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Bioremediation of explosives-contaminated soil using microbial and environmental enhancements: a field-simulated pilot study in Israel

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Abstract This pilot study evaluated a field-simulated bioremediation treatment for soils contaminated with military explosives as a precursor to a planned large-scale cleanup (~ 7,000,000 m^2) in Israel. Sandy-loam soil was amended with compost and a microbial and managed under controlled irrigation and aeration for 85 days. HPLC analyzed six sampling rounds collected at defined intervals. Temporal changes in RDX, HMX, and TNT, commonly found at military sites and posing risks to human and environmental health, were assessed with LMMS and start-vs-end comparisons with non-parametric tests. Initial mean concentrations were 120.46 ± 34.54 mg kg^{-1} (RDX), 144.73 ± 36.95 mg kg^{-1} (TNT), and < LOQ for HMX. RDX and TNT declined sharply from day 55 onward and remained at or near non-detectable levels through day 85 (GLMM contrasts, all $p < 0.001$ after day 55); start-to-end differences corroborated these reductions (RDX: W = 12, $p = 0.050$; TNT: W = 12, $p = 0.0319$). HMX exhibited a non-monotonic pattern, increasing at day 40 (mean 4.66 ± 1.06 mg kg^{-1}) and decreasing thereafter, with a small residual at day 85 (0.12 ± 0.25 mg kg^{-1}; start-to-end W = 0.01, $p = 0.0436$). Overall, under field-simulated conditions, the treatment rapidly and sustainably reduced RDX and TNT. However, HMX showed greater variability and may require extended treatment or complementary measures. These quantitative pilot results inform the design and risk management of forthcoming full-scale remediation in similar semi-arid settings.

Keywords Ammunition disposal sites · Explosive compounds · Soil contamination · Environmental bioremediation · Microbial degradation

Avi Ben Laish, Jakub Z. Kosicki and Reuven Yosef have share equal credit.

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Introduction

Soil contamination with explosive compounds is a significant environmental issue commonly associated with military training areas, ammunition manufacturing, and disposal sites. Compounds such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and 2,4,6-trinitrotoluene (TNT) are among the most frequently detected residues in these environments (Sunahara et al. 2009; Walsh et al. 2019). These

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nitroaromatic and nitramine explosives are persistent, toxic, and potentially carcinogenic, posing risks to soil health, groundwater quality, ecosystems, and human health (Lewis et al. 2004; Singh et al. 2012; Bhatnagar and Kumari 2013). For RDX, the U.S. EPA has set a chronic oral reference dose (RfD) of 0.003 mg kg^{-1} day^{-1} and a lifetime drinking-water health advisory of 0.002 mg L^{-1}, with neurotoxicity (including seizures) as the primary concern at higher exposures (EPA 2018). For HMX, the chronic oral RfD is 0.002 mg kg^{-1} day^{-1} (based on liver toxicity in animal studies), and the occupational TLV is 0.5 mg m^{-3} (EPA 2000; ATSDR 2014). For TNT, the chronic MRL is 0.0005 mg kg^{-1} day^{-1} and California's NSRL is 8.2 µg day^{-1} for adult cancer risk, with an occupational TLV of 0.1 mg m^{-3} (ATSDR 2009; OEHHA 2010). These benchmarks incorporate uncertainty/safety factors and are intended for long-term (chronic) exposure; however, sensitive populations may experience adverse effects at lower levels (Health Canada 2023). Given the chemical stability of these compounds and limited natural attenuation, developing effective and scalable remediation strategies remains a substantial challenge.

Bioremediation, particularly through microbial degradation, has emerged as a promising approach for addressing such contamination. This method offers a favorable balance between remediation efficiency and environmental safety, as it allows for the microbial breakdown of persistent explosive compounds without introducing additional, potentially hazardous chemicals into the ecosystem. It is also cost-effective, scalable, and suitable for in situ applications with minimal ecological disruption (Esteve-Núñez et al. 2001; Lotufo et al. 2009). Various microbial taxa have shown the ability to metabolize or transform RDX, HMX, and TNT under both aerobic and anaerobic conditions. However, degradation rates and pathways can vary significantly based on environmental conditions, the chemical structure of the compounds, and the composition of the microbial community (Bhushan et al. 2003; Srivastava et al. 2022).

