

# Differential analysis of soil using GC Orbitrap MS and Compound Discoverer

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## ABSTRACT

The objective of this work is to demonstrate the utility of gas chromatography-Orbitrap™ mass spectrometry and Thermo Scientific™ Compound Discoverer™ software for the differential analysis of soil samples. Specifically it will show the power of Thermo Scientific™ Compound Discoverer™ software for the processing of GC HRMS data and present statistical differences between soils from different locations and make compound identifications.

## INTRODUCTION

Environmental samples, (e.g., soil, sediments, or surface water) can contain a broad spectrum of volatile or semi-volatile contaminants, including polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants (BFRs), and pesticides. The combination of gas chromatography (GC) with quadrupole-based mass spectrometers is a common analytical setup for detection of these contaminants. GC-MS quadrupole-based instruments are well known for their robustness and ease of use.

High-resolution accurate mass (HRAM) mass spectrometry provides a very sensitive and selective non-target acquisition and surpasses quadrupole instruments in all non-targeted applications. However, to fully realize the benefits of a HRAM system, powerful software is essential to convert high quality data into scientific discovery. In this study, GC-Orbitrap technology and Compound Discoverer software were used to assess the chemical profile of soil sample extracts taken from three locations near Bremen, Germany. Data were acquired in full-scan with electron ionization (EI) mode. Positive chemical ionization (PCI) and negative chemical ionization (NCI) were used to confirm the elemental composition of the molecular ions using accurate mass information, isotopic match (measured versus theoretical), and presence of specific adducts.

## MATERIALS AND METHODS

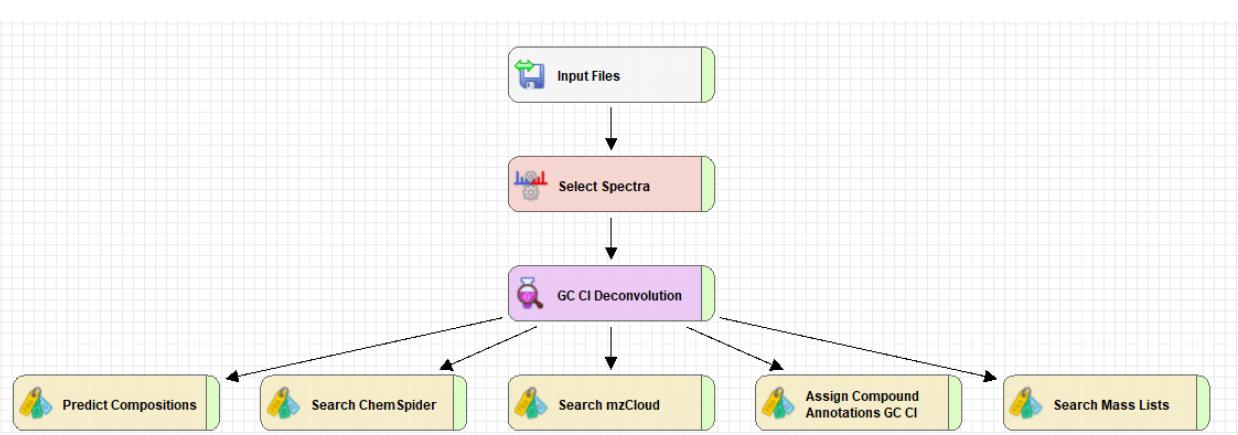
Three soil samples were taken from various locations in and near to Bremen in Germany. They were collected in proximity of a motorway junction, close to an airport, and in a stand-alone house neighborhood, respectively. The samples were extracted without any pretreatment. A 2 g portion of soil was weighed in a polypropylene tube followed by the addition of 4 mL of acetonitrile and vortexed for 5 minutes. Organic contaminants were transferred to the hexane phase.

Table 1 & 2. Thermo Scientific™ TRACE™ GC 1610 and mass spectrometer conditions.

