Novel GC-IRMS-Based Method for Compound-Specific Isotope Analysis (CSIA) of Explosives Contaminants in Soil

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Abstract: Military explosives are common environmental pollutants, contaminating soils and water. Nevertheless, assessing the biodegradation and transformation process of nitroaromatic explosives in situ is challenging. In this study, we developed a procedure for compound-specific analysis of stable C, N, and O isotopes (CSIA) in explosives contaminants and characterized biodegradation of 2,4,6-trinitrotoluene (TNT) and dinitrotoluene isomers in the soil of a contaminated site. It will lead to a more quantitative analyses about the biodegradation in explosives contaminant, and help to evaluate the risk management of military ranges.

1. Introduction

Explosives contaminants include the explosives, by-products, manufacturing impurities, and environmental transformation and degradation products.[1] It is the result of the manufacture of explosives, production of ordnance, and the disposal of out-of-date and off-specification material. Explosives contaminants can cause serious pollution to the soil, groundwater and other related environments, and will continuously and deeply threaten the lives and health of surrounding soldiers, residents, and wild animals. [2]

With its clear advantage in terms of remediation costs, in situ microbial degradation of these compounds may be a positive process. [3] The prerequisite for using this natural attenuation method is to understand the extent, rate, and pathways of relevant biodegradation. These are most frequently assessed by monitoring the shifts in compounds’ concentration. However, quantifying biodegradation rates of contaminants based on the decrease in the compound’s concentration is not always as conclusive as one would like. Since a decrease in a contaminant's concentration cannot be related to biodegradation alone, as it may also be due to other natural attenuation processes, such as dispersion, sorption or volatilization. [4]

Compound-specific isotope analysis (CSIA) may therefore represent a promising way of inferring and understanding natural attenuation of Explosives contaminants. This methodology is not based on monitoring shifts in concentration, but rather shifts in isotopic composition. The fundamentals for this approach imply that light isotopes \(^{14}\text{N}, \, ^{16}\text{O}, \, ^{12}\text{C}\) are converted slightly more rapidly than heavy isotopes...
(15N, 18O, 13C). Therefore, as biodegradation proceeds, reactants with a heavier isotopic composition are said to be enriched in the remaining pool of pollutants.[5] Explosives produced from different sources may have different N/O/C isotopic values.[6] Similarly, explosives produced from a unique source, but through different degradation processes, also have different isotopic values.[7] The use of isotopes in a 2D/3D plot, the so-called “dual/multi-isotopic approach”, increases the likeliness of source identification.[8]

In this study, 13C, 15N, and 18O isotope analysis methods of compound-specific isotope analysis is established by applying the monomer stable isotope testing technology in the research of military site pollution. This could trace the source of energetic compounds, and help to study the 13C, 15N, 18O isotopic fractionation mechanism and biodegradation reaction kinetics in the microbial degradation of explosives contaminants, explain the migration law and degradation mechanism of explosive compounds, and provide a technical basis for understanding their fate in the natural environment.

2. Materials and methods

2.1. Study area and sampling
The soil samples were collected from an ammunition destruction site, which has been in operation for nearly 50 years. The ammunition destruction site includes comprehensive destruction platforms and burning platforms. The samples were collected in 2018. A stainless steel shovel was used to collect soil samples from the surface 0-5cm deep. The soil samples are stored in tin foil sample bags at -20°C.

2.2. Extraction of explosive contaminants
The rapid solvent extraction method is used to extract the explosive contaminants in the soil. Specifically, the explosive components in 10g soil samples were extracted with a fast solvent extraction instrument, and then evaporated to dryness with a rotary evaporator. Add 800μl acetonitrile and 200μl internal standard (3,4-DNT) and mix well. The sample was passed through a 0.22um hydrophobic polytetrafluoroethylene filter membrane before testing.

2.3. Detection of explosive contaminants
Gas chromatography electron capture detector (GC-ECD) was used to detect the contents of TNT, RDX, HMX, 2,4-DNT, 2,6-DNT, 2-ADNT, 4-ADNT and TNB in the samples. During the experiment, 2,5-DNT and 3,4-DNT were used as substitutes and internal standards to ensure the accuracy and accuracy of the measurement results. The recovery rate of substitutes was 102.16±11.19%. The relative standard deviation of the internal standard is less than 10%. The recovery rate of soil samples is 80~120%.

2.4. Compound-specific isotope analysis

2.4.1. EA-IRMS analysis. In this study, elemental analyzer (EA) and stable isotope ratio mass spectrometry (IRMS) are used to detect TNT standards to obtain the true isotope values. FLASH EA is coupled with MAT 253 IRMS plus (Thermo Scientific) via a Conflco III. The analysis of δ18O is that 0.2g benzoic acid standard (IAEA-601) and TNT standard are wrapped in silver cups respectively, in which the oxygen is pyrolyzed to form CO at 1280 °C, and then pyrolyze N2 separated from CO through poraplot GC column at 40 °C. The stability of IRMS in the whole operation process is monitored, with three continuous CO pulses as reference before and after, the flow rate of carrier gas He of 100ml/min, reference gas CO of 100ml/min, the cycle time of 50s and the sampling delay of 15s.