Importantly, recent armed conflicts in Eastern Europe, the Middle East, and Africa have exacerbated the scale of soil contamination with explosives, amplifying the urgency to develop sustainable and efficient remediation technologies. Despite progress in laboratory studies, the temporal dynamics of compound-specific degradation under semi-controlled or field-simulated conditions remain poorly understood. This knowledge gap limits the ability to design site-specific interventions and hinders large-scale implementation.

Military installations in Israel are often located near both urban and rural communities, which differ from larger countries where such sites are more isolated. Although there is a common assumption that military sites are far from densely populated areas, in Israel a substantial share of the population may live near or adjacent to these facilities. Previous studies have shown that contaminants such as RDX, HMX, and TNT can migrate from training grounds and storage sites through groundwater and soil, posing risks to drinking-water sources and agriculture, especially where population centres lie close to these installations (Moshe et al. 2009; Ansenberg and Marom 2024; Mystrioti and Papassiopi 2024). Therefore, populations at risk in Israel include nearby urban residents, rural farming communities, and individuals directly dependent on local groundwater resources. Comprehensive assessments have documented detectable concentrations of explosives in water sources close to military areas, and regulatory agencies have acknowledged potential exposure among communities living within short distances of these contaminated sites. Thus, the risk to human populations is elevated because of the country's small geographic area and the relatively high density of settlements in relation to military infrastructure.

To address these challenges, we designed a pilot study to investigate the degradation trajectories of RDX, HMX, and TNT in soil over a defined bioremediation period. Our objective was to generate time-resolved data that would inform future full-scale remediation efforts and contribute to a better understanding of microbial treatment of explosive residues in arid or semi-arid regions.

Methods

Experimental design

Contaminated soil (400 m^2 working area, 30 cm treatment layer = 120 m^3) was supplied by Netzer Hasharon from a former military-industrial area and brought to the pilot site at Ramat Beka, southern Israel. It arrived air-dry, with moisture limited to hygroscopic water. An accredited ISO/IEC 17025:2017 laboratory classified the material as sandy loam with basic pH, high salinity, and elevated sodium adsorption ratio (SAR). The field capacity of the soil was approximately 20% moisture by weight. Baseline measurements included particle-size fractions only (sand/silt/clay: 64%/20%/16%). To improve uniformity, the soil was mixed with a loader in cross passes, coarse fractions were removed, beds were formed, and the upper 0–20 cm was rototilled (Fig. 1). Initial explosive residues were RDX = 120.46 ± 34.54 mg kg^{-1}; TNT = 144.73 ± 36.95 mg kg^{-1}; HMX < LOQ (at baseline)) with initial bacterial load was 2.2×10^5 CFU g^{-1}.

On 16.07.2024 we incorporated animal-waste compost at 2 kg m^{-2} (≈20 t ha^{-1}) to enrich organic matter and support microbial activity, and supplied a mineral nutrient mix (N:P:K = 36:6:6) to sustain biodegradation.

A microbial consortium (producer BGP) was applied twice (16.07.2024 and 21.07.2024) following the manufacturer's dose of 50 mL liquid and 14 g powder per m^3 of treated soil. For 120 m^3 this corresponds to 6.0 L of liquid and 1.68 kg of powder in total (≈ 3.0 L and 0.84 kg per application), delivered in a working solution to ensure even distribution.

A drip-irrigation system with Netafim GrowSphere™ control and fertigation was installed (four lines per bed; emitters at 15 cm spacing, Fig. 2), and moisture was managed at 0.8–1.0 of field capacity (~16–20% w/w) with short dry intervals to maintain aeration. Daily inputs were adjusted from sensor data rather than kept fixed. Each bed had two monitoring stations recording air temperature, soil temperature, and soil moisture at four depths; where available, soil EC was logged, with data streamed continuously to the platform for real-time adjustments. To enhance oxygenation and moisture distribution, beds were manually turned 2–3 times per week to 0–20 cm, and layout plus buffer strips prevented surface runoff. Sampling was conducted on 26.06, 05.08, 20.08, 28.08, 11.09, and 19.09.2024 from fixed, geotagged points, collecting 20 cm cores. Samples were cooled at 4 ± 2 °C and extracted within 24 h. RDX, HMX, and TNT were quantified by HPLC following US EPA 8330B after acetonitrile extraction with ultrasonic aid and SPE cleanup.

