

Organic Explosive Residues from Demining Sites in Amuru District, Northern Uganda

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Abstract

The study investigated the occurrence of explosive compounds at four demining sites in Amuru District, Northern Uganda. The concentrations of explosive compounds octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 2,4,6-Trinitrophenyl-*n*-methylnitramine (tetryl), 2,4,6-trinitrotoluene (TNT), 2,6-dinitrotoluene (2,6-DNT), cyclotrimethylenetrinitramine (RDX), nitroglycerin (NG) and Pentaerythritol tetranitrate (PETN) in soil samples collected from the demining sites were determined by liquid chromatography-tandem mass spectrometry with atmospheric pressure chemical ionization (LC-MSMS-APCI). The LC-MSMS-APCI method was optimized and validated. The validation parameters included were accuracy, precision, recovery, linearity, selectivity, limits of detection, and limits of quantification. The limit of detection and quantification of the target analytes ranged from 4.5 - 32.1 ng·mL⁻¹ and 17 - 102.9 ng·mL⁻¹, respectively. Recovery of the target analytes from the soil samples ranged from 76% - 96%. The intra- and inter-day accuracy and precision values of below 15% indicated that the target analytes could be determined with reasonable accuracy and precision using the optimized method. The target analyte concentrations in soil samples ranged from 24.6 to 551.8 µg·g⁻¹ with RDX and TNT being dominant. All the soil samples analyzed had explosive residue concentrations higher than the set chemical contamination values set by the US EPA PRGs Region 9 for Soil Screening Levels.

Keywords

Organic Explosives, Eco-Toxicity, Detonation, Chromatography, Validation

1. Introduction

Northern Uganda suffered a two-decade Lord's Resistance Army (LRA) insurgency. Consequently, abandoned munitions and unexploded remnants of war have been identified by civilian populations in many areas. These unexploded ordnances are often found by children who play with them as toys, hunters, and women gathering firewood or are disturbed unknowingly during farming activities resulting in explosions that cause fatalities and serious injuries. On recognition, these unexploded ordnances are collected by the bomb experts and detonated at designated sites. Contamination of soil with high explosives compounds has been reported in many demolition sites and military training ranges (Jenkins et al., 2001, 2006). The main environmental sources of high explosives contamination are residues from the detonation of military munitions which include mortar, artillery rounds, grenades, landmines, aerial bombs, and missiles (Pennington & Brannon, 2002; Ahmad et al., 2007).

According to previous researchers, the main common contaminants resulting from the detonation of military munitions are: the high explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 2,4,6-trinitrotoluene (TNT) (Ahmad et al., 2007; Chatterjee et al., 2017; Broomandi et al., 2020). Other explosive contaminants include nitroglycerine (NG), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT) (Broomandi et al., 2020; Clausen et al., 2004; Halasz et al., 2002). Many scholars have assessed and reviewed the risk associated with explosive compounds and their residues from military munitions and their harm to human health and the environment (Lima et al., 2011; Maser et al., 2024; Nemeikaitė-Čėnienė et al., 2006). Explosive compounds TNT, RDX, and HMX, for example, are reported to be neurotoxic and possible carcinogens to humans (Hoek, 2004). Exposure pathways of humans to explosive compounds are inhalation, ingestion, and dermal contact (McKone & Daniels, 1991).

In the event of demolition of munitions, the explosive residues are dispersed in soil and air, surface water through run-offs, and underground water through leaching (Sanderson et al., 2017). The most impacted media by explosive compounds after demolition are soil and sediments (Hewitt et al., 2007). This poses a threat to organisms in soil and water ecosystems. Numerous studies have reported soil contamination by explosive compounds and unexploded ordinance. In Ontario, Canada, the Petawawa demolition range was reported to be contaminated with HMX and RDX (Clausen, 2011). Similarly in the United States of America, the Holloman demolition center had soil contaminated with HMX and RDX at amounts of 11.4 and 1.84 mg.kg⁻¹, respectively (Pennington et al., 2006a).

In Uganda however, limited studies have been done to establish the occurrence and fate of explosives in soils despite a series of wars. There is inadequate literature on the levels of explosive compounds in environmental matrices in Uganda which poses potential risks for both aquatic and terrestrial ecosystems. A lot still needs to be done to quantify the amounts of explosive traces in soils at these sites. This

study established the amounts of selected organic explosives residues in soils at demining sites in Amuru district, northern Uganda. The data generated can help stakeholders and experts in the environmental sector to make informed decisions on the remediation strategies for the explosive contaminated sites, and the formulation of environmental and management policies.

2. Materials and Method

2.1. Study Area

The study was conducted at Amuru demining sites located in Northern Uganda (**Figure 1**). The sites were chosen because of their continuous use in the demolition of unexploded munitions.

