



CW Terahertz Spectroscopy of Heavy-Metal-Induced Alterations in *Arabidopsis halleri*

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Abstract

Soil pollution and the resulting accumulation of heavy metals in plants represent an increasing challenge for food safety and environmental monitoring. Traditional detection techniques are often destructive, labor-intensive, and poorly suited for in-situ or large-scale applications. This study explores whether continuous-wave (CW) THz spectroscopy can detect alterations in *Arabidopsis halleri* leaves induced by long-term exposure to heavy metals, and how different sample preparation states influence this. Five leaf sets, ranging from well-prepared (dried and pressed) to fresh and unprocessed, were analyzed using CW THz transmission spectroscopy in the frequency range from 0.1 to 0.8 THz. Under controlled sample conditions, differences in the log-linear slope of the normalized transmission spectra were observed between contaminated and control groups. These findings suggest a sensitivity of the THz transmission signal to changes in leaf composition, potentially associated with heavy metal uptake. However, the spectral contrast diminished under less standardized conditions (e.g., fresh or unpressed leaves), likely due to water-induced absorption and structural variability. In some cases, a difference in fit amplitude remained detectable, but the slope-based separation was not preserved. While the physical origin of the observed spectral differences remains to be fully resolved, possible contributing mechanisms include changes in water binding, dielectric properties, or cell wall organization. These results highlight both the promise and the current limitations of CW THz spectroscopy for non-destructive leaf analysis. Future efforts will focus on clarifying underlying mechanisms, improving robustness under variable sample conditions, and exploring the potential of multivariate and machine-learning methods for enhanced signal interpretation. The compact architecture of CW systems may ultimately support their integration into portable diagnostic tools.

Keywords Thz spectroscopy · Heavy metal detection · Plants

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1 Introduction

Heavy metal contamination of agricultural soils poses a serious threat to food safety and environmental sustainability [1, 2]. Plants growing in such soils can accumulate toxic metals like cadmium (Cd) and zinc (Zn), which may enter the food chain or alter plant physiology [3]. Conventional detection methods, such as atomic absorption spectroscopy or inductively coupled plasma mass spectrometry (ICP-MS), require destructive testing and are typically restricted to laboratory settings [4]. Non-destructive techniques that enable early detection of stress-related physiological changes would therefore be of great interest for environmental monitoring and precision agriculture [5, 6]. In recent years, terahertz (THz) spectroscopy has emerged as a potential method for probing the biochemical and structural properties of plant tissues [6, 7]. Due to their sensitivity to water content and molecular relaxation processes, THz waves interact with a range of biological features that may reflect environmental stress [8, 9]. However, many THz-based approaches rely on time-domain systems, which, while powerful, are often complex, bulky, and difficult to scale for portable applications. Among THz modalities, continuous-wave (CW) THz spectroscopy offers several practical advantages for near the field use cases. These include higher spectral resolution and spectral brightness, simplified optical architectures, and greater potential for miniaturization [10–12]. CW systems generate narrowband, tunable THz radiation via optical heterodyning of two lasers with slightly offset frequencies. This setup enables the construction of compact and cost-effective sensing systems, which may support future deployment in distributed or in-field diagnostics. This study investigates whether CW THz spectroscopy can detect structural modifications in plant leaves resulting from long-term heavy metal exposure under varying preparation states. Using genetically identical clones of *Arabidopsis halleri*, a model hyperaccumulator species, we compare transmission spectra of contaminated and non-contaminated samples across four different sets—from pressed and dried to fresh and unprocessed. We focus on two metrics: the log-linear slope of the transmission spectra and the amplitude of fitted signals. The aim is to assess the robustness of CW THz signals under increasingly realistic sample conditions and to explore the potential limitations of the method. Building on our earlier work, which focused exclusively on dried leaf samples [13, 14], this study extends the investigation to a broader range of preparation conditions and introduces a unified, slope-based comparison metric across all sets. While previous studies have linked THz responses to water content or mechanical properties [15, 16], recent work has further demonstrated the potential of THz spectroscopy in detecting water status changes in plants [17, 18], stress-induced physiological changes in *Arabidopsis thaliana* [19], internal leaf structural properties [20], and for characterizing leaf permittivity as a function of water content [21]. However, the sensitivity of CW THz measurements to subtle stress-induced changes remains poorly understood. Our approach aims to provide first insights into the feasibility and constraints of slope-based discrimination in CW THz leaf spectroscopy. Rather than attempting to map detailed biochemical mechanisms, we evaluate whether spectral slope and amplitude can serve as reliable indicators of stress exposure, subject to the influence of preparation method, tissue hydration, and surface irregularity.