Selected samples were confirmed by LC–MS/MS (MRM), and QA/QC followed accreditation procedures. There was no untreated control bed. To limit confounding we used fixed sampling points, controlled moisture near field capacity, prevented runoff, and maintained oxic conditions through frequent turning, as the aim was to test feasibility under field-simulated conditions typical of planned full-scale work. The timeline was: 09.06.2024—initial characterization and planning; 16.07.2024—compost addition and first biological application; 21.07.2024—start of

Fig. 1 The plots of the explosive contaminated soil with the irrigation drip system

Fig. 2 Monitoring units in the explosive contaminated soil plots

irrigation and continuous monitoring; sampling continued until 19.09.2024 under the guidance of Netzer Hasharon and the Ministry of Environmental Protection of Israel with appropriate safety procedures.

Statistical analysis

To quantify temporal changes in soil concentrations of the three explosive compounds (RDX, HMX, TNT) during bioremediation, we fitted generalized linear mixed models (GLMMs) with a Gamma distribution and log link. Gamma was chosen because concentrations are positive and strongly right-skewed (overall moment skewness, γ_1: RDX = 1.29, HMX = 1.57, TNT = 1.79), which also justifies the log link (Limpert et al., 2001). Time was modeled as a categorical factor with six levels (26.06.2024 [day 1], 05.08.2024 [day 40], 20.08.2024 [day 55], 28.08.2024 [day 63], 11.09.2024 [day 77], 19.09.2024 [day 85]); the first date served as the reference. To account for repeated sampling within locations, we included the sampling identifier as a random intercept. Models were estimated by maximum likelihood (Laplace approximation). Because the only predictor was the time factor, multicollinearity was not an issue. When the random-intercept variance converged to (near) zero (singular fit), the random effect was non-informative and inference reduced to the fixed-effects (time) component. Inference was two-sided with $\alpha = 0.05$. We report coefficient estimates ± SE and *p*-values. Marginal R^2 was used to measurements of explained variance.

For transparency and robustness, we complemented model-based inference with start–end comparisons (26.06.2024 vs. 19.09.2024) using the Wilcoxon rank-sum (Mann–Whitney U) test (Zuur et al., 2009). The *p*-values were computed exactly where possible, and by normal approximation with continuity correction when ties or sample size precluded exact calculation. All analyses were conducted in R

(R Core Team) using the packages lme4, lmerTest and e1071 (for skewness calculations).

Results

Over the course of the experiment, we observed differences in the concentrations of TNT, RDX, and HMX (Table 1). Our model-based approach revealed that, for RDX, there was no change to day 40 of experiment relative to baseline (Estimate = 0.046 ± 0.797, $p = 0.954$, Fig. 3), but concentrations were substantially lower after day 55 (− 11.699 ± 0.712, $p < 0.001$, Fig. 3), day 63 (− 7.485 ± 0.797, $p < 0.001$, Fig. 3), day 77 (− 6.799 ± 0.797, $p < 0.001$, Fig. 3), and day 85 (− 11.699 ± 0.745, $p < 0.001$, Fig. 3). R-square of the model was 0.95 (Fig. 3). For HMX, concentrations were higher in 40 day of experiment (8.448 ± 0.839, $p < 0.001$, Fig. 3) and day 85 (4.836 ± 0.785, $p < 0.001$, Fig. 3), no significant differences at days 55, 63, or 77. R-square of model = 0.9. For TNT, there was no difference after day 40 of experiment (Estimate = − 0.380 ± 1.064, $p = 0.721$, Fig. 3). After day 55, concentrations were zero (laboratory-reported as 0 mg kg^{-1}; see Table 1), and the GLMM indicated a very large reduction after 40 day of experiment (Estimate = − 11.70 ± 0.71, $p < 0.001$, Fig. 3). R-square of the model was 0.92. In all mixed models, the random-intercept variance was ~ 0 (singular fit), so inference rests on the fixed time effects.

As a direct start–end check (26 June 2024 vs. 19 September 2024), RDX showed a marginal decrease (Wilcoxon rank-sum: W = 12, $p = 0.057$, Fig. 4), TNT decreased significantly (W = 12, $p = 0.0319$, Fig. 4), and HMX differed significantly with higher end-of-study values (W = 0, $p = 0.0436$, Fig. 4). These pairwise results align with the model-based findings, indicating substantial reductions for RDX (from day 55 onward) and TNT, and a distinct trajectory for HMX with increases at days 40 and 85.

