg) were noted. In moss, elevated levels of arsenic (As), barium (Ba), nickel (Ni) and tin (Sn) were observed. In sediment, the presence of cobalt (Co) and manganese (Mn) was remarkable.

The contamination by **Substances of Very High Concern (SVHC)** such as dioxins, PFAS and heavy metals – is not limited to eggs or moss but reflect a broader issue of persistent organic pollutant (POP) deposition in the environment of Zubieta. This rising pollution trend underscores the urgent need for a thorough investigation into the semi-continuous flue gas measurement data from the Zubieta incinerator. Such an investigation is essential to determine whether the facility complies with legal obligations to eliminate or at least minimise emissions of hazardous substances - including dioxins (PCDD/F/dl-PCB), heavy metals and PFAS - into the environment.



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List of figures

FIGURE 1: WASTE-TO-ENERGY INCINERATOR ZUBIETA, PROVINCE GIPUZKOA, BASQUE COUNTRY.....	5
FIGURE 2: (BIO)MATRICES LOCATIONS IN THE SURROUNDING ENVIRONMENT OF WTE INCINERATOR ZUBIETA, MAY 2024 ..	6
FIGURE 3: CHEMICAL PFAS ANALYSIS VS. DIFFERENT (BIO)ASSAY PFAS ANALYSIS	7
FIGURE 4: DIOXIN (PCDD/F/DL-PCB) BY DR CALUX ANALYSIS IN EGGS OF BACKYARD CHICKEN 2019-2024	8
FIGURE 5: COMPARISON OVERVIEW LOCATIONS OF DIOXIN RESULTS IN BACKYARD CHICKEN EGGS, 2019 - 2024	9
FIGURE 6: LOCATION 1 "PRESS", DIOXIN PATTERN (DR CALUX) 2019-2024.....	10
FIGURE 7: EXCEEDING DIOXIN-LIKE PCB IN EGGS OF BACKYARD CHICKENS (LOC. 9 AND 3), ZUBIETA 2019-2024	10
FIGURE 8: RESULTS DIOXINS (PCDD/F) IN MOSSES (BRYOPHYTES), 2024	11
FIGURE 9: DIOXIN IN MOSSES (BRYOPHYTES) MARKED BY TW COLOR INDICATIVE SCALE (88% DRY WEIGHT)	12
FIGURE 10: CHEMICAL ANALYSIS PFAS ON EGGS OF BACKYARD CHICKEN 2022-2024.....	13
FIGURE 11: PFAS IN WATER STREAM 2021, DUE TO CALAMITIES IN SAME WATER STREAM OF RESEARCH IN MAY 2024.....	15

List of tables

TABLE 1: RESULTS DIOXINS (PCDD/F/DL-PCB) RESULTS IN MOSSES CONCENTRATIONS AND HEATMAP BY DR CALUX .	12
TABLE 2: CHEMICAL PFAS ANALYSES EGGS OF BACKYARD CHICKEN 2022-2024	13
TABLE 3: PFAS ANALYSES IN MOSSES.	14
TABLE 4: CHEMICAL PFAS ANALYSIS MOSSES (BRYOPHYTES), SEDIMENT, WATER 2021-2024.....	14
TABLE 5: HEAVY METALS IN WATER	15
TABLE 6: CONCENTRATION HEAVY METALS IN SOIL, ZUBIETA 2024	16
TABLE 7: HEATMAP OF EXCEEDING FACTORS IN SOIL, ZUBIETA 2019 - 2024	16
TABLE 8: CONCENTRATIONS HEAVY METALS IN MOSS, 2019 - 2024 ZUBIETA	17
TABLE 9: HEATMAP HEAVY METALS IN MOSSES, 2019 - 2024 ZUBIETA	17



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