For δ15N and δ13C analysis, 0.2g of urea standard and TNT standard are wrapped in tin cup, in which the carbon and nitrogen elements are converted into CO2 and N2 in an oxidation-reduction furnace at 960 °C, and then sent to IRMS for isotope detection after separated through poraplot GC column at 70 °C. Taken three continuous CO pulses as reference before and after, the flow rate of He is 180 ml / min, the flow rate of oxygen is 200 ml / min, and the reference flow rate is 70 ml / min. The cycle time
is 50s, the sampling delay is 15s, and the oxygen injection end is 3s. All EA-IRMS isotopic values are more than 5 replicated analyses.

![Diagram](image)

**Figure 1.** Instrumentation for GC-IRMS isotope analysis of energetic compounds. GC 1 is for separating compounds in the reaction mixture. The HTC (high temperature convertor) pyrolytically converts each eluate from GC 1 to N\(_2\) and CO gases, which are then separated by GC 2, before being swept to IRMS for isotope ratio measurements.

2.4.2. GC-IRMS analysis. The GC-IRMS combined system was used to carry out the monomeric compound \(\delta^{18}O\), \(\delta^{15}N\) and \(\delta^{13}C\) on soil explosive extracts containing TNT, 4-ADNT, 2-ADNT, RDX analysis. A Trace GC 1300 GC interfaced to a MAT 253 Plus IRMS via an Isolink conversion unit (pyrolysis reactor, Thermo Scientific) for determination on \(\delta^{18}O\) and \(\delta^{15}N\). As shown in Figure 1, 1.5 μl sample dissolved in CH\(_2\)Cl\(_2\) is injected into the Trace TR-8095 (Thermo Scientific, USA) capillary column (GC1, 12m×0.32mm×0.25μm, with 8% phenyl polycarbon atesiloxane stationary phase, which is suitable for EPA 8095). The oxygen and nitrogen in the analyte is pyrolyzed into CO and N\(_2\) at 1280°C, which are then carried to the IRMS by He (99.995%) via a Conflo IV universal interface in a splitless mode. To prevent the pyrolytically generated N\(_2\) from isobarically interfering with the isotope ratio measurement of CO, a HP-PLOT molecular sieve column (GC2, 10 m×0.43 mm×50μm, P/N:19095P-MS0, isothermally maintained at 25 °C) is inserted between the exit of the pyrolysis furnace and the entry of the IRMS [9].

During the operation of GC-IRMS, five contiguous CO reference pulses are first performed, followed by the elution of the analyte peaks under the following chromatographic conditions: carrier gas He flow rate 1.2 mL/min, injector temperature 200 °C. The temperature program of TR-8095 column and oven maintains at 50 °C for 2 min, warming up to 290 °C at the rate of 10 °C/min for 4 min. At the end of each run, three CO pulses are introduced to monitor the stability of IRMS during the whole operation. In the experiment, DCM (CH\(_2\)Cl\(_2\)) as a blank for ensuring the anaerobic environment in the reactor, minimizes the carbon surplus effect. Blank is usually lower than 80 mV. According to the actual concentration of TNT in the sample, concentrate or dilute the IRMS sample (the injection concentration of IRMS is about 1mg / ml) ensure that the injection volume is close and within the linear range. Use peak area and \(\delta^{18}O\) to correct the drift \(\delta^{18}O\) value to reduce the impact of injection volume when necessary.

For the determination of monomers \(\delta^{13}C\) in soil explosive extracts by using GC-IRMS, five continuous CO\(_2\) pulses as reference, the sample injection volume is 1μl, the injector temperature is 200 °C, and the flow rate of He is 0.6ml/min. The carbon in the analyte is converted to CO\(_2\) in an oxidation-reduction reactor at 1000 °C. The temperature program of TR-8095 column and oven is set to 50 °C for 2 min, warming up 290 °C at rate of 10 °C/min for 4 min. At the end of each run, three CO\(_2\) pulses are introduced to monitor the stability of IRMS over the entire run.
2.4.3. Data Correction

Based on the test value of standard of Benzoic acid (IAEA-601, δ¹⁸O = 23.3‰) and urea (Urea-standard, δ¹⁵N = 0.32‰, δ¹³C = -41.3‰), the δ¹⁸O, δ¹⁵N and δ¹³C value of TNT standard (purity 99. 9%) in EA-IRMS detection are corrected. Then taken the EA-IRMS results of TNT standard as the benchmark to correct the value of δ¹⁸O, δ¹⁵N and δ¹³C in GC-IRMS. By monitoring the stability of the reference gas before and after the experiment, all non-reference peaks are corrected by the drift of the isotope value of the reference gas. Each sample is detected 3-5 times on GC-IRMS, and 3-5 TNT standards are inserted between each two samples to correct the baseline drift of GC-IRMS.