To complement the modeling results and provide a direct comparison between the initial and final stages of the bioremediation process, we examined changes in compound concentrations between the first (26 June 2024) and last (19 September 2024) sampling dates. For RDX, the analysis indicated a marginally significant reduction in concentration (W = 12, $p = 0.050$, Fig. 4). A statistically significant decrease was observed for TNT (W = 12, $p = 0.0319$, Fig. 4), while HMX exhibited a significant change as well, although with an increase in concentration at the end of the experiment (W = 0.01, $p = 0.0436$, Fig. 4). These pairwise comparisons support the overall degradation patterns observed in the full model and underscore the compound-specific responses to the bioremediation treatment.

Discussion

This pilot study indicates that bioremediation under field-simulated conditions can rapidly and substantially reduce soil concentrations of selected military explosives. Model-based analyses (GLMMs) showed large declines in RDX and TNT from day 55 onward, with values remaining at or near non-detectable levels in subsequent samples, whereas HMX followed a non-monotonic trajectory with increases at day 40 and

Table 1 Mean concentrations (± standard deviation, SD) of RDX, HMX and TNT (mg kg^{-1}) in soil samples collected at six time points during the bioremediation experiment (from 26 June to 19 September 2024). Additional measurements of soil physicochemical parameters (electrical conductivity, chloride, sodium, and total nitrogen) are provided where available

Date of sample (no of experiment days)	mean ± SD of RDX (mg kg^{-1})	mean ± SD of HMX (mg kg^{-1})	mean ± SD of TNT(mg kg^{-1})	Electric conductivity mS/cm	Chloride mS/cm	Sodium (meq/L)	General_ nitrogen (meq/L)
26.06 (1)—start	120.46 ± 34.54	0	144.73 ± 36.95	110.6	2044.9	58.75	1925.7
05.08 (40)	126.1 ± 33.98	4.66 ± 1.06	98.95 ± 102.0	–	–	–	2396.36
20.08 (55)	0.08 ± 0.11	0	0	–	–	–	2214.53
28.08 (63)	0.06 ± 0.11	0	0	–	–	–	–
11.09 (77)	0.13 ± 0.13	0	0	–	–	–	–
19.09 (85)—end	0	0.12 ± 0.25	0	965.6	8.24	550.2	–