TRACE 1610 GC		Orbitrap Exploris GC 240 mass spectrometer in EI mode	
Injector		Transfer line (°C)	300
Injection volume (μL)	1	Ion source (ionization type)	Thermo Scientific™ ExtractaBrite™ (EI) source
Liner	Single goose-neck with glass wool	Ion source (°C)	280
	Thermo Scientific™ LinerGOLD™ (PN 453A1925-U)	Electron energy (eV)	70
Inlet temperature (°C)	300	Emission current (μA)	50
Inlet module and mode	SSL, Splitless	Acquisition mode	Full scan (FS)
Splitless time (min)	1	Mass range (m/z)	50–550
Septum purge flow (mL/min)	5	Resolving power	120,000
Oven and column		AGC target	Standard
Carrier gas, flow rate (mL/min)	He, 1.2	Maximum injection time	Auto
Column	Thermo Scientific™ TraceGOLD™ TG-5SiMS 30 m x 0.25 mm i.d. x 0.25 μm (PN 26096-1420)	Lock masses	133.01356; 207.03235; 225.04292; 281.05114; 299.06171; 355.06993
Oven temperature program		Temperature 1 (°C)	40
Temperature 2 (°C)	300	Rate (°C/min)	10
Hold time (min)	2	Hold time (min)	7
Total GC run time (min)	35		

