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Comparative sampling methodologies for detecting and quantifying 2,4,6 trinitrotoluene post-blast traces in water

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ABSTRACT

This study addresses the analytical challenges associated with recovering explosive residues, focusing on the identification of 2,4,6-trinitrotoluene (TNT) in water samples. It evaluates the practicality, efficiency, and representativeness of three sampling methodologies: traditional grab sampling (GS), composite sampling (CS), and 3-D multi-increment sampling (3D-MIS). High-Performance Liquid Chromatography (HPLC) was employed for explosive identification. Post-blast sampling of TNT residues from high-order and low-order deflagrations was conducted to assess each method's efficacy and limitations in detecting trace and bulk contaminations. The experiments were conducted at the Alford Technologies Group range in Broadmead, UK, with analysis performed at the Defence Academy in Shrivenham, UK. Key findings highlight the varying effectiveness of each sampling method, with implications for enhancing detection sensitivity and accuracy in post-blast scenarios. This study underscores the importance of selecting appropriate sampling strategies tailored to different contamination scenarios, thereby informing more effective response protocols in CBRNe incidents involving water environments.

1.0 INTRODUCTION

Sampling and analysis are commonly employed in various industries to monitor workplace pollutants and compliance with health and safety protocols. Chemical analysis is important to all verification tasks, as it provides clear evidence of the presence or absence of specific chemicals in a sample. This is particularly important in Chemical, Biological, Radiological, Nuclear, and high-yield Explosives (CBRN(e)) incidents, where accurate detection and identification are vital (Andrewes, 2023; OPCW, 2024; Petersen et al., 2005). The use of extremely poisonous nerve agents, explosives and other chemical weapons by terrorist organisations and state actors as seen in conflicts like those in Syria and Ukraine, as well as in domestic attacks such as the Salisbury poisoning, demonstrates the real and ongoing risk of exposure of civilian or military, despite the Chemical Weapons Convention's (CWC) prohibition (de Koning et al., 2023; Skripal et al., 2018). Accurate identification and recovery of hazardous contaminants are essential to assess and mitigate risks, limit exposure and casualties.

For accurate and successful identification of both trace and bulk contamination, representative, economical and most importantly reliable sampling is essential. However, representative sampling is difficult to achieve due to the heterogeneous nature of explosive contaminations and environmental conditions. Traditional sampling methods often produce highly variable results from sample to sample (Walsh et al., 2014). To achieve accurate sampling, any given sample should reflect the distribution and composition of the residues in the environment. Non-uniform or heterogenous distribution within the sample and environment as well as non-uniform residues, are caused by both distributional and constitutional heterogeneity (Walsh et al., 2014). According to the North Atlantic Treaty Organisation (NATO) accuracy in samples can be achieved through training, standard operating procedures (SOP), rules of procedure (ROP) and quality control (QC) samples, such as field blanks (NATO, 2015, p. 59). However, the chosen sampling method is equally important. Grab sampling has been reported as the least representative way of sampling (Estoppey et al., 2019; Madrid & Zayas, 2007; Minkkinen & Esbensen, 2009). Therefore, a comprehensive sampling plan is essential, while time-consuming and reliant on available information and intelligence, which may not always be present in a CBRN (e) incident. This study compares three sampling methodologies—grab, composite, and 3-D multi-increment sampling—

based on literature and published CBRN (e) guidance from the MOD and NATO. Two experiments were conducted simultaneously in water to investigate the performance of these methods for both trace and bulk contamination: one involving high-order detonations and the other low-order deflagrations. High order detonation, the most widely utilised approach, entails detonating an explosive donor charge around the unexploded ordinance (UXO), causing any live explosive material within the UXO to detonate with it. While effective, this method produces loud underwater blasts that harm marine ecosystems (Fox, 2022). In contrast, low order deflagrations use smaller donor charges, which do not detonate the UXO, thus minimizing the environmental damage. This method pierces the UXO shell, allowing it to burn out without detonation. Despite its advantages, there is limited information about the effectiveness of low-order detonations. To address these sampling and analysis issues, the study compared grab, composite, and multi-increment sampling methodologies, assessing their reliability, efficacy, and representativeness, as well as their limitations, for both high-order detonations and low-order deflagrations.