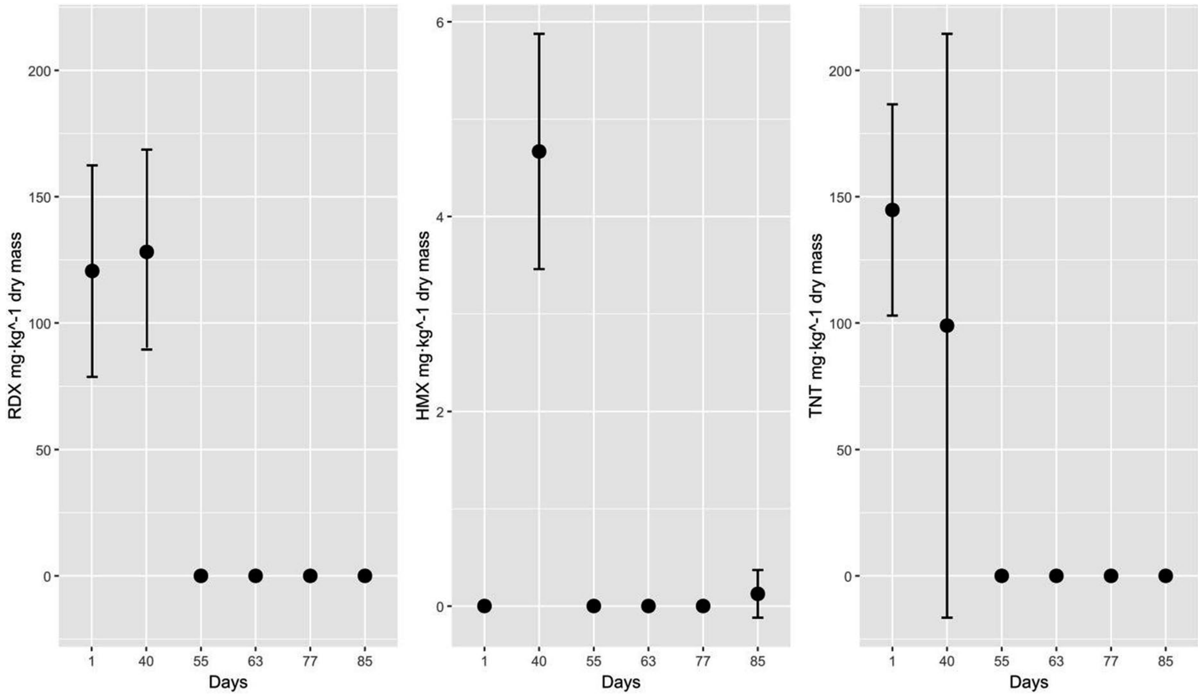

Fig. 3 Mean soil concentrations (±95% CI) of RDX, HMX, and TNT over the bioremediation period. Y-axis: Concentration (mg kg^{-1}, dry mass). Number of X-axis represents sampling day and calendar date (Day 1 = 26 Jun 2024; Day 40 = 05 Aug 2024; Day 55 = 20 Aug 2024; Day 63 = 28 Aug 2024; Day 77 = 11 Sep 2024; Day 85 = 19 Sep 2024)

day 85. Start–end comparisons (26 June vs. 19 September) were consistent with these patterns, showing decreases for RDX (marginal) and TNT and higher end-of-study values for HMX. While these reductions compare favorably with other study—where complete degradation often requires longer operation under optimal conditions (Bhushan et al. 2003; Singh et al. 2012; Bhatnagar and Kumari 2013)—our inference is limited to the monitored period and generaly small analytical resolution.

A more cautious interpretation is that the rapid declines most likely reflect the managed process conditions we imposed—organic-matter addition (compost) and balanced nutrients to supply substrates and electron donors/acceptors, drip irrigation that kept moisture near field capacity with intermittent aeration, and frequent shallow turning that improved oxygen diffusion and moisture distribution. Such conditions are well known to stimulate indigenous microbial communities capable of transforming nitroaromatic and nitramine explosives (Lewis et al. 2004; Esteve-Núñez et al. 2001). Because we did not directly quantify community composition or enzymatic pathways in this pilot, this mechanistic link remains speculation rather than measured.

Process conditions likely supported the observed kinetics. Compost and a balanced N:P:K supplement provided organic substrates and nutrients; drip irrigation maintained moisture near field capacity with intermittent aeration; and shallow, frequent turning improved oxygenation and moisture distribution. The sandy-loam texture and near-neutral pH may also have facilitated oxygen diffusion and colonization (Srivastava et al. 2022). Together, these factors created a favorable environment for biotransformation.

The bioremediation effect of HMX can be interpreted as potenctialy contradictory ecause its concentration increased at certain time points (notably around day 40 and again at day 85). Howeaver this tendency is consist with its known lower solubility and bioavailability relative to RDX and TNT (Lotufo et al. 2009). Plausible contributing processes include early-phase mobilization from microzones as the water regime stabilized, desorption/