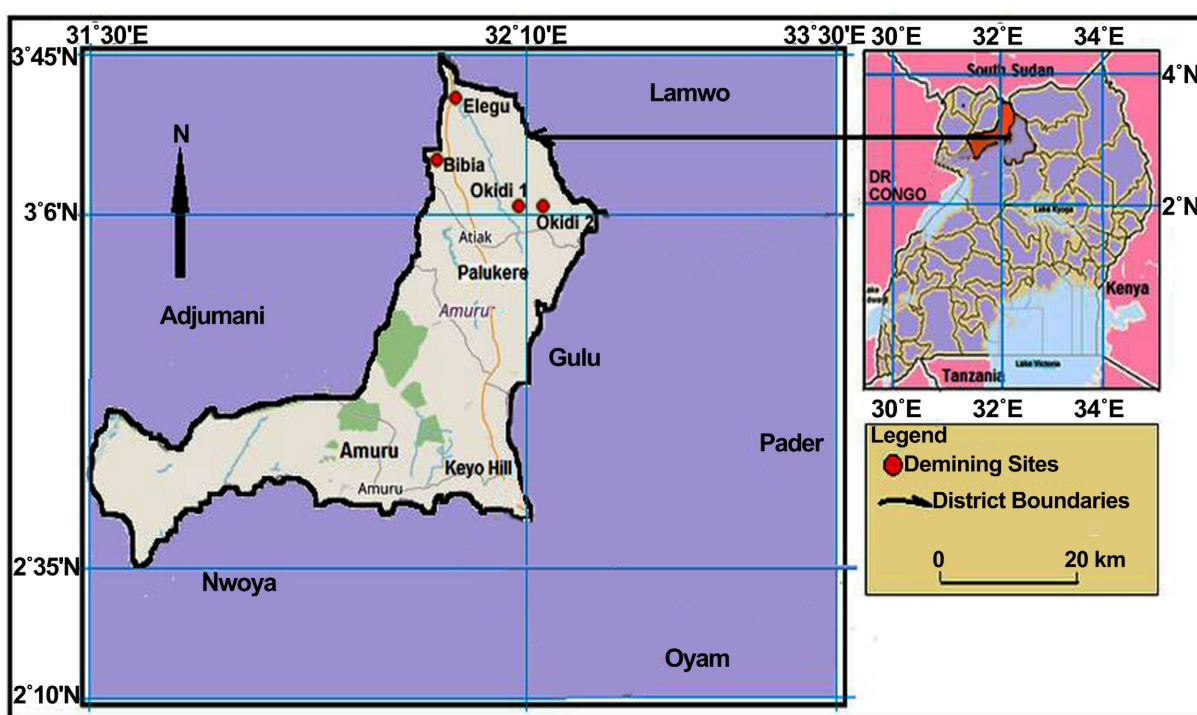


Figure 1. A map showing four demining sites in Amuru district, Northern Uganda.

2.2. Reagents, Solvents, and Standards

All chemicals used were analytical grade. Explosive standards RDX, HMX, TNT, 2,6-DNT, Tetryl, PETN, and NG were purchased from Accu Standards (New Haven, CT, USA) at a certified concentration of 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ in acetonitrile: methanol (1:1). LCMS-grade methanol, LCMS-grade acetonitrile, LCMS-grade organic-free reagent water, ammonium acetate, ammonium formate, and formic acid were purchased from Kobian Scientific, Uganda.

2.3. Preparation of Standard Solution

Standard stock solutions of seven target compounds (HMX, RDX, Tetryl, PETN, NG, TNT, and 2,6-DNT) were prepared in methanol: acetonitrile (50:50% v/v) at

a concentration of 1000 $\mu\text{g}\cdot\text{mL}^{-1}$. Intermediate stock solutions for the target compounds were prepared in methanol to a concentration of 100 $\mu\text{g}\cdot\text{mL}^{-1}$ and stored at 4°C until use. Picric acid the internal standard was prepared at 25 $\mu\text{m mL}^{-1}$. Working solutions were prepared from the intermediate stock solution to a concentration of 10 $\mu\text{g}\cdot\text{mL}^{-1}$. Solutions were prepared at concentrations 5, 20, 50, 100, 250, 500, 750, and 1000 $\text{ng}\cdot\text{mL}^{-1}$ for the calibration curve for each target compound.

2.4. Method Optimization

The precursor ion and product ion transition of the target compounds were optimized. The Flow Injection Analysis-Tandem mass spectrometry (FIA-MS/MS) was performed on a Shimadzu Liquid Chromatography tandem mass spectrometer (LC-MS/MS) by method as reported by Ostrinskaya et al. (2019). The mobile phase used were water and methanol for the target compounds which resulted in good ionization and peak shape. The isocratic elution mode for the mobile phase composed of a methanol and water mixture (50:50, v/v) with 0.1% formic acid, delivered at a flow rate of 0.3 $\text{mL}\cdot\text{min}^{-1}$ for a run time of 1 min. Sample aliquots of 10 μL were injected while maintaining the autosampler temperature at 4°C. The LC-MS/MS was equipped with an atmospheric pressure chemical ionization (APCI) ion source operated at dual polarity of positive and negative and set at a voltage of 550V. The MS data were obtained by collision-induced dissociation (CID) employing argon as the collision gas. Multiple reaction monitoring (MRM) was selected in the scan mode to monitor the analytes precursor and product ions. The optimal detection parameters for each target analyte are listed in Table 1.

Table 1. MS conditions of the analytical instrument.

<i>Nebulizing gas flow:</i>	3 L/min
<i>Drying gas flow:</i>	10 L/min
<i>Heating gas flow:</i>	10 L/ min
<i>Interface temperature:</i>	300°C
<i>CID gas:</i>	270 Kpa
<i>Interface current:</i>	0.1 μA
<i>Heating block temperature:</i>	400°C
<i>DL temperature:</i>	250°C

The column of the high-performance liquid chromatography (HPLC) system was then connected and the HPLC conditions were optimized. The HPLC parameters that were optimized included the retention time of target analytes, buffers, flow rate, mobile phase elution mode, oven temperature, column selection, and ionization source selection. The optimized HPLC conditions are in Table 2. The optimized MS and HPLC conditions were validated before their applications in sample analysis.

Table 2. HPLC conditions of the analytical instrument.

<i>Analytical column:</i>	Phenomenex kinetic XB-C18 (2.1 mm I. D × 100 mm L, 2.6 µm Thickness)
<i>Guard column:</i>	Phenomenex security Ultra C18 2.1mm I. D
<i>Mobile phase A:</i>	10 mmol/L formic acid + ammonium formate-water
<i>Mobile phase B:</i>	10 mmol/L formic acid + ammonium formate-Methanol
<i>Flow rate:</i>	0.3 ml/min.
<i>Injection volume:</i>	10 µL
<i>Column oven:</i>	40°C
<i>Elution mode:</i>	Gradient

2.5. Method Validation

2.5.1. Limits of Detection (LOD) and Quantification (LOQ)

LOD and LOQ of the target analytes were determined by measuring signals from samples with known low concentrations of analytes to those of blank samples and thereafter establishing the minimum concentration at which the analytes have an acceptable signal-to-noise ratio of 3:1 and 10:1 for LOD and LOQ, respectively (Desimoni & Brunetti, 2015; Evard et al., 2016).