Grab sampling involved collecting a lump of material from a lot in a single operation. Each primary increment is processed and analysed separately, which increases both time and analysis costs. While this method is a very simple, approximative and cost-effective, it is far from ideal due to several inherent sampling errors, failure to address heterogeneity and its representation limited to a specific moment and location (Minkkinen & Esbensen, 2009). Despite these drawbacks, grab sampling is outlined as the preferred method for water sample collection in the SIBCRA (Sampling and Identification of Biological, Chemical, and Radiological Agents) guidance (NATO, 2015). To address the limitations of grab sampling and enhance representativeness, composite sampling is also investigated. Unlike grab sampling, which requires individual analysis of each sample, composite sampling combines multiple samples into a single bulk sample, reducing the time and materials needed for analysis (France et al., 2015; Huizer et al., 2021; Minkkinen & Esbensen, 2009; Patil, 2011). Composite sampling has however limitations too. Increased volume from combining liquid samples can lower detection sensitivity and potentially produce false negatives. However, this issue can be mitigated by concentrating the analytes using standard filtration methods (France et al., 2015).

In CBRN (e) studies, composite sampling has proven to be a viable method for producing representative samples, while the number of analyses required for remediation without compromising data integrity (France et al., 2015; Patil, 2011). Standard procedure involves collecting a set of grab samples, retaining half as individual samples and pooling the other half into a composite sample. This approach allows for initial composite testing to determine contamination, followed by re-testing individual grab samples if the composite tests positive, thus enabling precise hazard mapping (Mesilaakso, 2005).

Multi-increment sampling (MIS) is considered the most representative method discussed in this paper. MIS uses systematic sampling, pooling several primary increments into one overall sample. The sampling area is divided into a grid, and a single grab sample is taken from approximately the same location within each square to form the pooled sample (Walsh, Walsh, Gagnon, et al., 2014). To ensure MIS is truly representative of a body of water, it must incorporate a spatial aspect and be conducted three-dimensionally. This method enhances the representativeness of the samples and provides a more comprehensive assessment of contamination.

2.0 MATERIALS AND METHODS

Six pipe bombs were manufactured using seamless mild steel S235 J tubes supplied by Precision Profiles, each filled with 100 g TNT and 20 g PENO (80% Pentaerythritol tetranitrate (PETN) and 20% marker) donor fill. Three devices were detonated as high order explosions and three as low order deflagrations. Each was placed in 1000 L water filled intermediate bulk containers (IBC).

Following detonation or deflagration, and after the area was declared safe, samples were collected in order of ease of sampling. Therefore, grab samples were collected and sample split in situ to form the composite sample first, then the multi-increment samples were taken. The experiment was carried out at Alford Technology's explosive range in Broadmead, UK. To prevent pressure, build up, the IBC roofs were removed using a handsaw. To enhance water collection post-detonation/deflagration in the IBC, four commercially available paddling pools were placed around the elevated IBC (Figure 1). The 1000 mm x 1160 mm x 1200 mm IBCs were constructed from a galvanized steel cage, with a high-density polyethylene body.

Figure 1:Experimental set up at Alfords range showing all four paddling pools A, B, C and D as well as the IBC filled with 1000l of water suspended on top of two solid wood beams.

2.1 Quality Control measures

Procedural blank controls, also known as field blanks, were collected at the range. Additionally, background samples were collected before detonations and duplicate samples post detonations to prevent contamination during sampling, transportation (NATO, 2015). For quality control, NATO guidelines recommend including a field duplicate, a field blank, and a background sample for every ten samples collected (NATO, 2015, p. 59).

2.2 Composite and grab sampling through sample splitting

Sampling involved composite and grab techniques using sample splitting which are endorsed by institutions such as the OPCW and NATO. This method allows the reduction of sampling materials required. Essentially, grab samples were split into two portions: one retained as an individual grab sample and the other used to create a composite sample. To execute this, 100-ml grab samples were split immediately after collection (Figure 2). One 50-ml aliquot was stored in a 50-ml amber vial as an individual grab sample for hazard mapping. The remaining 50-ml aliquot was placed in a 500-ml amber bottle to form the composite sample. Sampling occurred promptly after detonation once safety was assured.