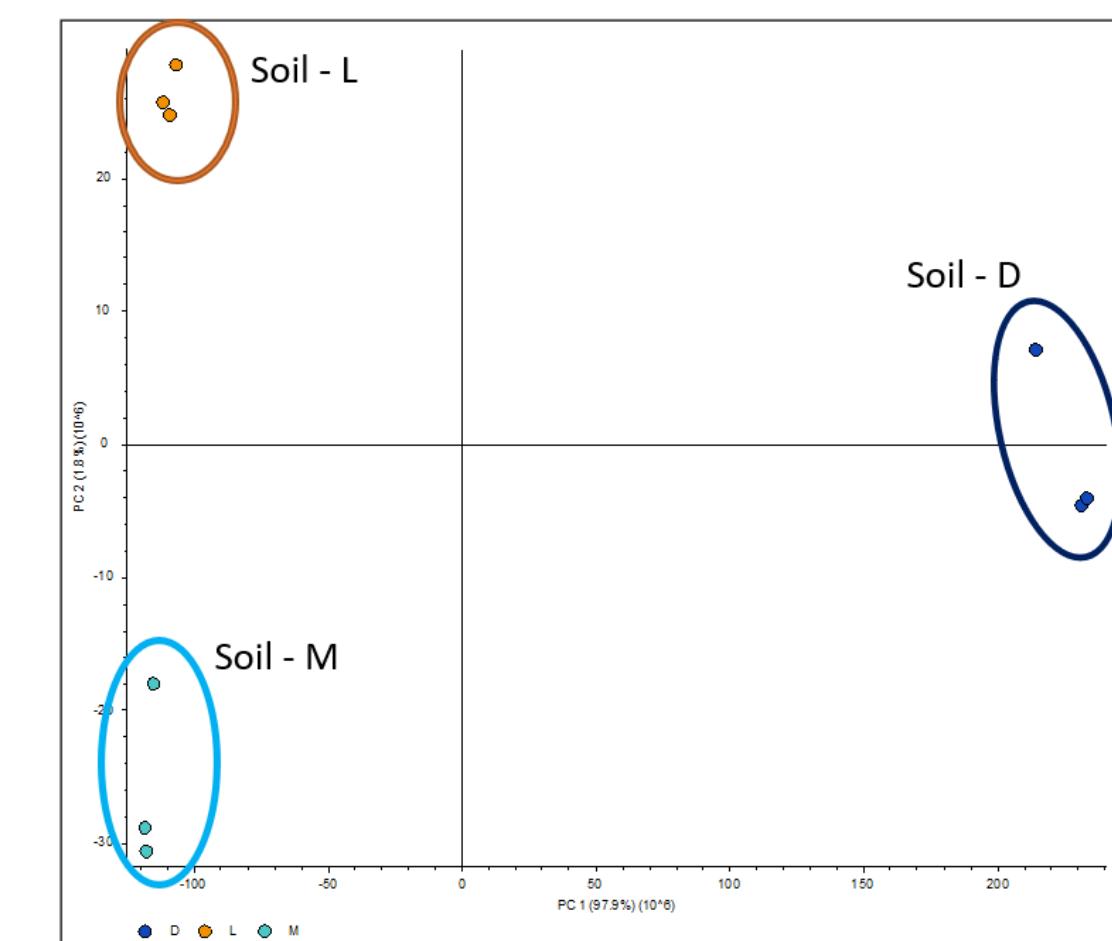
## RESULTS

Figure 1. The PCI Compound Discoverer software workflow used to confirm the compounds identified in the EI workflow.



Compound Discoverer software contains template workflows for GC EI, as well as GC PCI data. In this study, the EI data were used for statistical analysis and compound identification, whereas the PCI data were used for the confirmation purposes. Figure 1 shows the workflow applied for the PCI data processing. In this study, the following identification nodes were applied: Predict Composition, Search ChemSpider™, Search mzCloud™, Search Mass List: serves to a databases search (this node is also available for EI workflows).

Figure 2. PCA score plot of the volatile compounds that differentiate the three soil samples from different locations. A complete separation between the sample groups was observed and good agreement between replicate injections.



The first objective was to identify if there was any significant difference between the three soil samples at locations D, L, and M. This was achieved through a PCA plot of the replicate injections of each sample. Figure 2 shows the PCA plot that demonstrates that there are clear differences between the samples and good agreement of the replicate injections.

Figure 3 shows a volcano plot for the samples D and L. The volcano plot is a type of scatter plot for replicate data where the x axis represents the log2 of the fold change between two sample groups (generated ratio), and the y axis represents the negative log10 of the p-value (test of significance) of the fold change. In other words, when a point (compound) is more on the right (positive values on x axis), the peak area of that compound is much higher in the sample D than in the sample L. Whereas, points that are higher on the graph are statistically more significant. The following step is to make identification as shown in figure 4 for pyrene.

Figure 3. V-plot scatterplot showing the statistical significance (P value) versus magnitude of change (fold change) when comparing the soil sample D (right) versus the soil sample L (left). The main chemical components that are responsible for sample diversity between two sample groups are located in the upper right and left sides of the plot.