2 *Arabidopsis Halleri*

Arabidopsis halleri is a well-established model for studying heavy metal hyperaccumulation and tolerance, offering genetic uniformity and well-characterized physiological responses to metal exposure [22]. This includes mechanisms such as enhanced root-to-shoot translocation, metal chelation, and vacuolar sequestration, which are likely to affect water distribution, cell wall structure, and internal morphology, factors potentially influencing THz transmission. *A. halleri* is a facultative metallophyte and hyperaccumulator, capable of tolerating and storing unusually high concentrations of zinc (Zn) and cadmium (Cd) in its tissues. These physiological traits are linked to specific adaptations in metal transport, detoxification, and compartmentalization mechanisms, as well as to enhanced resistance against oxidative stress [7]. Because of these characteristics, *A. halleri* is frequently used in phytoremediation research and plant physiology studies related to heavy metal stress. In this work, the species serves as an ideal biological platform for exploring spectral signatures associated with heavy metal exposure. To minimize biological variability, we employed vegetatively propagated, genetically identical clones. Two groups of plants were cultivated under otherwise identical greenhouse conditions (light, temperature, irrigation):

- The **control group** was grown in uncontaminated soil with background levels of Zn and Cd.
- The **contaminated group** was grown in soil supplemented with elevated concentrations of Zn and Cd.

This controlled design ensures that differences observed in THz spectral behavior can be attributed primarily to the presence or absence of heavy metal exposure and resulting differences in the magnitudes of metal concentrations in the leaves, rather than to genetic or environmental variability. The clear separation between treatment groups provides a robust basis for assessing the sensitivity of CW THz spectroscopy in detecting metal-induced changes in plant tissue.

2.1 Leaf Sampling and Preparation

To systematically assess the influence of leaf condition and sample handling on THz spectral behavior, five distinct sample sets were prepared from genetically identical clones of *Arabidopsis halleri*. All plants were cultivated under controlled greenhouse conditions with either uncontaminated or Zn/Cd-contaminated soil. To minimize positional or developmental biases, leaf samples were randomly selected from different locations on each plant. The preparation workflow for each set is illustrated in Fig. 1.

- **Set 1 (Sept. 2022):** Leaves were harvested in late summer, air-dried at room temperature for seven days, and then gently pressed to reduce surface variability. This set represents consistently prepared samples with relatively uniform structure.
- **Set 2 (Jan. 2023):** Leaves were prepared in the same manner as Set 1 but originated from plants that had been exposed to soils containing different levels of metals for a longer time period. The aim was to assess whether extended exposure time correlates with spectral differences.

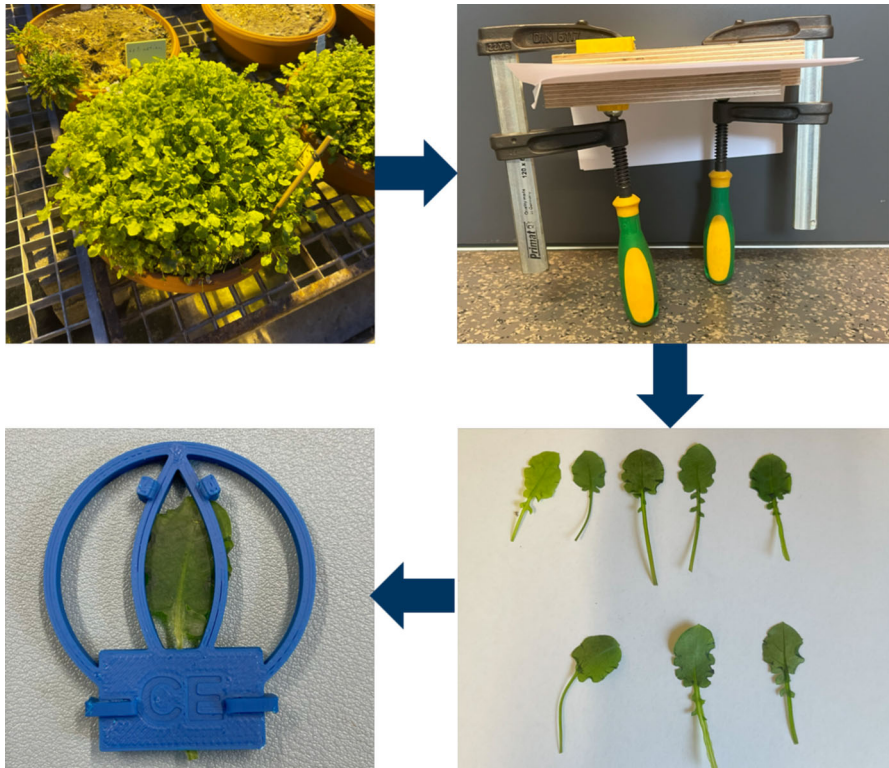


Fig. 1 Preparation steps of plant leaf samples used in the THz experiments

- **Set 3 (Jan.–Apr. 2023):** Harvested alongside Set 2 but stored cool and measured after three months. This set provides insight into possible changes resulting from post-harvest storage, under otherwise similar preparation conditions.
- **Set 4 (Aug. 2023):** Fresh leaves were analyzed directly after harvest without drying or pressing. Due to the absence of mechanical preparation, increased structural variability (e.g., natural curling or folding) is expected. This set reflects a more native state. *Note: A newly propagated clone was used for the contaminated group in this set, as the original plants had senesced.*
- **Set 5 (Aug. 2023):** The same leaves as in Set 4 were oven-dried at 80°C for two days. Pressing was omitted, preserving the original surface geometry. The purpose was to examine the influence of water content independently from sample flattening.