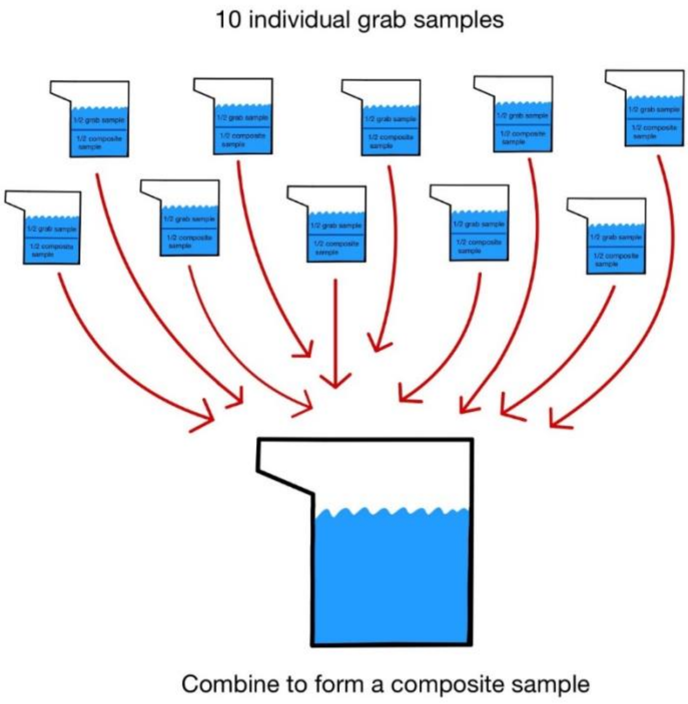

Figure 2: Illustration of composite and grab sampling through sample splitting.

Random selection of the grab samples was determined by the sampler rather than utilising an algorithm or software, reflecting common procedures in CBRN (e) protocols. Duplicates were taken resulting in 20 grab samples and two composite samples, each composed of 10 subsamples, collected for each of the three detonations and three deflagrations. Six of these samples were collected from the surface of the IBC and one sample was taken each from the four paddling pools to make up the 10 grab samples and composite sample (Figure 1).

2.3 3-D multi-increment sampling (3-D MIS)

3-D multi-increment sampling (3-D MIS) was employed by sectioning the lot within the metal cage surrounding the IBC into 12 sub-lots across two layers. Each sub-lot was sampled from approximately the same positions (Figure 3). In total, 12 subsamples, six from layer one and six from layer two, were collected. All samples were taken in duplicates.

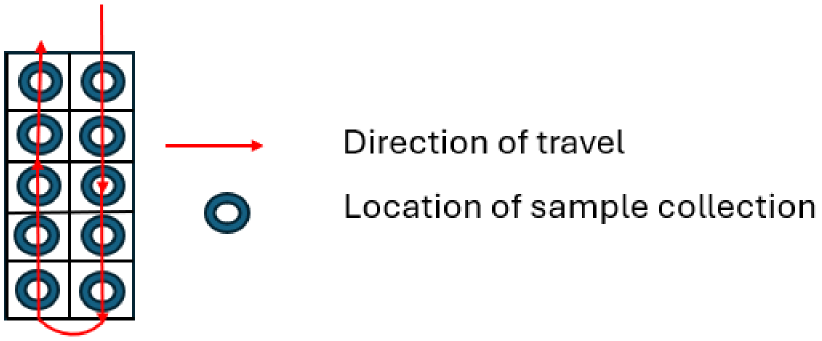

Figure 3: Illustration of multi-increment sampling.

2.4 Low order deflagration

To achieve low order deflagration, the Vulcan method, used by the British military, was used (Parliament, 2020). The method utilises a Magnesium Jet Forming Cone disc activated by 20 g PENO donor charge and a Davey Bickford Electric Detonator. The Vulcan device was taped to the pipe bomb using steel leg attachments and electrical tape. The assembly, including the pipe bomb with attached Vulcan, was suspended centrally within the IBC using wire, aiming to evenly distribute the blast force and minimise damage to the IBC with equal amounts of water on each side (Figure 4).

Figure 4: Pipe bomb suspended in 1000l IBC tank for low order deflagration.

2.5 High order detonations

To initiate the high order detonations, one cap of each pipe bomb was removed and 20 g of PENO, a PETN based plastic explosive, was placed directly in contact with the TNT (Figure 5, left image). The detonator was then directly placed into the PENO. Like the LO disposals, the pipe bomb was placed as centrally within the IBC as possible (Figure 5, right image).

Figure 5: Left image: Pipe bomb rigged for high order detonation with PENO donor charge visible.

Right Image: Suspended pipe bomb in IBC tank for high order detonation with detonator attached

2.6 HPLC analysis

The HPLC used is Agilent 1260 with a LoD of > 1ppm. To ensure quick, efficient analysis a HPLC method was set up to allow fast analysis at 3 minutes with a flow rate of 1.50 ml and an injection volume of 10μl. The 3-minute runs allowed for the TNT (RT≈2.4 min) to be detected. A C18 column from Agilent was used in reverse phase with a mobile phase made up of 60:40 ACN: Water. Following each complete run (up to 91 samples) two washing steps were completed taking approximately 20 minutes. One with water and one with acetonitrile. The first 5 samples run consisted of the standards, that were freshly manufactured and filtered for analysis.

3.0 RUSULTS AND DISCUSSION

3.1 Preliminary results

To determine the optimal sample preparation method for HPLC analysis, an initial experiment was carried out to compare two mobile phase compositions: 100% water and 50:50 Acetonitrile (ACN): Water. Each comparison utilised a single grab sample from each IBC. The results indicated that filtering the water samples through a 0.2μm Nylon filter significantly improved the detection of low concentrations of TNT (Table 1).