2.5.2. Evaluation of Method Selectivity

The ability of the method to accurately measure the target analytes of interest in the presence of other interfering sample components was assessed. Selectivity of the analytical method was established by comparing the chromatograms of uncontaminated extracts of blank soil samples from six different soil samples spiked with the target analytes and internal standard to ensure that it is free of interferences at the retention times of each target analyte (Xu et al., 2014, Şener et al., 2017).

2.5.3. Accuracy and Precision

The intra-day precision and accuracy were determined within one day by analyzing five sample replicates within one day at concentrations 50 ng·mL⁻¹ and 250 ng·mL⁻¹ for the respective target compounds. The inter-day precision and accuracy were determined on five separate days at identical concentrations. The accuracy of the target compounds analyzed was defined as the absolute value of the ratio of the calculated mean values of the Quality Control (QC) samples to their respective nominal values expressed as percentages (Xu et al., 2014, Şener et al., 2021).

2.5.4. Recovery

To quantify the loss of target analytes during sample preparation and extraction processes, the recovery of the method was evaluated as the amounts of extracted QC samples after a full sample preparation process to that of direct injection of equivalent concentration of compounds in methanol (Xu et al., 2014, Şener et al., 2021).

2.5.5. Linearity

The results obtained from the calibration curve were used to evaluate the linearity range of quantification, set to the lowest point of the calibration curve. A good linearity was obtained with R^2 always larger than 0.995 (Xu et al., 2014, Şener et al., 2021).

2.6. Sample Collection and Handling

Soil samples were collected in October 2024 from the study sites, Okidi 1, Okidi 2, Bibia, and Elegu demining sites located in Amuru district northern Uganda. Access to the demining sites considered in the study was granted by the bomb experts and the security leaders of the area. Systematic random sampling design as described by Jenkins et al. (2004) was used to sample soils from the selected demining sites. Each of the study sites was divided into grids of three meters square each and half a kilogram of soil were randomly picked from each grid of a given study site. 10 kg of soil samples from each of the selected sites were collected and mixed thoroughly and sub-samples were used for analysis. Only a horizon up to a maximum depth of 30 cm was collected using an auger and placed in labeled plastic bags with zip-locks. The plastic bags were placed in plastic buckets and transported to the laboratory for analysis.

2.7. Sample Preparation

Before analysis, the collected soil samples were air-dried at room temperature. The dried soil sample was sieved to particle size ≤ 2 mm using a mechanical sieve shaker. The sieved soil samples were carefully homogenized by turning several times in a plastic bag before a sub-sample was taken for analysis. Extraction was performed by the method reported by Numbera (2006) with slight modifications. Briefly, 2 g of each soil sample was mixed with 10 mL of methanol-acetonitrile mixture (50:50 v/v) and extracted on an overhead shaker for 18 hours. Extracts were centrifuged at 5000 g for 5 minutes and the supernatant was filtered through 0.22 μ m polytetrafluoroethylene filters. Filtrate from each sample (1 mL) was pipetted and placed in a vial for liquid chromatography-tandem mass spectrometer (LC-MS/MS) analysis.

2.8. Instrumental Analysis

The Sample analysis was done in triplicate using the Shimadzu LCMS 8060NX following the method reported by Sener et al. (2021) with slight modification after optimization and validation. The mobile phase used contained formic acid ammonium formate as buffers. The LC-MS/MS instrument was operated in the APCI mode of ionization. The method had a run time of 13 minutes with the mobile phase starting at 5% B which was increased to 95% B within 7.5 minutes, maintained for 2.5 minutes, dropped back to 5% B in the next 0.1 minutes, and finally maintained at 5% for the next 2.9 minutes before the next injection starts. The HPLC and MS conditions of the analytical instrument used for the analysis of the

target analytes are shown in **Table 1** and **Table 2**, respectively.

2.9. Eco-Toxicological Risk Assessment

The eco-toxicity of the soil for the target analytes was evaluated by US EPA Region 9 Preliminary Remediation Goals (PRGs) for Soil Screening Levels (SSL) (Orel et al., 2022). SSLs are reference threshold values of contaminants in soil required by environmental laws and designed to be protective against exposure in a residential or industrial setting. **Table 3** shows the US EPA Region 9 PRGs for residential and industrial SSLs of the explosive compounds.

Table 3. US EA Region 9 PRGs for Residential and Industrial SSLs values of the target analytes.

Analyte	Environmental	Sector
	Residential	Industrial
RDX	6.1	28
HMX	1.3	13
Tetryl	0.96	6.4
PETN	2.2	15
NG	1.4	10
TNT	0.72	5.4
2,6-DNT	0.48	3.2

Adopted from Orel et al. (2022).

2.10. Statistical Analysis

The statistical analyses of the data generated were performed using Microsoft Excel 2016 (v16.0) and SPSS software version 20. A one-way ANOVA test was used to test for statistical differences in the means of the target analytes from the study sites.

2.11. Ethical Consideration

The study was conducted after ethical approval by the Gulu University Research Ethical Committee (GUREC-2024-898) and the Uganda National Council for Science and Technology (UNCST-NS858ES). The bomb disposal experts helped to show the demining sites with brief information on vital aspects that are of great help in the amounts of explosive compounds in soils. The sample collected was strictly for this study and not for any other purpose. All information on the study sites was kept private and treated with the confidentiality it deserved.

3. Results and Discussion

3.1. Optimization for the Mass Spectrometer Transitions for the Explosive Compounds

Mass Spectrometer (MS) Multiple Reaction Monitoring (MRM) transitions of the explosive compounds are shown in **Table 4**.

Table 4. Retention time and MS-MRM of the explosive compounds.