All samples underwent visual inspection to ensure basic sample integrity. In addition, Optical Coherence Tomography (OCT) was performed on all leaves to quantify spatial variations in leaf thickness. Although these thickness measurements were not directly used in the THz data evaluation, they provided important context for interpreting structural variability across sets. After completion of the THz measurements, all leaf samples were subjected to Inductively Coupled Plasma Optical Emission

Spectrometry (ICP-OES) to validate the accumulation of Zn, Cd, and other relevant elements in the respective plant tissues. This compositional analysis served as a reference standard for assessing the sensitivity and specificity of the THz spectroscopy approach.

This preparation strategy ensures that differences observed in THz spectral behavior can be attributed primarily to structural changes resulting from long-term heavy metal exposure rather than secondary effects such as water content, surface morphology, or aging.

2.2 Leaf Thickness Measurements

To characterize structural differences across all sample sets, the optical thickness of each leaf was measured using Optical Coherence Tomography (OCT, Thorlabs CAL110C1). OCT provides high-resolution, non-destructive imaging of biological tissues and was employed to assess local thickness variations with micrometer precision.

For each leaf, a surface area of 5 mm × 5 mm was scanned, yielding 16,384 A-scans per sample. The resulting thickness distributions are illustrated in the boxplots in Fig. 2, which compare optical thicknesses between preparation sets and soil treatment groups.

The data reveal the following trends:

- **Set 1:** Broad variation in leaf thickness due to biological heterogeneity and seasonal influences during the September 2022 harvest.
- **Set 2:** Narrower distributions with more consistent thickness, reflecting uniform summer growth and identical preparation (January 2023).
- **Set 3:** Slightly increased deviations compared to Set 2, likely influenced by storage and delayed measurement post-harvest.
- **Set 4:** Sample thickness was relatively consistent across all measurements.

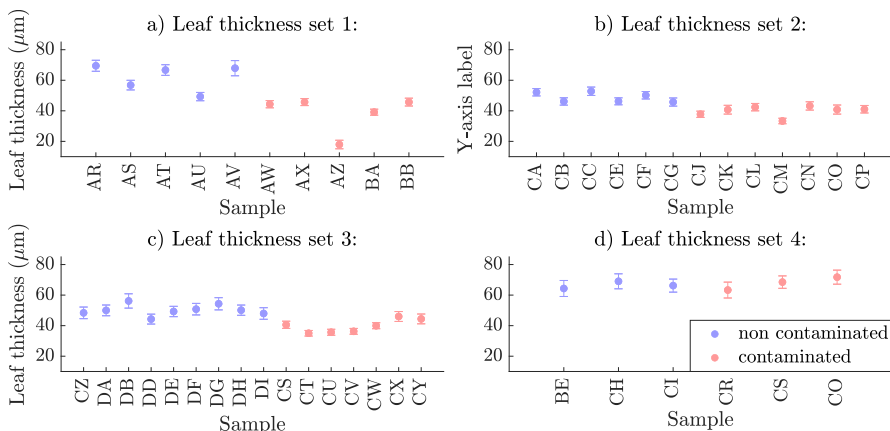


Fig. 2 Boxplots of the optical thickness of leaves from Sets 1 to 4 measured using OCT. For each leaf, 16,384 A-scans were analyzed

Across Sets 1 and 2, leaves from contaminated soil tended to be thinner than those from uncontaminated soil. This observation supports the hypothesis that prolonged exposure to heavy metals can affect plant morphology and structural development. In contrast, in Sets 3 and 4, thickness differences between treatment groups were less pronounced, likely masked by increased surface complexity and measurement variability.

While OCT measurements offer important structural insight, they confirm that leaf thickness alone does not suffice to reliably distinguish contaminated from non-contaminated samples. This reinforces the complementary value of THz spectroscopy, which captures compositional contrasts beyond geometric variation.

3 Experimental

The choice of a CW THz system is motivated by its inherent advantages for compact and potentially field-deployable instrumentation. Compared to time-domain THz systems, CW implementations offer higher spectral resolution and simpler optical layouts, which make them particularly attractive for future miniaturization and robust integration. To investigate the impact of heavy metal accumulation in *Arabidopsis halleri* leaves, we employed two complementary techniques: CW THz transmission spectroscopy and inductively coupled plasma optical emission spectrometry (ICP-OES). Five distinct leaf sets, differing in hydration state and preparation method, were analyzed to evaluate the robustness of the spectroscopic response under varying conditions.

3.1 CW THz Spectroscopy

THz measurements were performed using a CW THz system based on optical heterodyning. Two near-infrared lasers with slightly offset wavelengths generate a tunable difference frequency in the THz range. The resulting beat signal is amplified using an Erbium-Doped Fiber Amplifier (EDFA) and then split into two arms (see Fig. 3).