Both EA-IRMS and GC-IRMS isotopic data are recorded and processed by ISODAT 3.0 software (Thermo Scientific). Report according to the standard of V-SMOW δ Symbol: δ¹⁸O (mUr) = (R(sample) − R(V-SMOW)) / R(V-SMOW), where R(sample) and R(V-SMOW) are the ¹⁸O/¹⁶O ratios of the sample and IAEA V-SMOW standard, respectively. δ¹⁵N (mUr) = (R(sample) − R(V-SMOW)) / R(V-SMOW), where R(sample) and R(VPDB) are the standard ratios of ¹³C/¹²C for sample and urea respectively.

![Figure 2](image_url)

**Figure 2.** (a) Concentrations of different explosive pollutants; (b) Average concentration of explosive pollutants; (c) Correlation between TNT and 4-AND or 2-ADNT in surface soil of the destruction site.

3. Results and Discussion

The main energetic compounds in the surface soil of the destruction site contain NG, 1,3-DNB, 2,6-DNT, 2,4-DNT, TNB, TNT, RDX, 4-ADNT and 2-ADNT, with great varied concentrations. For example, the average TNT concentration is 288049 ng/g, while the average concentration of 1,3-DNB and 2,6-DNT is less than 100 ng/g, as shown in Figure 2b. The high concentration of various energetic...
compounds in the soil of ammunition destruction sites potentially threaten the ecological environment and human health. Taking the TNT concentration distribution as an example, the high TNT concentration in soil samples is mainly distributed in the destruction point and burning point, but with the water transport and atmospheric transmission, it also has a serious impact on the surrounding soil. In addition, the positive correlation between 4-ADNT and 2-ADNT and the content of TNT in the soil indicates that 4-ADNT and 2-ADNT are the degradation and transformation products of TNT (Figure 2c).

![Figure 2c](image_url)

**Figure 2c.** The spatial variation of the TNT isotope ratios $\delta^{18}O(a)$, $\delta^{15}N(b)$ and $\delta^{13}C(c)$ in the soil of the weapons destruction site; (d-f) Two-dimensional plot of TNT isotope value.

Two kinds of isotopic detection methods, EA-IRMS and GC-IRMS, were used in this study. Among them, EA-IRMS has higher precision for the pure sample, but requires large sample, while the injection volume usually above 100 μg, then reduced to 10 μg when using the Confluo II interface. GC-IRMS is used for the sample of impure substance. The liquid sample is gasified and separated by gas chromatography, in which the oxygen, nitrogen and carbon elements are pyrolyzed or redox to form CO, N₂, CO₂ respectively, which are detected by IRMS. GC-IRMS needs less samples, about 1 μg, slightly lower than EA-IRMS precision.

Due to the low content of target compounds in soil samples, and high detection limit of GC-IRMS, it is difficult to determine the isotope of explosive pollutants in soil samples. Only $\delta^{18}O$, $\delta^{15}N$ and $\delta^{13}C$ value of TNT in most soil samples is detected. The $\delta^{18}O$ value of TNT contaminant in soil of explosive
ranges from 25.94 ‰ to 41.75 ‰, the higher values found in the central area of the destruction site (fig. 3a). The $\delta^{15}N$ value of TNT contaminant in soil of explosive ranges from $-2.526\%$ to $-2.154\%$, the higher $\delta^{15}N$ values found in the soil of ammunition burning site (Fig. 3b). The $\delta^{13}C$ value of TNT contaminant in soil of explosive ranges from $-33.27\%$ to $-21.86\%$, the higher $\delta^{13}C$ values found in the soil at the edge of the contaminated area (Fig. 3c). The $\delta^{18}O$, $\delta^{15}N$ and $\delta^{13}C$ value detected is close to the TNT isotope value reported in the literature.

4. Conclusion

In this study, compound-specific isotope analysis (CSIA) was used to establish a stable isotope diagnosis method for explosive pollutants in a military range, and analyzed the spatial distributions of explosive $\delta^{18}O$, $\delta^{15}N$ and $\delta^{13}C$ pollutants. Due to the high detection limit of GC-IRMS, the quality of extracted soil samples should be increased in future research and the chromatographic conditions should be optimized. In order to serve the military range pollution diagnosis investigation and precise repair in a better degree, the 2-ADNT, 4-ADNT, RDX and other more explosives-contaminated soil monomer compounds $\delta^{18}O$, $\delta^{15}N$ and $\delta^{13}C$ isotope data should be obtained to carry out the monitoring of the biological attenuation of explosive pollution on military ranges.

Acknowledgments

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