Explosive	Precursor ion	Product ion	Q1Pre-Bias (V)	CE (V)	Q3Pre-Bias (V)	Polarity	Retention Time (Mins)
RDX	223.05	149.1 ^Q	−26	−19	−29	Positive	6.393
		177.1 ^q					
		121.05 ^q					
HMX	340.05	183.05 ^Q	22	9	15	Negative	9.077
		147 ^q					
		174 ^q					
2,4,6-TNT	271.75	233.95 ^Q	18	12	16	Negative	5.056
		205.9 ^q					
2,6-DNT	180.85	137.15 ^Q	20	14	24	Negative	5.269
		93.1 ^q					
		113.0 ^q					
NG	271.70	235.80 ^Q	18	13	15	Negative	5.760
		233.9 ^q					
		167.19 ^q					
PETN	314.95	268.9 ^Q	11	9	18	Negative	3.570
		126.65 ^q					
		135.2 ^q					
Tetryl	305	111.2 ^Q	−16	−14	−20	Positive	5.291
		200.2 ^q					
		112.2 ^q					
Picric acid	227.90	182.2 ^Q	25	18	18	Negative	5.321
		198.0 ^q					
		63.1 ^q					

Q—Quantifier m/z and q—Qualifier m/z, Picric acid was used as internal standard.

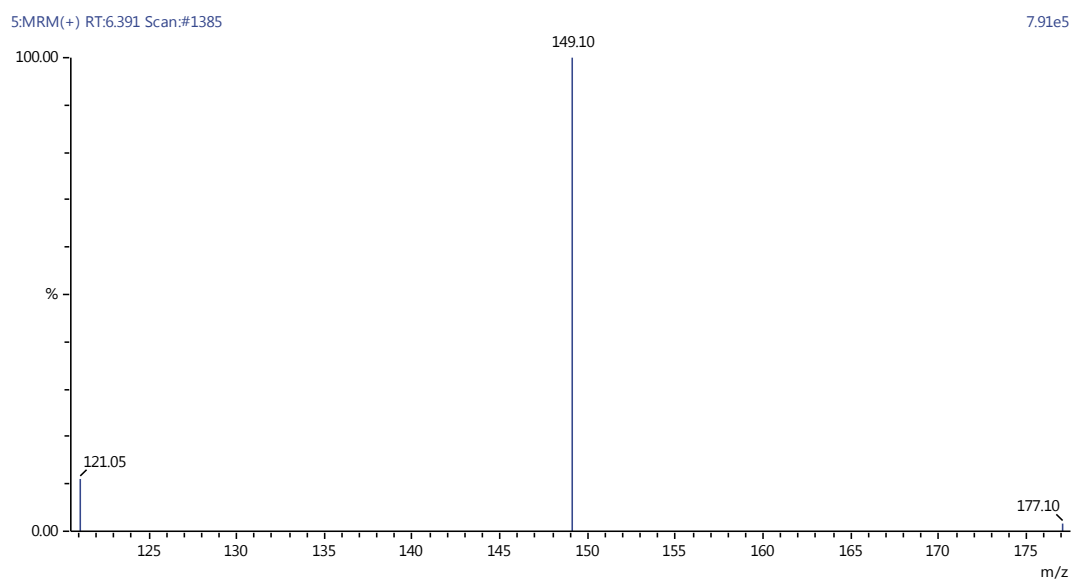
**Figure 2.** MRM spectrum of RDX.

Figure 2 and **Figure 3** show the MRM spectrum and product ion scan of RDX, respectively generated by the Shimadzu LC-MSMS 8060NX.

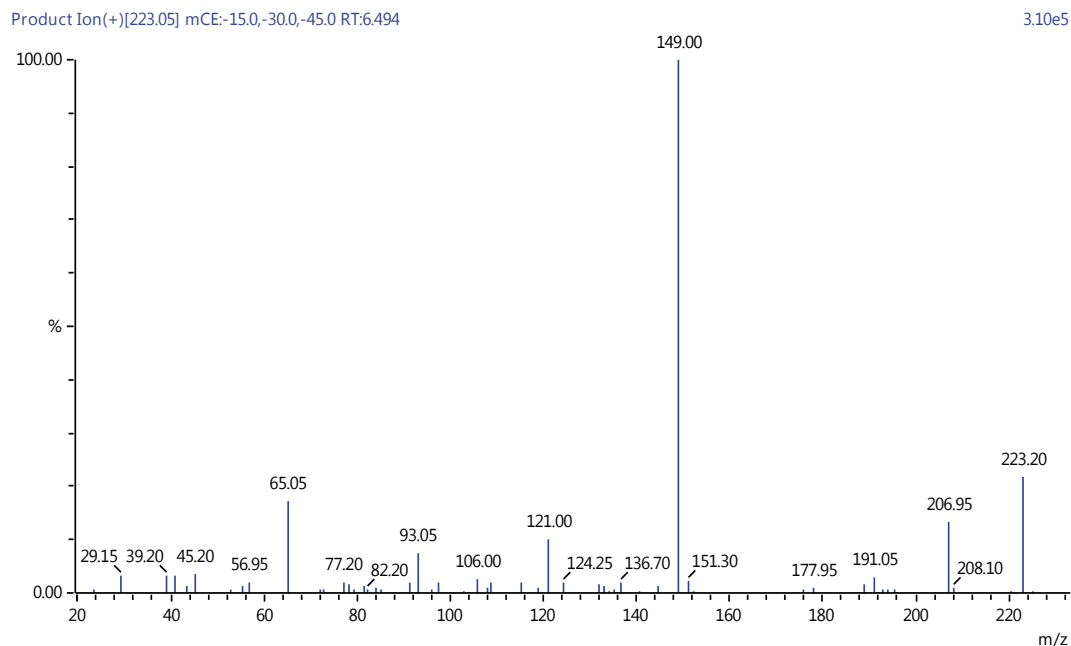


Figure 3. Product ion scan of RDX.

3.2. Method Validation

The optimized LC-MS/MS method for the estimation of the amounts of explosive compounds in the demining sites was validated. Parameters for validation included selectivity, sensitivity, recovery, linearity, accuracy, and precision.

3.2.1. Selectivity

The optimized analytical method used for the analysis of the target analytes had appreciable selectivity. **Figure 4**, for selectivity, shows the representative chromatogram of HMX.