A 50:50 splitter evenly divides the optical signal between the transmitter and receiver paths. The transmitter consists of an InGaAs p-i-n photodiode, which is biased with a sinusoidal signal and serves as the THz emitter [23]. The receiver comprises an InGaAs:Fe-based photomixer acting as the detecting antenna [24]. Both antennas are integrated into a homodyne detection scheme. Sampling is done using a mechanical delay line. The photocurrent from the receiver is amplified using a Transimpedance Amplifier (TIA), and the signal is demodulated by a Lock-In Amplifier (LIA) referenced to the modulation of the emitter. Each leaf sample was placed in the focused beam spot of the THz setup and was measured over the frequency range of 0.1 to 0.8 THz. All measurements targeted the central region of each leaf (including portions of the midrib), ensuring consistent probe positioning relative to biological structure. Multiple time-domain periods were recorded and post-processed using MATLAB. Spectra were averaged across repetitions, and results were normalized to mitigate

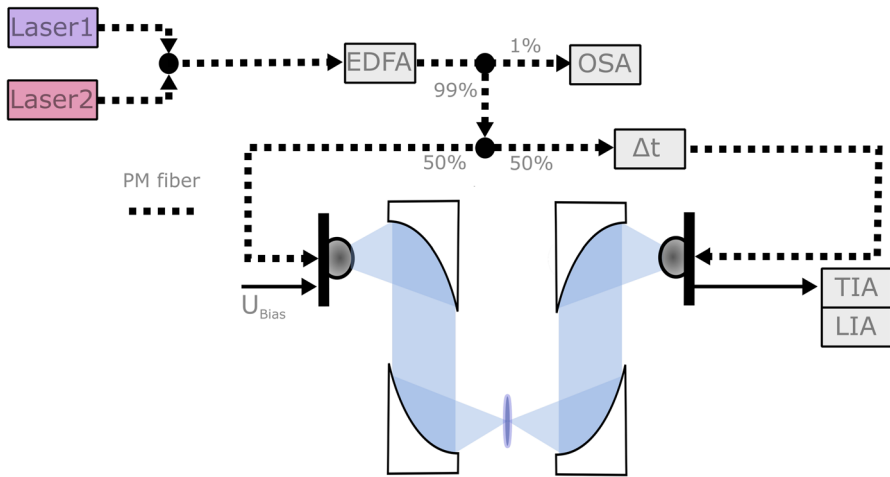


Fig. 3 CW THz homodyne system. Erbium-Doped Fiber Amplifier (EDFA), Optical Spectrum Analyzer (OSA), Transimpedance Amplifier (TIA), Lock-In Amplifier (LIA). Mechanical delay line (Δt)

effects of thickness variability and structural heterogeneity. Sets 1–3 were dried and pressed prior to measurement to minimize water-related absorption; Sets 4 and 5 included fresh and non-pressed dried leaves, respectively, enabling comparison under more natural conditions.

3.2 Spectral Slope and Amplitude Analysis

To evaluate differences in THz transmission spectra, each measurement was analyzed within the frequency range from 0.1 to 0.8 THz, discretized into 32 points. For each leaf at every frequency point, the amplitude of a sinusoidal fit to the time-domain signal was computed. These amplitudes were smoothed to reduce noise and normalized to the range [0,1]. To quantify the spectral shape, the normalized amplitudes were plotted on a semi-logarithmic scale (linear x-axis, logarithmic y-axis) (see Fig. 4). A linear fit was applied to the $\log_{10}$ -transformed amplitudes within the linear portion of the spectrum to capture the overall spectral decay (log-linear slope). The resulting fit coefficient corresponds to the spectral slope in dB per decade. This procedure was applied to all measurements from dried leaf Sets 1–3. For each sample group (contaminated vs. non-contaminated), the distribution of spectral slopes was visualized using boxplots. A two-sided unpaired t -test with unequal variances was conducted to test for statistically significant group differences. A p -value below 0.05 is considered significant.

3.2.1 Spectral Contrast Hypothesis

The observed differences in THz spectra are hypothesized to originate from compositional or microstructural changes induced by heavy metal accumulation. These may include alterations in cell wall composition, dielectric constant variation due to

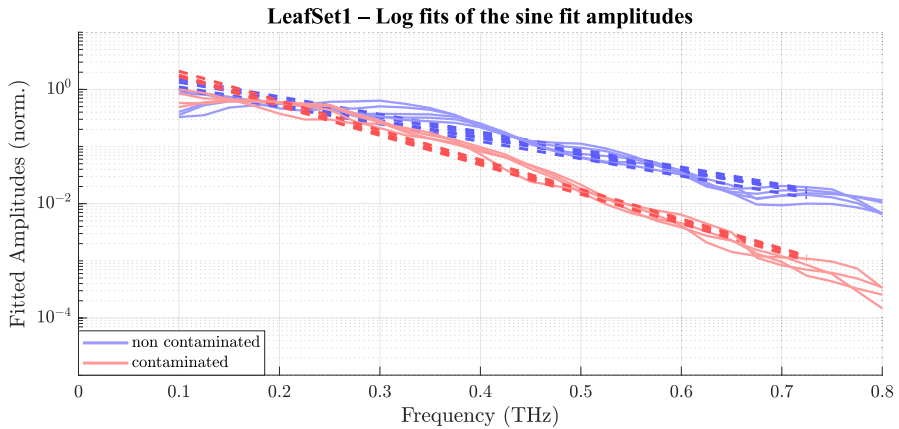


Fig. 4 Representative spectral fit example for leaf Set 1. Normalized amplitude spectra (solid lines) and corresponding linear fits in the log-domain (dashed lines) are shown. Contaminated samples (red) exhibit steeper slopes and lower amplitudes than non-contaminated controls (blue), indicating reduced THz transmission