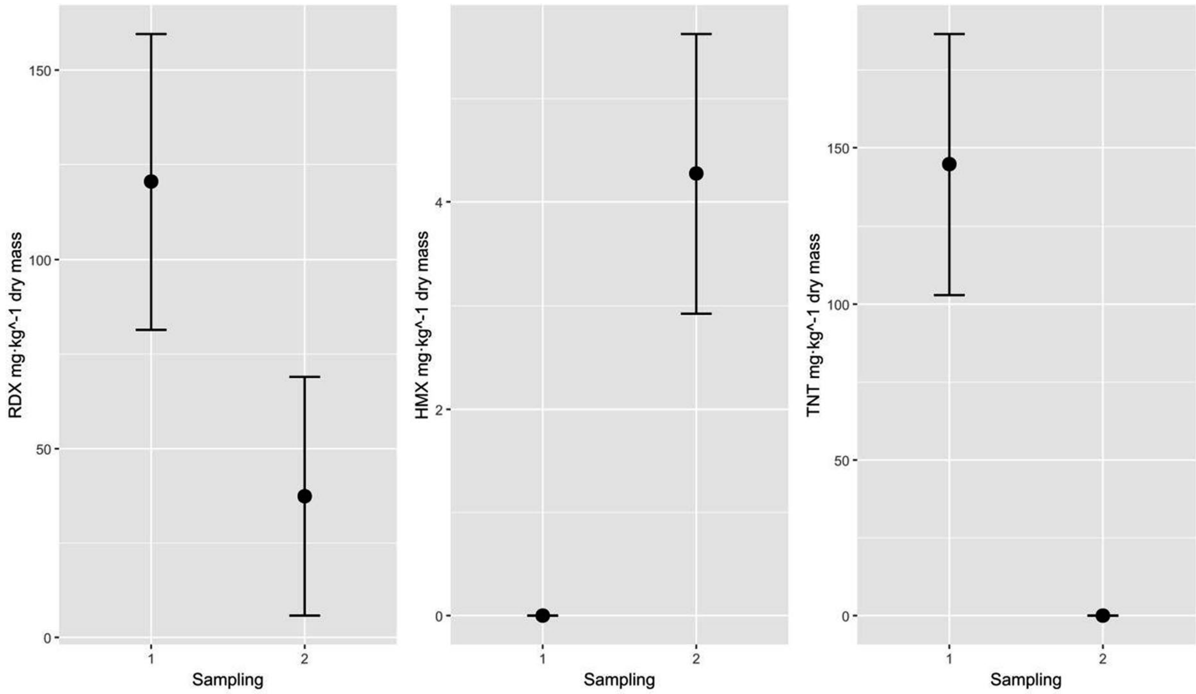

Fig. 4 Changes in soil concentrations of RDX, HMX, and TNT between the initial (1 = 26 June 2024) and final (2 = 19 September 2024) sampling dates of the bioremediation experiment. Points represent mean concentrations, and error bars indicate 95% confidence intervals

dissolution of crystalline particles, and slower microbial adaptation to HMX substrates. Given the residual seen at day 85, HMX may require extended treatment time or targeted process adjustments (e.g., staged redox regimes, co-substrate management or bioavailability enhancement) in future implementations.

These findings are practically relevant in Israel, where military sites can be close to urban and agricultural areas. The approach tested here may be applicable at munitions factories, firing ranges, former training grounds, ammunition storage, border zones, and other legacy sites. More broadly, bioremediation can be deployed in buffer zones protecting groundwater and in semi-arid regions where moisture control is feasible and critical.

The limitation of the study shod be also noted. First, the pilot lacked an untreated control, so we cannot fully separate biodegradation from photolysis or physical migration (e.g., under drip irrigation). Second, homogenization and bed management reduce variance but under-represent field heterogeneity and hot spots. Third, we did not employ lysimeters. Hence, no mass balance was performed and downward transport cannot be excluded. Fourth, microbial data were limited to CFU counts, with no taxonomic or functional profiling to link pathways to organisms. Finally, we did not analyze transformation products (e.g., MNX/DNX/TNX for RDX; ADNTs/DANTs for TNT; HMX intermediates), so the persistence and potential toxicity of by-products are unknown. These constraints are typical of feasibility pilots but this does not change the fact that our experimental approach showed a significant reduction in explosive residues.

Thus future study should (i) include proper controls or staggered starts; (ii) extend runtime to capture long-tail kinetics, especially for HMX; (iii) monitor leachate (lysimeters) for mass balance and groundwater protection; (iv) measure key transformation products by LC–MS/MS alongside parents; (v) characterize the microbiome (metagenomics/functional genes) to connect process conditions with pathways; and (vi) explicitly compare free versus immobilized bacterial systems and bioavailability-management options for HMX.

In summary, under field-simulated conditions the treatment produced rapid and durable reductions in RDX and TNT from day 55 onward, while HMX exhibited a more complex trajectory and will likely need extended or tailored remediation. These quantitative pilot results, together with the identified limitations, provide a foundation for designing and de-risking the forthcoming full-scale remediation while ensuring environmental safety.

Author contribution Conceptualization—ABL, RY; Methodology—ABL, JZK; Software JZK; Validation ABL, RY; Formal analysis—JZK; Investigation—ABL; Data curation—RY, JZK; Writing original draft—RY, JZK, ABL; Writing review and editing—RY, JZK, ABL; Visualization—JZK; Supervision—ABL, RY.

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

Declarations

Conflict of interests The authors declare no competing interests.

Ethical guidelines According to the guidelines supplied by, and supervision of, the Netzer Hasharon Company, Ministry of Environmental Protection of the State of Israel.

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