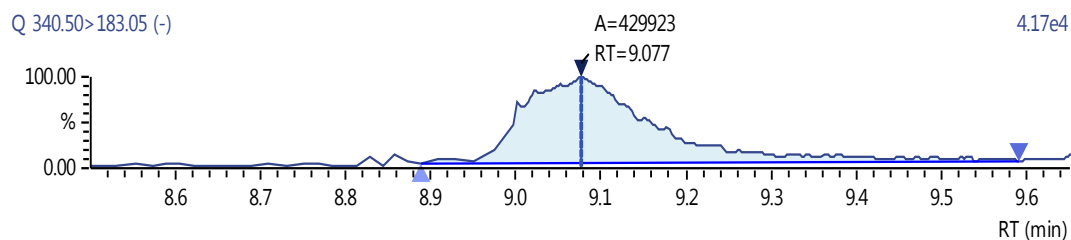


Figure 4. Chromatogram of HMX.

3.2.2. Limits of Detection (LOD) and Quantification (LOQ) of the Target Analytes

The LOD and LOQ of the target analytes ranged from 4.5 - 32.1 and 17 - 102.9 ng·mL⁻¹, respectively. 2,6-DNT had the lowest LOD and LOQ values of 4.5 and 17 ng·mL⁻¹, respectively. The highest LOD and LOQ values were observed for RDX

at values of 32.1 and 102.9 ng·mL⁻¹, respectively. The limits of detection and quantification of the explosive compounds are shown in **Figure 5**. Samples were considered detected when the concentration of the analytes studied had values \geq LOD.

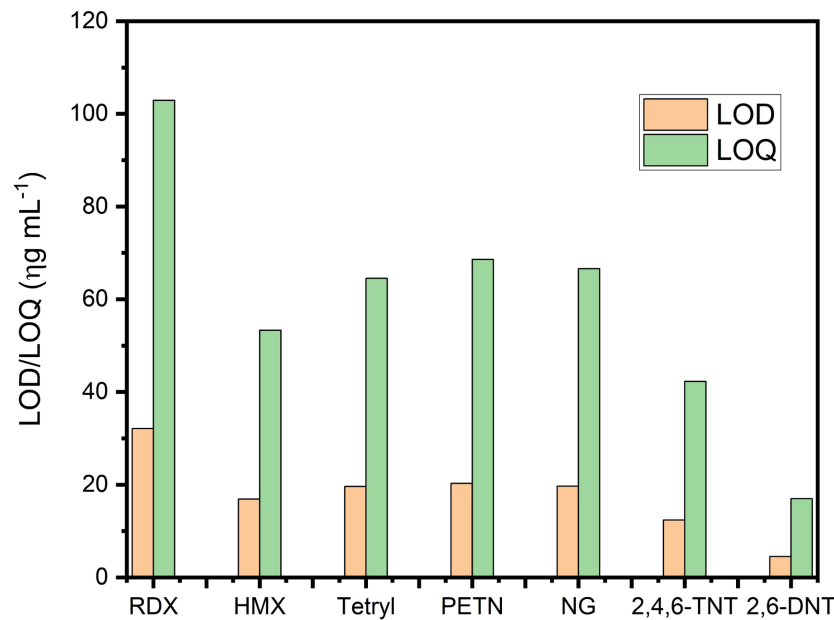


Figure 5. Limits of detection and quantification of the explosive compounds.

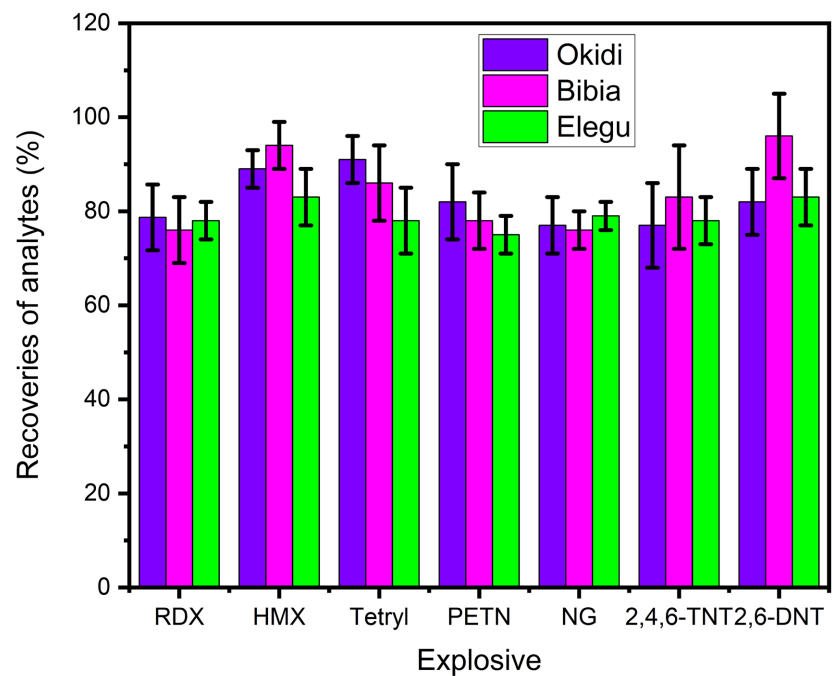


Figure 6. Percentage recoveries of the target analytes from the soil samples.

3.2.3. Average Percentage Recoveries

Figure 6 presents the recoveries obtained by spiking the uncontaminated blank

soil samples close to each study site at $1 \mu\text{g}\cdot\text{mL}^{-1}$ of each explosive compound. The recoveries varied from 77% - 91%, 76% - 96%, and 78% - 83% for blank soil samples of Okidi, Bibia and Elegu, respectively. Since recoveries ranged from 70% - 120% (within acceptable limits for routine analysis), the results were not corrected for recovery. The recoveries were meant to ensure that the performance of the method for each sample was within acceptable limits for routine analysis.

3.2.4. Accuracy and Precision

For target concentration at $50 \text{ ng}\cdot\text{mL}^{-1}$, the intra-day precision (CV%) and accuracy (RE%) ranged from 1.26 to 5.10 and 0.24 to -8.30, respectively. The intra-day precision and accuracy for target analytes at $250 \text{ ng}\cdot\text{mL}^{-1}$ ranged from 1.61 to 2.86 and 0.02 to 0.69 $\text{ng}\cdot\text{mL}^{-1}$, respectively. Intra-day and inter-day precision and accuracy for target analytes at concentrations of 50 and $250 \text{ ng}\cdot\text{mL}^{-1}$ are shown in **Table 5** and **Table 6**, respectively. The intra- and inter-day precisions and accuracies below 15% indicated that the target analytes can be determined with reasonable precision and accuracy and that the optimized method can be used to analyse the target analytes.