bound ions, or differences in water-binding behavior. Although the exact mechanism remains under investigation, preliminary results from this and other studies suggest that such effects are detectable in the THz frequency range. To further isolate the spectral contributions, future work will include measurements on compressed metal salt tablets as inorganic references. Compared to techniques such as Laser Induced Breakdown Spectroscopy or hyperspectral Near Infrared imaging, THz spectroscopy does not resolve elemental composition directly. Instead, it provides a holistic view of structural and dielectric properties, making it a potentially complementary tool for environmental monitoring, especially under in-field conditions where preparation is minimal and water content varies.

3.3 ICP-OES

To validate the presence and magnitude of heavy metal accumulation, all leaf samples underwent quantitative elemental analysis via ICP-OES. Following THz measurements, the leaves were acid-digested and analyzed for cadmium (Cd) and zinc (Zn) concentrations, with select samples also assessed for additional macro- and micronutrients. Table 1 shows the Cd and Zn concentrations for Set 1, highlighting a clear distinction between the control and contaminated groups. *p*-values below 0.05 confirm statistically significant group separation. To assess specificity, Table 1 also lists concentrations of additional elements (Al, Ca, Fe), none of which showed statistically significant differences between groups.

Table 2 provides the Cd and Zn concentrations measured in Set 2. As in Set 1, the data confirm a clear accumulation of heavy metals in the contaminated group.

Table 3 shows the Cd and Zn levels for leaf Sets 4 and 5. As with Set 1, a clear group separation is evident despite differences in measurement timing and preparation.

Table 1 Heavy metal concentrations (Cd and Zn) and concentrations of selected elements in $\mu\text{g/g}$ in Set 1 leaf dry biomass, measured by ICP-OES. Significant group differences just for Cd and Zn were observed

Group	Sample	Cd	Zn	Al	Ca	Fe
Non — contaminated soil	AR	0.28	177.30	7.87	20,460.00	46.00
	AS	0.34	166.40	8.35	23,645.69	67.25
	AT	0.36	168.60	2.78	16,990.00	57.47
	AU	0.10	374.40	3.73	29,070.00	132.90
	AV	0.21	323.40	20.19	20,580.00	66.63
Contaminated soil	AW	137.10	14,620.00	6.30	37,140.00	88.35
	AX	99.06	8824.00	8.61	29,590.00	79.66
	AZ	43.85	4678.00	3.46	14,580.00	37.90
	BA	63.48	6084.00	0.31	18,740.00	77.90
	BB	37.28	3799.00	2.40	10,760.00	44.32
<i>p</i> -value		0.0036	0.034	0.2386	0.9953	0.6579

Table 2 Heavy metal concentrations (Cd and Zn) for Set 2 leaf dry biomass, measured by ICP-OES

Group	Sample	Cd ($\mu\text{g/g}$)	Zn ($\mu\text{g/g}$)
Non — contaminated soil	CA	0.20	280.81
	CB	0.17	587.83
	CC	0.13	448.21
	CE	0.29	755.71
	CF	0.22	605.02
Contaminated soil	CG	0.16	721.75
	CK	66.19	12,144.27
	CL	51.74	13,536.27
	CM	61.87	14,590.66
	CN	64.85	12,003.24
	CO	68.87	14,018.18
	CP	65.13	16,464.22
<i>p</i> -value		0.0019	8.3×10^{-07}

Table 3 Heavy metal concentrations (Cd and Zn) for Sets 4 and 5 leaf dry biomass measured by ICP-OES

Group	Sample	Cd ($\mu\text{g/g}$)	Zn ($\mu\text{g/g}$)
Non — contaminated	BE	1.2	398
	CL	1.28	718
Contaminated	CH	1.15	223
	CO	56.30	14,428
	CR	32.55	8446
	CS	49.37	12,056
<i>p</i> -value		0.024	0.023

4 Results

4.1 Dried Leaves

Figure 5 displays the transmission spectra of Set 1, comprising air-dried and mechanically pressed leaves. The normalized spectra show a consistent group-specific difference: while samples from non-contaminated plants closely follow the reference signal in both amplitude and spectral slope, the contaminated group exhibits a stronger spectral decay.

To quantify this observation, a linear fit was applied to the $\log_{10}$ -transformed amplitude spectra over the range 0.1–0.75 THz. The resulting slope values reveal a significantly steeper decline in contaminated leaves, indicating increased absorption or scattering. Since all samples were identically prepared, the observed differences are unlikely to result from residual water content or surface variation.

A two-sample t-test comparing the fitted slope parameters confirmed statistical significance between the groups ($p < 0.001$), supporting the hypothesis that CW THz spectroscopy is sensitive to structural changes induced by prolonged heavy metal exposure.