Table 1: Initial results for six grab samples comparing two sample preparation methods-Samples A-F were only filtered, whereas A.1-F.1 was diluted with ACN in a 50:50 mix.

Detonation type	Samples	Peak Area	Concentration (ppm)	Retention time (RT)
LO	A	60.8	5.067	2.317
LO	B	177.2	10.725	2.319
LO	D	418.2	22.439	2.321
HO	C	0.56	2.139	2.499
HO	E	0.52	2.137	2.241
HO	F	0.5	2.136	2.243
LO	A.1	37.5	6.569	2.318
LO	B.1	117.4	14.817	2.319
LO	D.1	198	23.137	2.315
HO	C.1	0	0.000	/
HO	E.1	0	0.000	/
HO	F.1	0	0.000	/

This can be attributed to the 50% dilution with 99.9% pure acetonitrile, resulting in concentration falling below the limits of detection (LoD) of the HPLC (≥1ppm). Samples A through F were prepared by only filtering the water, whereas samples A.1 through F.1 were diluted with ACN in a 50:50 mix (Table 1; Figure 4). Initial results also showed significantly more residues remain after low order deflagrations, than high order detonations where only ≈2 ppm out of 100 ppm originally remained. For further analysis all samples were filtered with a 0.2 µm Nylon filter only.

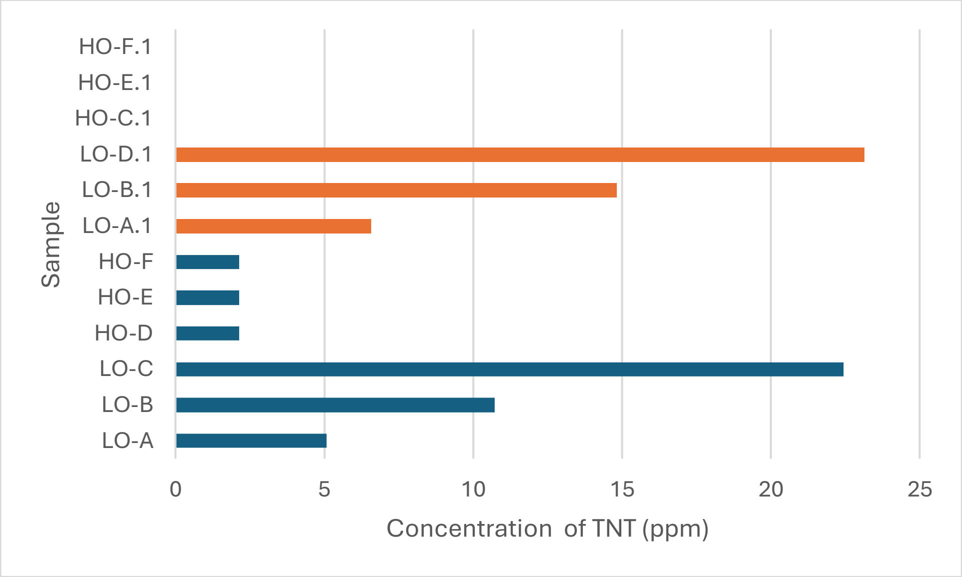

Figure 6: Comparison of two sample preparation methods: only filtration and filtration and dilution with ACN. Blue-only filtration with 0.2 µm Nylon filter. Orange-filtration and dilution with 99.9% ACN to 50:50 mix with water.

From the limited data collated up to date, an assessment was conducted on the efficacy and practicality of the sampling methodologies. Planning a comprehensive sampling plan requires extensive time and resources, which contrasts sharply with the urgent nature of most CBRN (e) incidents. Therefore, a differentiation must be made between preliminary sampling and comprehensive sampling, as outlined in NATO SIBCRA guidance. External calibration was performed to determine TNT concentration. Results show that low order deflagrations leave behind more residue than high order detonations. The mean values for the low order deflagrations ranged between 6.12and 22.9 ppm, while the mean values for the high order detonations range between 1.4 and 2.16 ppm. In high order 'E' samples concentrations were below the LoD of the HPLC and could not be calculated through external calibration. However, it is important to note that a TNT peak was observed in the chromatogram, confirming the presence of the analyte. Key findings suggest the closer the concentration of the analyte of interest is to the LoD of the equipment, the greater the number of samples required for reliable detection.