Table 5. Intra-day accuracy and precision of the target analytes.

Explosive	50 $\text{ng}\cdot\text{mL}^{-1}$			250 $\text{ng}\cdot\text{mL}^{-1}$		
	Intra-day mean	CV (%)	RE (%)	Intra-day mean	CV (%)	RE (%)
HMX	53.95	5.10	0.24	249.39	2.48	0.24
Tetryl	53.31	3.46	-6.62	249.95	1.61	0.02
NG	54.14	5.37	-8.30	251.86	2.42	-0.75
RDX	53.15	2.98	-6.31	248.27	1.61	0.69
PETN	51.63	1.26	-3.26	250.58	2.86	-0.23
TNT	48.22	2.72	3.56	248.12	1.01	0.75
2,6-DNT	51.63	2.37	-3.27	251.18	1.84	-0.504

Table 6. Inter-day accuracy and precision of the target analytes.

Explosive	50 $\text{ng}\cdot\text{mL}^{-1}$			250 $\text{ng}\cdot\text{mL}^{-1}$		
	Inter-day mean	CV (%)	RE (%)	Inter-day mean	CV (%)	RE (%)
HMX	52.86	2.85	-5.72	248.27	2.44	0.69
Tetryl	53.35	3.42	-6.71	250.24	1.37	-0.09
NG	54.52	3.41	-9.03	252.08	3.06	-0.83
RDX	55.1	3.47	-6.74	250.84	4.29	-0.34
PETN	51.31	2.02	-2.63	242.11	4.05	3.15
TNT	47.6	6.59	4.79	246.49	1.47	1.4
2,6-DNT	55.18	8.32	-10.36	255.75	6.69	-6.29

3.2.5. Linearity

Good linearity with R^2 values greater than 0.995 was obtained for four target analytes. The calibration equation, R^2 value, and the target compounds obtained from the calibration curve are shown in **Table 7**.

Table 7. Calibration equation, R^2 value, and linearity range of the explosive compounds.

Compound	Calibration equation	R^2 Value
HMX	$y = 0.0284x + 0.2805$	0.9864
Tetryl	$y = 0.0223x + 0.0765$	0.9965
NG	$y = 0.0471x + 0.6741$	0.9802
RDX	$y = 0.5044x + 0.4999$	0.999
PETN	$y = 0.0277x + 0.2296$	0.9862
TNT	$y = 0.0328x + 0.2912$	0.9966
2,6-DNT	$y = 0.0132x + 0.1771$	0.9961

3.3. The Concentration of Extractable Explosive Compounds in Soils from the Study Sites

Target analytes were detected in all soils sampled from the selected study sites. The concentration of extractable explosive compounds in soil sampled from the demining sites of Amuru District is shown in **Table 8**.

Table 8. Extractable quantity of explosive compounds in soil sampled from the study sites.

Explosive	Concentration of extractable explosives ($\text{mg}\cdot\text{kg}^{-1}$)			
	Okidi 1	Okidi 2	Bibia	Elegu
RDX	410.5 ± 9.5	365.5 ± 12.1	448.3 ± 12.7	24.6 ± 0.6
HMX	132.6 ± 9.7	221.4 ± 1.8	198.2 ± 10.8	25.8 ± 2.5
TETRYL	139.8 ± 7.8	307.7 ± 9.8	334.9 ± 6.8	70.6 ± 7.9
PETN	158.2 ± 10	97.6 ± 7.6	95.6 ± 6.0	19.9 ± 0.9
NG	287.6 ± 6.4	329.6 ± 10.7	329.2 ± 10.6	34.2 ± 1.5
TNT	551.8 ± 6.4	246.1 ± 13.0	184.8 ± 10.3	220.8 ± 1.0
2,6-DNT	103.7 ± 14.6	113.6 ± 12.3	223.3 ± 8.6	32.5 ± 1.4

Values are shown as mean \pm standard deviation, and $n = 3$.

3.4. Ecotoxicity of the Soil Sampled Contaminated with the Explosive Compounds

The residential and industrial soil screening levels (SSLs) for the target analytes obtained from the study sites were evaluated. SSLs are reference threshold values of contaminants in soil required by environmental laws and designed to be protective against exposure in a residential or industrial setting. The SSL values revealed that the soil contamination levels are higher than the set threshold values by EPA Region 9. **Table 9** shows the US EPA Region 9 PRGs evaluation for resi-

dential and industrial SSLs of the sampled soil contaminated with explosive compounds.

Table 9. Residential and industrial SSLs of the sampled soils from the demining sites contaminated with the explosive compounds.

Analyte	Environmental Sector	Study sites			
		Okidi 1	Okidi 2	Bibia	Elegu
RDX	Residential	High	High	High	High
	Industrial	High	High	High	Low
HMX	Residential	High	High	High	High
	Industrial	High	High	High	Low
Tetryl	Residential	High	High	High	High
	Industrial	High	High	High	High
PETN	Residential	High	High	High	High
	Industrial	High	High	High	High
NG	Residential	High	High	High	High
	Industrial	High	High	High	High
2,4,6-TNT	Residential	High	High	High	High
	Industrial	High	High	High	High
2,6-DNT	Residential	High	High	High	High
	Industrial	High	High	High	High