4.1.1 Comparison Across Sets 1–3

Figure 6 summarizes the group-wise mean spectra for Sets 1 to 3. Despite seasonal, aging, and preparation-related variations, all datasets consistently show a stronger spectral decay in contaminated samples. The slopes, shown in Fig. 7, were derived from quadratic polynomial fits in the log-lin domain. A two-sample t-test revealed significant differences between groups in each set (Set 1: $p = 0.0159$, Set 2: $p = 0.0131$, Set 3: $p = 0.0026$), confirming that spectral slope is a robust contrast metric under dried conditions.

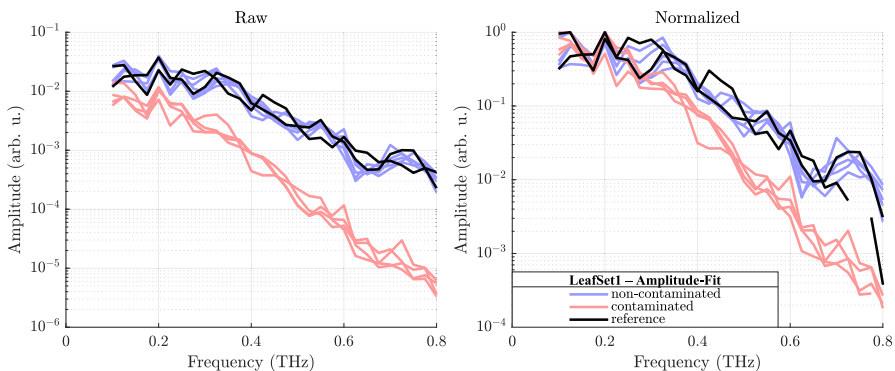


Fig. 5 Raw and normalized transmission spectra of dried and pressed leaf Set 1. The spectra of the non-contaminated group are shown in blue, the contaminated group in red. Black curves denote reference spectra acquired with an empty sample holder

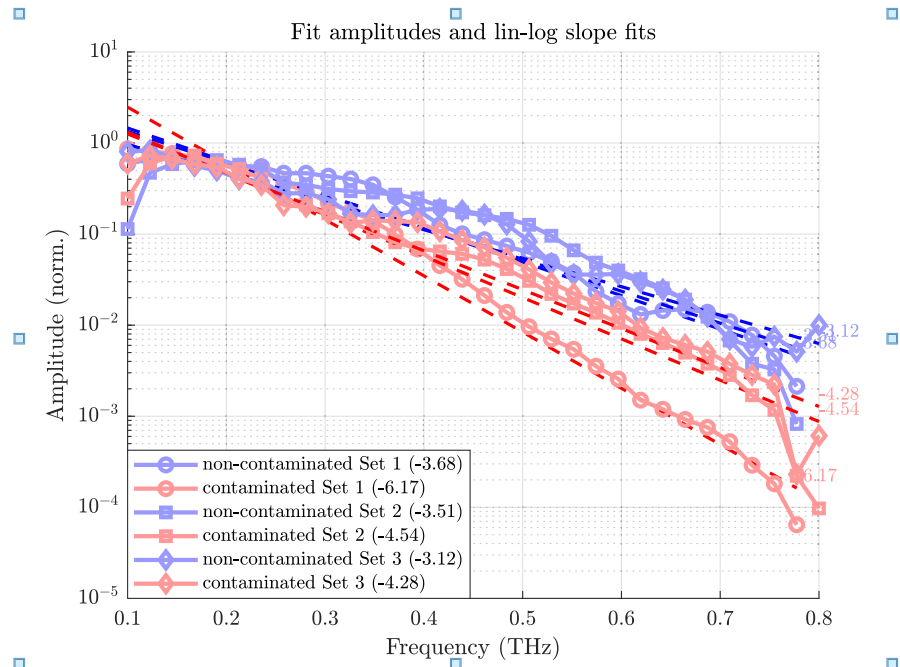


Fig. 6 Mean normalized spectra of dried leaf Sets 1–3. Non-contaminated samples (blue), contaminated samples (red), and corresponding fitting of the slope (dotted). Differences in spectral slope are visible across all sets

4.2 Unprepared Leaves

Figures 8 and 9 summarize the results from the unpressed leaf sets (Set 4 and Set 5). These measurements extend the study to less standardized plant material and evaluate the robustness of CW THz spectroscopy under conditions that resemble potential field use. However, the results illustrate clear limitations of the technique in such contexts.

4.2.1 Fresh Leaves (Set 4)

Figure 8a shows that the overall transmission is strongly attenuated across the entire frequency range. This is primarily due to the high water content in the fresh leaves, which introduces substantial absorption in the THz regime. In addition, each measurement takes approximately four hours per leaf, during which the leaves dehydrate and deform. As a result, each measurement reflects a different hydration state and geometry, impairing comparability, contributing to spectral variability, and highlighting the impracticality of long acquisition times for real-world or high-throughput settings.