3.2 Grab samples

Twelve grab samples were collected in two batches of six each. The samples were stored at 5◦C until analysis. Before extracting triplicate subsamples, sampling vials were inverted three times to mitigate any distributional heterogeneity. Subsamples were taken using single use plastic and filtered using 0.2µm Nylon filters before storing at 5◦C until HPLC analysis. Out of 36 samples analysed for all three low order deflagrations 100% were found to contain TNT (Figure 7). However, for the post high order detonations, only 44% of grab samples from HO 'C', 72% from HO 'E' and 53% from HO 'F' could be integrated using the HPLC software. However, concentrations could only be calculated for HO 'C' and 'E' (Figure 7).

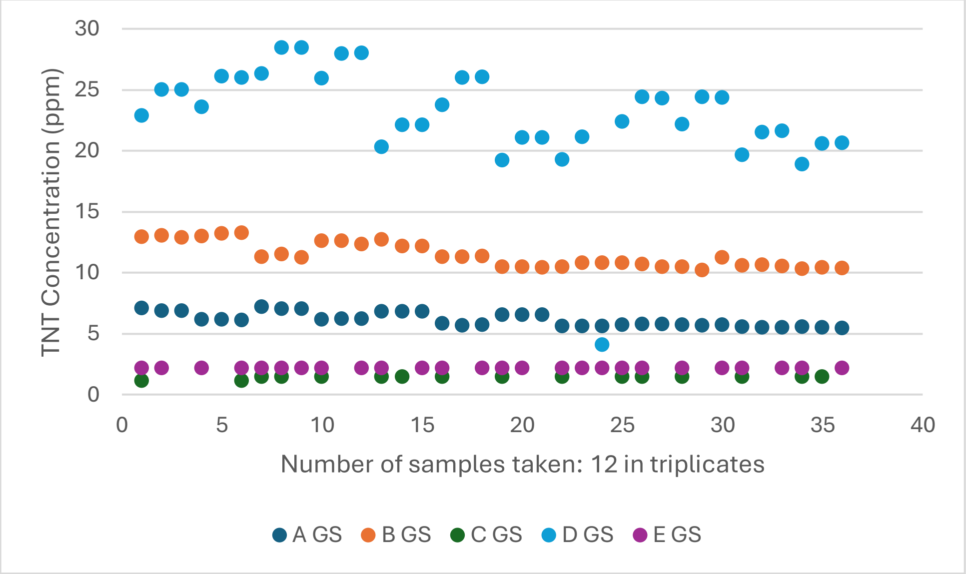

Figure 7: Visual comparison of all grab samples (GS) within the intermediate bulk container (IBC) where concentrations could be calculated by use of HPLC.

Results also showed, that grab samples taken from outside the IBC, the paddling pools, had a higher variance and an overall lower concentration of TNT (Figure 8). Furthermore, it was observed that the higher the residual concentration post-clearance operation correlated with increased variance in the grab samples both inside the IBC and in the paddling pools (Figure 7,8; Table 2).

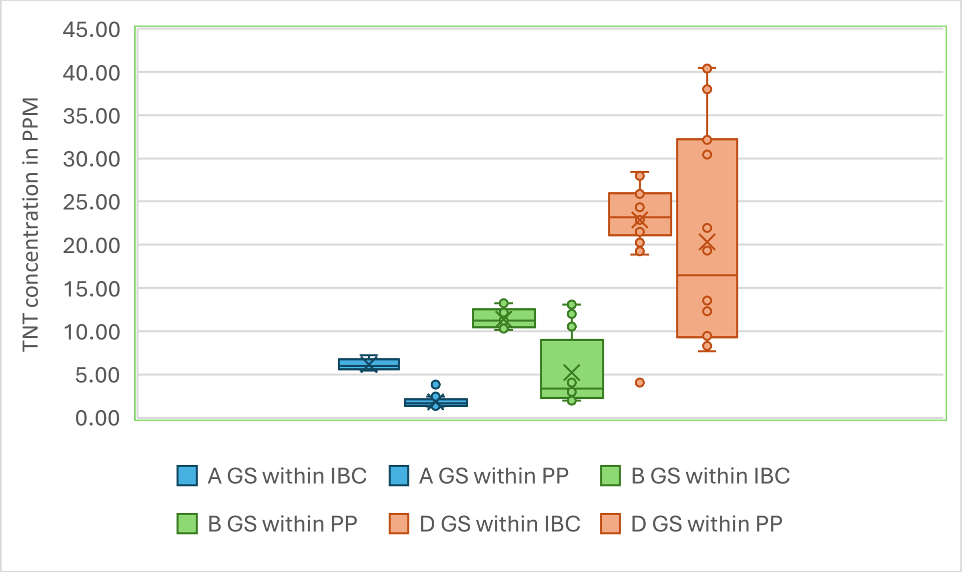

Figure 8: Visual comparison of grab samples (GS) taken within the intermediate bulk container (IBC) and within paddling pools (PP) for all low orders (LO).