3.5. Discussions

RDX was detected in all the study sites at a concentration ranging from 24.6 - 448.3 mg·kg⁻¹ (with a mean of 312.35 mg·kg⁻¹). Soil samples from sites Okidi 1, Okidi 2, Bibia, and Elegu had RDX concentrations at 410.5, 365.5, 448.3, and 24.6 mg·kg⁻¹, respectively. The highest and lowest contributors of RDX at 38% and 2% were recorded for Bibia and Elegu study sites, respectively. The RDX contamination of the soil samples is attributed to release during demolition of unexploded ordinance. RDX has been reported to be mobile in soil and highly persistent with low degradation rates hence posing neurotoxic effects to both terrestrial and aquatic organisms (Khan et al., 2021). To minimize the toxic effects of organic explosives, the Agency for Toxic Substances and Disease Registry (ATSDR) of the USA has set the Maximum Residue Limit (MRL) of 0.2 mg/kg/day for an acute duration of 14 days or less for RDX (Orel et al., 2022). The USA Environmental Protection Agency (EPA) has set the Soil Screening Level (SSL) of RDX at 6.1 mg·kg⁻¹ and an industrial SSL at 28 mg·kg⁻¹ for RDX (Orel et al., 2022). The study by Kalsi et al. (2021) reported the RDX concentration in soil from the Iowa Army munition demolition plant, in the USA at 7000 mg·kg⁻¹, which is twenty-fold higher than that of the present study (Kalsi et al., 2021). In the USA, the concentration of RDX in soil from a Louisiana army munition plant was reported by

Clark & Boopathy (2007) at $1900 \text{ mg}\cdot\text{kg}^{-1}$, which is six-fold higher than that of the present study (Clark & Boopathy, 2007). A study from Dunhua and Taiyuan, in China by Zhang et al. (2022) reported RDX amounts in soil from the munition demolition sites at 7.8 and $158 \text{ mg}\cdot\text{kg}^{-1}$ which are lower than in the present study.

HMX was detected in all demining sites in the study with a mean of 144.4 (and range of 25.8 - $221.4 \text{ mg}\cdot\text{kg}^{-1}$). The highest HMX concentration was recorded from the Okidi 2 demining site at a concentration of $221.4 \text{ mg}\cdot\text{kg}^{-1}$. The percentage contribution of HMX was 38%, 34%, 23%, and 5% for Okidi 2, Bibia, Okidi 1, and Elegu study sites, respectively. The presence of the compound in the soil is attributed to release during the demolition of unexploded ordinance and non-point source pollution from settling dust particles containing the explosive compound. Other sources may be from some explosive formulation of TNT or RDX, which contain HMX. Jenkins et al. (2006) in a study on grenade ranges in Canada and the USA at 23 military installations reported HMX concentrations at a range of 1 - $9.1 \text{ mg}\cdot\text{kg}^{-1}$, which is a magnitude lower than that of the present study. Another study by Clark & Boopathy (2007) on the soil samples from a Louisiana Army ammunition plant in the USA reported HMX concentrations at $900 \text{ mg}\cdot\text{kg}^{-1}$, which is six-fold higher than the present study (Clark & Boopathy, 2007). The authors attributed the elevated levels of explosive compound contamination in the soil to accumulation due to the incineration of explosives. Hewitt et al. (2005) reported the HMX amounts in the soil of Fort Hood, USA at a concentration of $59 \text{ mg}\cdot\text{kg}^{-1}$, which is two-fold lower than the present study.

TNT was detected in all the soil samples from the study sites. The mean concentration of the chemical from the study sites was $300.8 \text{ mg}\cdot\text{kg}^{-1}$ (with a range of 184.8 - $551.8 \text{ mg}\cdot\text{kg}^{-1}$). Okidi 1 was the site that registered the highest amount of TNT in soil at $551.8 \text{ mg}\cdot\text{kg}^{-1}$. The percentage contribution of TNT in soil samples from the study sites Okidi 1, Okidi 2, Bibia, and Elegu were 46%, 21%, 18%, and 15%. The source of TNT contamination of the soil samples is attributed to the demolition of unexploded ordinance. In a similar study at Fort Bliss, New Mexico, USA. Pennington et al. (2006b) reported TNT levels in soil samples from the munitions demolition sites at $2520 \text{ mg}\cdot\text{kg}^{-1}$, which is eight-fold higher than the present study. The Royal Canadian Air Force has been using the Cold Lake Air weapons range to drop for over 20 years. The soil TNT concentration reported by Pennington et al. (2005) was in the range of 0.12 - $778 \text{ mg}\cdot\text{kg}^{-1}$ (with a mean of $31.8 \text{ mg}\cdot\text{kg}^{-1}$), which is ten-fold lower than of present study.

2,6-DNT a degradation product of TNT was also detected in all the soil samples from the study sites. The concentration of the compound was in the range of 32.5 - $223.3 \text{ mg}\cdot\text{kg}^{-1}$ (with a mean of $118.3 \text{ mg}\cdot\text{kg}^{-1}$). The percentage contribution of 2,6-DNT in the soil samples was 47%, 24%, 22%, and 7% for Bibia, Okidi 2, Okidi 1, and Elegu sites. The study site Bibia has TNT and 2,6-DNT in the soil at the concentrations 184.8 and $223.3 \text{ mg}\cdot\text{kg}^{-1}$, showing that the former degraded to the latter. The soil sample from site Okidi 1, had the highest TNT concentration at $551.8 \text{ mg}\cdot\text{kg}^{-1}$ implying that either its recent input or lower degradation led to the

lower concentration of $103.7 \text{ mg}\cdot\text{kg}^{-1}$ of 2,6-DNT. Pennington et al. (2006a) reported the amounts of 2,6-DNT from Yakima Training Centre, Washington, USA in the soil at an artillery firing range at $0.4 \text{ mg}\cdot\text{kg}^{-1}$, which is a magnitude lower than of present study. A study by Jenkins (1998) reported the mean levels of 2,6-DNT in soil from the munitions demolition site from Fort Ord, California, the USA at $0.9 \text{ mg}\cdot\text{kg}^{-1}$ which is a magnitude lower than of present study (Jenkins, 1998). A similar study at the Bangor Naval Submarine base, in Washington, USA by Jenkins et al. (2001) reported the concentration of 2,6-DNT in soil samples analyzed at $0.41 \text{ mg}\cdot\text{kg}^{-1}$, which is a magnitude lower than the present study (Jenkins et al., 2001). Pichtel (2012) reported the levels of 2,6-DNT in soil from an explosive open-burning and detonation site at Welden Spring, Missouri, the USA at $8.1 \text{ mg}\cdot\text{kg}^{-1}$, which is fourteen-fold lower than the present study.