Despite these challenges, no consistent difference is visible between contaminated and non-contaminated samples in this dataset. The strong water absorption, coupled with scattering effects from natural curvature and uncontrolled deformation, appears to mask any subtle compositional contrast. The overall sample size for this group was

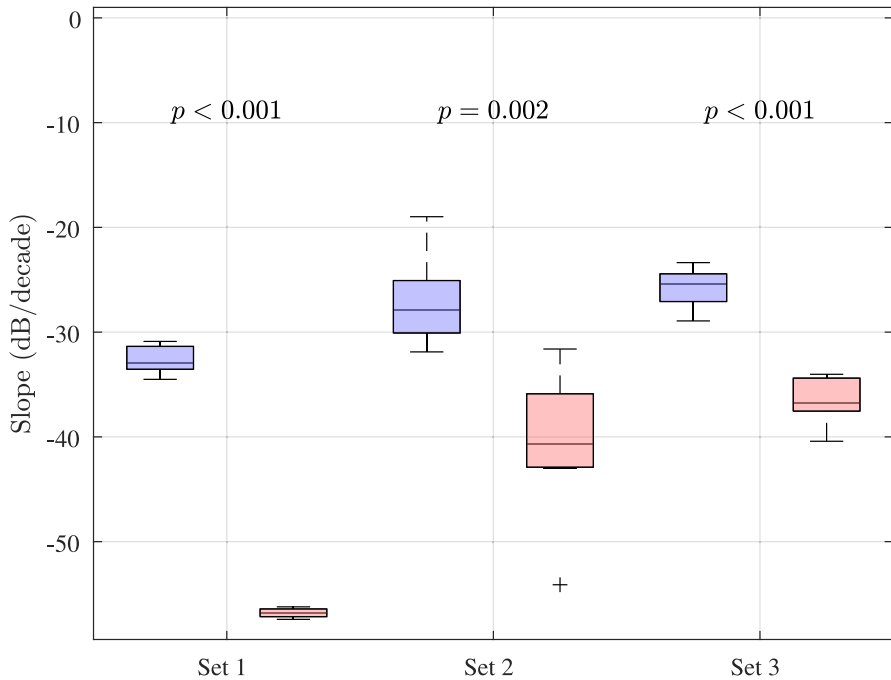


Fig. 7 Boxplot of log-lin spectral slope values for Sets 1–3 (dried leaves). Statistically significant differences are observed in all sets

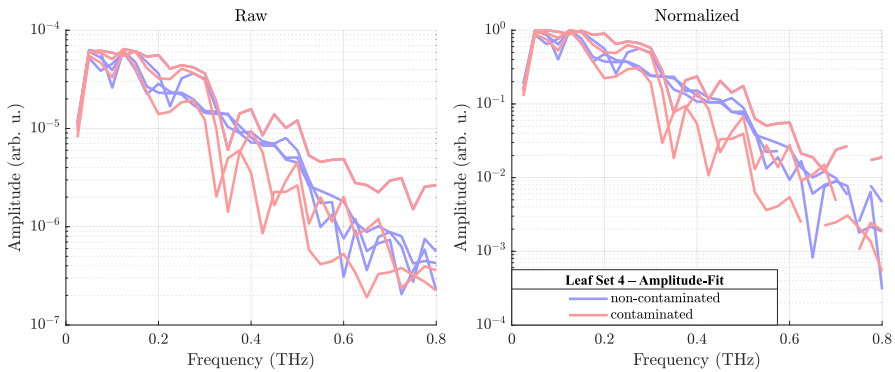
deliberately kept small due to the long acquisition time and progressive instability of the fresh samples.

4.2.2 Dried Leaves (Set 5)

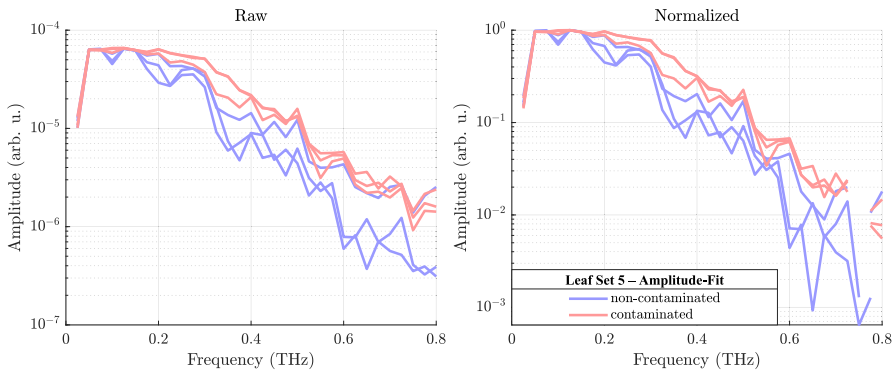
After oven-drying, a modest increase in overall signal amplitude is observed (see Fig. 8b). This is consistent with the reduction in water-related absorption. However, since the leaves were not mechanically pressed, they exhibited surface irregularities and shrinkage (see Fig. 9), introducing additional scattering and variability.

While the transmission improves slightly compared to fresh leaves, the spectral shape remains noisy, and no pronounced group-specific separation is evident by visual inspection. A statistical analysis of the mean fitted amplitudes across frequency steps revealed a significant difference ($p = 0.0172$), suggesting that amplitude may still serve as a useful—though limited—metric in dried, unpressed leaves. However, the observed contrast is clearly weaker and less reliable than in dried and pressed sets.