Table 2: Mean and standard deviation for all grab samples (GS) taken within the IBC and within the IBC and paddling pools (PP).

IBC	Sampling method	Mean	Mean (w/ PP)	SD	SD (w/ PP)
LO-A	GS	6.12	4.55	0.57	2.16
LO-B	GS	11.41	8.92	1.02	4.08
LO-D	GS	22.9	22.05	4.25	7.38
HO-C	GS	1.4	1.28	0.11	3.84
HO-E	GS	2.16	1.59	0.01	1.04
HO-F	GS	-0.22	-0.024	0.06	0.05

3.3 Composite samples

As 40% of the composite sample originating from the paddling pools, the results were notably biased. Subsequently, the samples underwent triplicate subsampling after the bottle was inverted three times. The same sample preparation procedure was followed, and samples were refrigerated until HPLC analysis. Concentrations could only be calculated for the LO samples (Table 3), consistent with findings in the literature (France et al., 2015).

Table 3: Mean and standard deviation of composite samples.

IBC	Sampling method	Mean	SD
A	CS	3.67	0.9
B	CS	9.11	0.82
D	CS	20.31	2.16
C	CS	-0.28	0
E	CS	-0.26	0.02
F	CS	/	/

Thus, a limiting factor is the further dilution of the trace contamination in post high order detonations. Figure 9 illustrates that composite sampling remains a valid method for estimating overall contamination levels particularly in in higher concentrations.

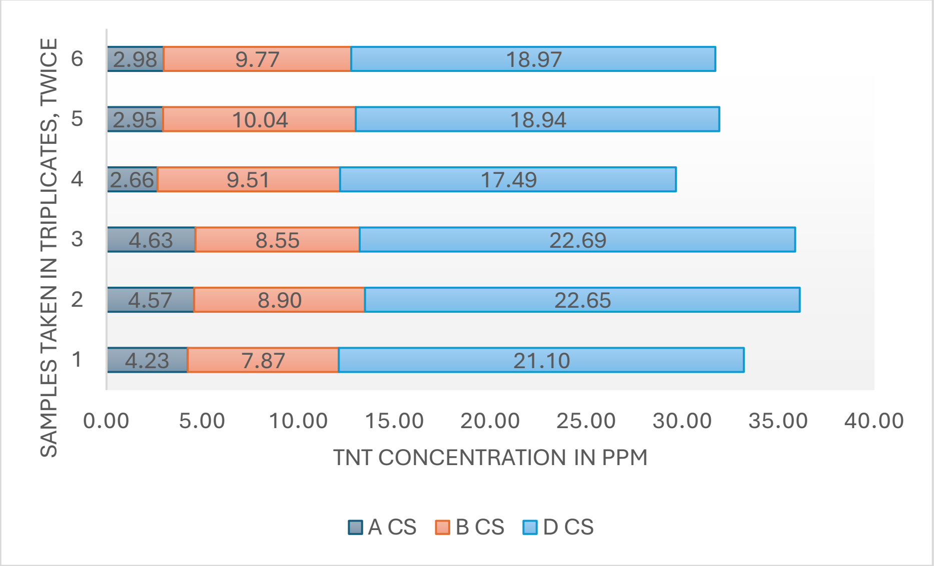

Figure 9: Visual comparison of duplicate composite sampling (CS) for all low order (LO) deflagrations.

Finally, a trend emerged showing that with increasing concentrations in post-deflagration samples, the standard deviation also increased (Figure 10).