NG was detected in all soil samples from the study sites at a mean concentration of $245.2 \text{ mg}\cdot\text{kg}^{-1}$ (with a range of $34.2 - 329.6 \text{ mg}\cdot\text{kg}^{-1}$). Percentage contribution to total NG from the study sites Okidi 2 and Bibia were 34% each, while Okidi 1 and Elegu sites contributed 24 and 3%, respectively of the explosive compound. The lowest concentration of NG recorded from the study sites in soil was from the Elegu demolition site. The compound contaminates the soil after the demolition of unexploded remnants of war with formulations that contain NG. The concentration of NG was reported in soil by Pennington et al. (2006a) at $4.6 \text{ mg}\cdot\text{kg}^{-1}$ from the artillery firing range of Yakima Training Centre, USA. Pennington et al. (2006a) reported the amounts of NG in soil from an anti-tank range in Ontario and crater bomb soil in Alberta, Canada at 227 and $2240 \text{ mg}\cdot\text{kg}^{-1}$, respectively. The amounts of the compound from Ontario are similar to the present study while the crater bomb soil from Alberta is nine-fold higher than that of the present study. A study conducted in an anti-tank range in New Brunswick, Canada by Thiboutot et al. (2004) reported NG levels in soil samples analyzed at a mean concentration of $6560 \text{ mg}\cdot\text{kg}^{-1}$, which is twenty-six-fold higher than that of the present study.

Tetryl was detected in all the soil samples analyzed with a mean concentration of $213.2 \text{ mg}\cdot\text{kg}^{-1}$ (with the range of $70.6 - 334.9 \text{ mg}\cdot\text{kg}^{-1}$). The highest amount of the explosive compound was registered at the Bibia demolition site at $334.9 \text{ mg}\cdot\text{kg}^{-1}$. The percentage contribution of Tetryl in the study sites Bibia, Okidi 2, Okidi 1, and Elegu were 39%, 36%, 17%, and 8%, respectively. The presence of Tetryl in the soil from the demining sites is attributed to contamination during the demolition of the unexploded ordnance. Bordeleau et al. (2008) reported Tetryl concentration in soil from Cold Lake Air weapons range, in Alberta, Canada at $3390 \text{ mg}\cdot\text{kg}^{-1}$, which is sixteen-fold higher than in the present study.

PETN was detected in all the soil samples from the demining sites. The amounts of the explosive compound in the analyzed soil samples were of a mean concentration of $92.8 \text{ mg}\cdot\text{kg}^{-1}$ (with the range of $19.9 - 158.2 \text{ mg}\cdot\text{kg}^{-1}$). The highest amount of the explosive compound was registered at the Okidi 1 demolition site at $158.2 \text{ mg}\cdot\text{kg}^{-1}$. The percentage contribution of PETN in the study sites Okidi 1, Okidi 2, Bibia, and Elegu were 43%, 26%, 26%, and 5%, respectively. The presence

of PETN in the soil from the demining sites is attributed to contamination during the demolition of the unexploded ordnance.

RDX and TNT were predominant in the soil samples analyzed from the study sites and contributed to 22% and 21%, respectively to the total summation of explosive compounds in the study. 2,6-DNT the degradation product of TNT contributed to 8% to the total summation of explosive compounds analyzed from the demining sites. PETN contributed the lowest at 7% to the total summation of explosive compounds from the study sites. One-way Analysis of variance revealed that the mean concentration of the target analytes from the study sites is likely to be equal ($P > 0.05$). **Figure 7** shows the percentage contribution to the total summation of the explosive compounds from the study sites.

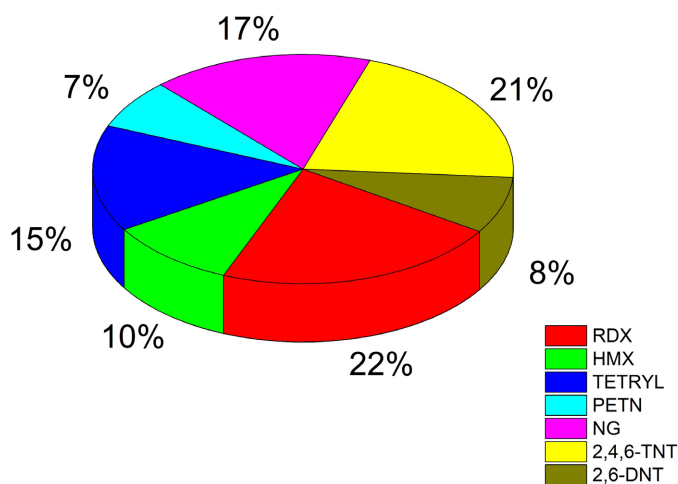


Figure 7. Percentage contribution to total summation of the explosive compounds detected in soil samples from Amuru demining sites, Northern Uganda.

4. Conclusion

The study determined the concentration of explosive residues of RDX, HMX, TNT, 2,6-DNT, NG, PETN, and Tetryl in soil samples from the demining sites in Amuru district, northern Uganda. The concentration of explosive compounds in the soil samples ranged from 19.9 - 551.8 mg·kg⁻¹ which are higher than the Soil Screening Level values set by EPA Region 9. TNT and RDX were the predominant explosive compounds from the demining sites. The presence of the explosive compounds is attributed to contamination due to the demolition of unexploded ordnance and unexploded remnants of war. It can be concluded that the soil from all the demining sites poses an Ecotoxicological risk to humans, soil micro-organisms, and the aquatic ecosystems of the nearby wetlands, and streams. Remediation of the explosive contaminated soils and water leads to a cleaner environment. Extensive studies should be carried out to evaluate the remediation of explosive contaminated sites. Some abiotic (physical and chemical) techniques are available and include application of biochar to explosive contaminated soils. Bioremediation techniques have been reported to be effective in the treatment of explosive

contaminated soils though a lot is still desired to explore further for better efficacy and application of microbial remediation technologies at explosive contaminated sites.

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Conflicts of Interest

The authors declare no conflict of interest.

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