Taken together, the findings from Sets 4 and 5 indicate that CW THz spectroscopy is currently not well suited for fresh, unprepared leaf samples due to dominant water absorption, irregular surface structures, sample deformation during leaf desiccation, and long acquisition times. Although partial contrast can be recovered after drying, mechanical flattening appears essential for consistent and reproducible group discrimination.



(a) Normalized transmission spectra of the fresh leaf Set 4. The spectra of the non-contaminated soil group are shown in blue while the spectra of the contaminated soil group are shown in red.



(b) Normalized transmission spectra of the dried leaf Set 5. The spectra of the non-contaminated soil group are shown in blue while the spectra of the contaminated soil group are shown in red.

Fig. 8 Comparison of normalized transmission spectra from Set 4 (fresh leaves) and Set 5 (oven-dried, unpressed leaves). Both sets show low overall transmission and limited spectral contrast

5 Interpretation and Hypothesis

The clear spectral distinction between contaminated and non-contaminated samples observed in Sets 1–3 underlines the sensitivity of CW THz spectroscopy to compositional differences in well-prepared plant material. These differences, evident through altered transmission behavior, are probably rooted in physical, chemical, and biochemical modifications accompanying the differential accumulation of Zn and Cd.

Potential mechanisms include changes in the dielectric response due to altered cell wall composition, shifts in bound water dynamics, or microstructural rearrangements, all of which influence the complex permittivity of leaf tissues in the THz regime. Recent

Fig. 9 Photograph of a dried, unpressed leaf after measurement. Shrinkage and surface deformation contribute to increased scattering and signal attenuation



studies confirm that THz dielectric properties of leaves vary with physiological status and can be exploited for stress monitoring [25].

Measurements of less standardized samples (Sets 4 and 5), however, proved less robust. In fresh leaves, dominant water absorption and uncontrolled dehydration during the lengthy acquisition period obscured compositional signatures. This can be seen in Fig. 8a, in particular, the spectra of leaves grown in contaminated soil are very noisy and show high variance. This behavior decreases after drying, which suggests that the individual samples had different water contents. Similarly, the unpressed dried leaves exhibited considerable structural variability, likely causing increased scattering and noise.

These results suggest that without rigorous control over hydration and geometry, the contrast mechanisms become unreliable. To extend CW THz spectroscopy toward real-world applications, technical improvements are necessary. Stabilization of environmental parameters—such as ambient humidity and temperature—as well as mechanical constraints to limit leaf deformation could mitigate current limitations.

Further research should target the physical basis of the contrast mechanism. Specific recommendations include:

- Microscopic imaging (SEM/TEM) to visualize structural differences.
- Dielectric analysis of dehydrated metal salts and aqueous metal solutions to isolate element-specific contributions.

Moreover, the incorporation of machine learning or multivariate classification methods could help recover subtle group differences in future studies, especially under less controlled sample conditions. Future studies may also explore the parametric fitting of spectral data to dielectric models of biological tissues, as demonstrated in earlier work on hydrated plant leaves [21], to further disentangle structural from compositional contributions to the THz response.

6 Conclusion

This study investigated the feasibility of continuous-wave (CW) Terahertz spectroscopy for detecting heavy metal accumulation in plant leaves. Using genetically identical clones of *Arabidopsis halleri* grown under controlled conditions, four sample sets comprising leaves contrasting in metal concentrations were analyzed, which differed in preparation and hydration state.

In dried and pressed leaves (Sets 1–3), statistically significant differences in spectral characteristics between leaf groups (cultivated on contaminated and uncontaminated soil) were observed. These differences, reproducible across several datasets, demonstrate that CW THz spectroscopy can reflect compositional changes related to Zn and Cd uptake, provided that structural and moisture-related factors are tightly controlled.

By contrast, fresh and unpressed dried leaves (Sets 4 and 5) showed limited spectral separability. Water absorption in fresh tissue dominated the spectra, while progressive dehydration during the approximately four-hour-long measurements introduced additional variability. Similarly, unpressed dried leaves exhibited scattering effects due to surface irregularities and shrinkage. Although some group-specific amplitude differences persisted after drying, the signal quality was markedly reduced.

These findings emphasize the critical role of sample preparation and currently preclude practical use of CW THz spectroscopy for in-field or live-plant applications without substantial methodological improvements. Water removal and mechanical flattening are essential for generating interpretable and consistent THz transmission spectra. Advanced evaluation techniques, such as machine learning, spectral decomposition, or multivariate models, can enhance interpretability under less ideal conditions but cannot compensate for strong physical signal degradation.

In conclusion, CW THz spectroscopy offers potential as a qualitative, non-destructive tool for assessing heavy metal stress in plants, especially when applied to structurally controlled samples. For broader deployment, future work should focus on reducing acquisition time, improving environmental stability, and elucidating the material-specific origins of spectral contrast.

Author Contributions L.C.K. wrote the main manuscript text and prepared the figures. F.B. and P.D. performed and evaluated the ICP-OES measurements. L.C.K., S.T.G., and N.S. performed the experiments and laboratory work. M.D. and R.B.K. developed and provided the antennas. C.B., U.K., and M.R.H. contributed to the conceptualization.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethical Approval Not applicable

Competing Interests The authors declare no competing interests.

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