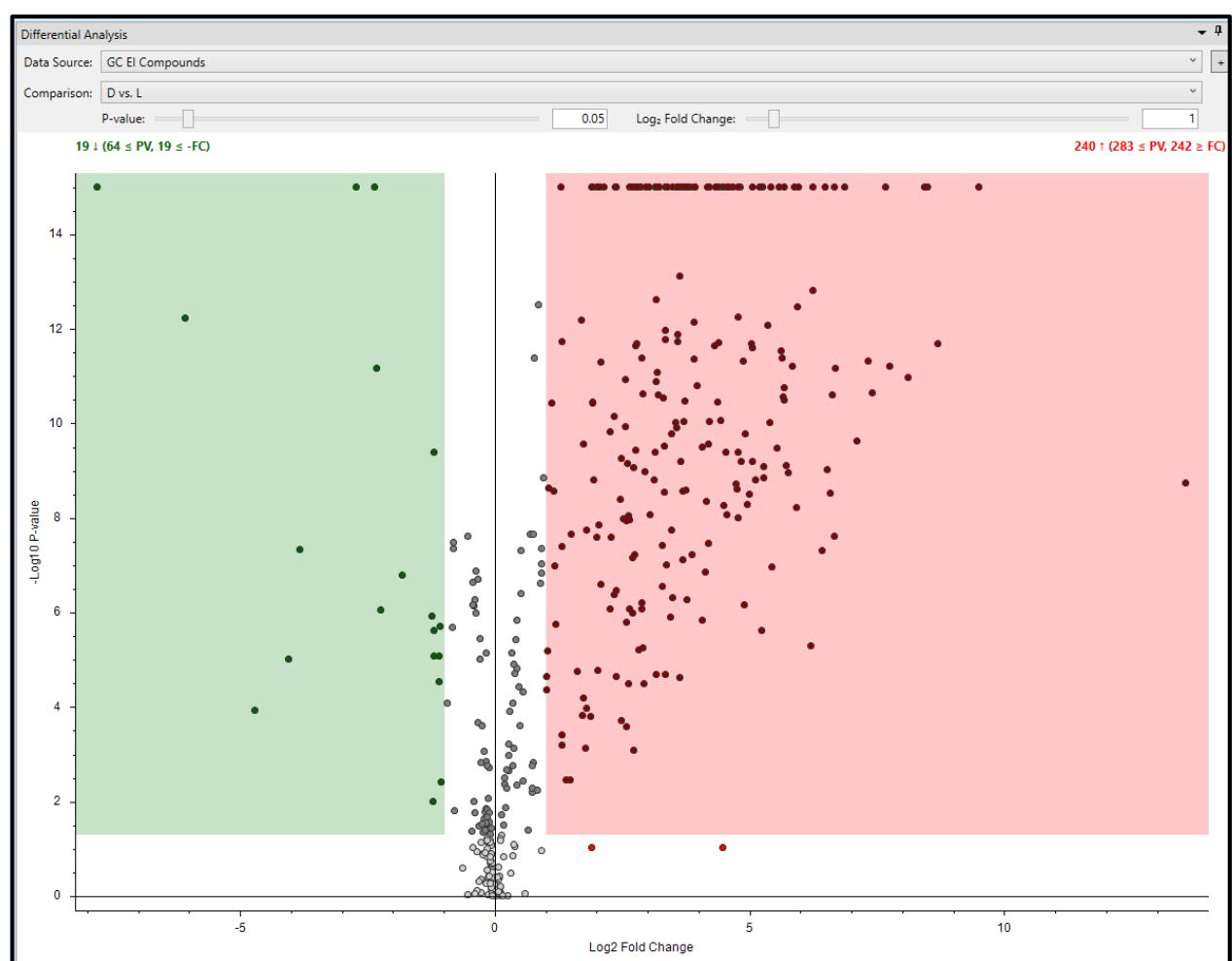
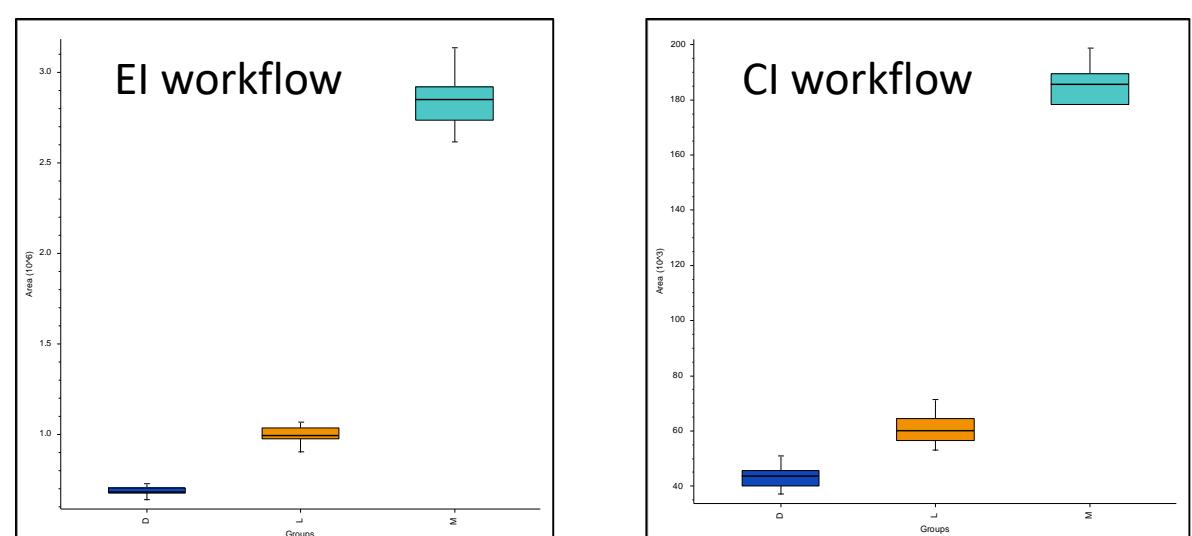