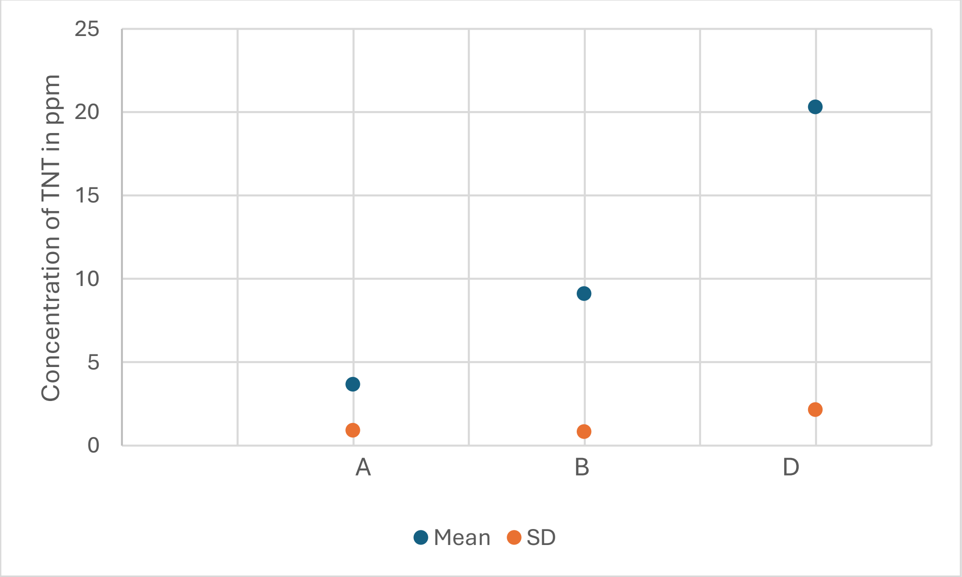

Figure 10: Mean and standard deviation for composite samples from IBC tanks, A, B, D of the low order deflagrations.

3.4 3-D multi-increment sampling

Multi-increment sampling was only performed for the low order deflagrations. This decision was influenced by the fact that only a quarter of the water remained in the split IBC after the high-order detonation, which did not accurately represent the entire water body. Multi-increment sampling is the most labour-intensive method explored in this study, requiring substantial planning, equipment, and time. This contrasts with the urgency typically associated with CBRN (e) incidents, therefore being identified as a limiting factor for this method. However, it is clear from the results that spatial sampling most definitely needs to be investigated further for the representation of bodies of water.

Noticeable was however that out of the three experiments one showed a large variance from sample one to sample two. Deflagration 'D' in fact had a standard deviation of 10.74 suggesting the concentration calculated is not reliable and severely skewing the mean calculated (Table 4, Figure 11).

Table 4: Mean and standard deviation of all multi-increment samples taken in the low order deflagration from IBC tanks A, *B, D.*

IBC	Sampling method	Mean	SD
A	MIS	10.76	1.72
B	MIS	14.83	0.71
D	MIS	32.03	10.74

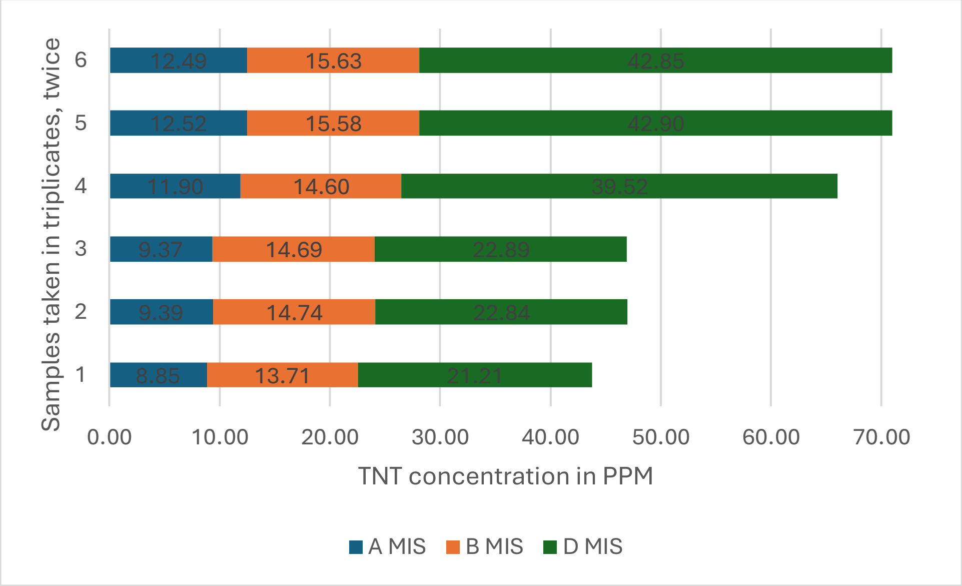

Figure 11: Visual comparison of duplicate multi-increment samples (MIS) taken for all low order (LO) deflagration.

3.5 Overall comparison

Generally, the CS showed the lowest concentration, while the GS was found to be in the middle and the MIS showed the highest detected concentrations of TNT (Figure 12). This disparity can be attributed to the three-dimensional sampling approach across two layers. The absence of spatial sampling in CBRN (e) incidents could therefore be a potential concern. However, it is crucial to acknowledge that this comparison involves 36 samples versus 6 samples and 6 samples, introducing a potential source of error that should be noted for clarity to the reader.