Figure 4. Identification of the peak at 22.18 minutes as pyrene. Table data (A) showing search index (SI) at 897 and reverse SI (932). The compound molecular mass was detected with excellent mass accuracy of 0.1 ppm (B). The spectrum mirror plot (C) comparison to the library.

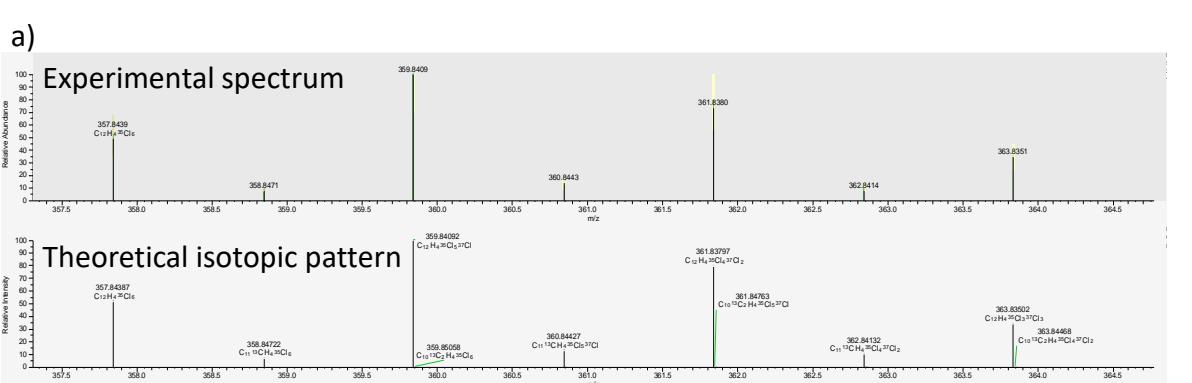


Figure 5. Box plot view from Compound Discoverer software showing how pyrene peak area varies across the three soil samples. The response was significantly higher in sample M.



Another interesting contaminant discovered in the investigated samples was a PCB containing six chlorine atoms. Sample D showed the highest levels of contamination; however, the analyte was also present in the two remaining soil samples. Thanks to the presence of six chlorine atoms, the isotopic pattern is very characteristic and can aid in the confirmation of the molecular formula (figure 6).

Figure 6. Comparison between experimental and theoretical isotopic pattern for C12H4Cl6 in EI.



## CONCLUSIONS

The Thermo Scientific™ Orbitrap Exploris™ GC 240 mass spectrometer in combination with Compound Discoverer software is an excellent tool for environmental sample analysis. Even in such a complex matrix as soil, the software detected and identified untargeted contaminants. The identification in EI was confirmed in CI. Moreover, the statistical analysis and graphical visualization tools facilitated the results interpretation.

## REFERENCES

1. Thermo Fisher Scientific Technical Note 10730: Mass resolving power of 240,000: for confident compound identification, 2021. <https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-10730-gc-ms-power-confident-compound-identificationtn10730-en.pdf>

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