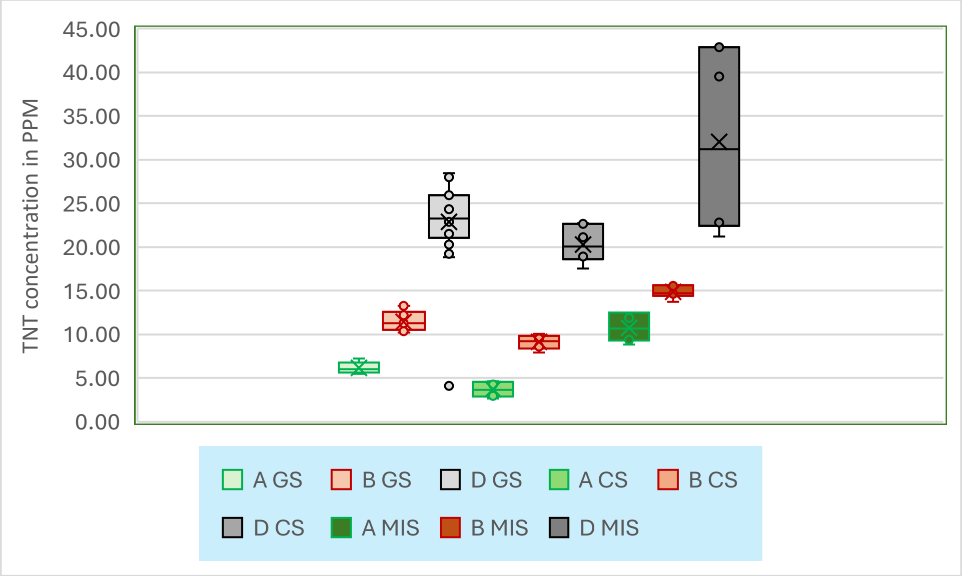

Figure 12: Statistical comparison of grab (GS), composite (CS) and multi-increment sampling (MIS) for low order (LO) deflagration 'A, B and D'.

Table 5: Comparison of grab (GS), composite (CS) and multi-increment sampling (MIS) mean and standard deviation for high-order (HO) detonation and low-order (LO) deflagration.

Type of detonation	IBC	Sampling method	Mean	SD
LO	A	GS	6.12	0.57
LO	A	CS	3.67	0.9
LO	A	MIS	10.76	1.72
LO	B	GS	11.41	1.02
LO	B	CS	9.11	0.82
LO	B	MIS	14.83	0.71
LO	D	GS	22.9	4.25
LO	D	CS	20.31	2.16
LO	D	MIS	32.03	10.74
HO	C	GS	1.4	0.11
HO	C	CS	-0.28	0
HO	C	MIS	/	/
HO	E	GS	2.16	0.01

HO	E	CS	-0.26	0.02
HO	E	MIS	/	/
HO	F	GS	-0.22	0.06
HO	F	CS	/	/
HO	F	MIS	/	/

4.0 CONCLUSION

In conclusion, this study highlights the potential benefits of exploring unconventional sampling methods for CBRN (e) recovery under specific conditions. It emphasizes the need to select appropriate sampling methods based on the specific objectives and scope of the sampling.

To obtain a comprehensive understanding of the environment hazard mapping through grab sampling is essential, but is prone to high variation, necessitating analysis in triplicate at minimum to mitigate false negatives. The closer the concentration of the sample is estimated to be to the LoD of the analytical equipment used, the more samples should be analysed to ensure effective mitigation of false negatives. However, GS has limited use beyond hazard mapping, particularly in a water, where it may inaccurately evaluate hazards due to its non-representative nature.

Composite sampling significantly reduces the time taken for analysis, while requiring meticulous planning and coordination. An alternative approach involves creating composite samples in the lab through sample splitting from grab samples taken in situ. However, composite sampling may not effectively recover low concentrations of post-blast residues without prior concentration. The inclusion of 40% of the sample from the paddling pools could have underestimated the true concentration, as well as possibly due to dilution effects as noted by France et al. (2015).

3D-MIS generally showed the highest recovery of TNT suggesting heterogeneous dispersion of post-blast materials within the IBC and showing promise for assessing contamination in bodies of water. However, its implementation is hindered by complexity, requiring rigorous planning and extensive equipment.

The main distinction between composite and multi-increment sampling lies in spatial and randomness factors. While grab and composite sampling employ random sampling, multi-increment sampling utilises systematic sampling, proving more effective in assessing true water contamination post-blast. This study also emphasizes the potential for false negatives with grab and composite sampling at low concentrations, underscoring the importance of comprehensive training for first responders in intelligence sampling of heterogeneous substances. Current assumptions of homogeneity in sampling may not hold true, as evidenced by results from multi-increment, composite, and grab sampling methods. In future research, ensuring triplicate sampling at minimum across all stages of experimentation is recommended to enhance result reliability and confidence.

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