



A novel strategy for high-throughput sample collection, analysis and visualization of explosives' concentrations for contaminated areas

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Abstract

The use of explosives has led to a widespread distribution of 2,4,6-trinitrotoluene (TNT) and its by- and degradation products in the soil on former production and testing sites. The investigation of those large contaminated sites is so far based on a few selected soil samples, due to high costs of conventional HPLC and GC analysis, although huge differences in concentrations can already be found in small areas and different collection depths. We introduce a novel high-throughput screening system for those areas, which combines a smartphone-based collection of GPS data and soil characteristics with a fast MALDI-TOF MS quantification of explosives in soil sample extracts and finally a heatmap visualization of the explosives' spread in soil and an analysis of correlation between concentrations and soil characteristics. The analysis of a 400 m² area presented an extensive contamination with TNT and lower concentrations of the degradation and by-products aminodinitrotoluenes (ADNT) and dinitrotoluenes (DNT) next to a former production facility for TNT. The contamination decreased in deeper soil levels and depended on the soil type. Pure humus samples showed significantly lower contaminations compared to sand and humus/sand mixtures, which is likely to be caused by an increased binding potential of the humic material. No correlation was found between the vegetation and the concentration of explosives. Since the results were obtained and visualized within several hours, the MALDI-TOF MS based comprehensive screening and heatmap analysis might be valuable for a fast and high-throughput characterization of contaminated areas.

Keywords 2,4,6-trinitrotoluene · Explosives · Heatmap · MALDI-TOF MS · Risk analysis · Smartphone

Introduction

Explosives are used for several applications as breaking rocks in mining, demolition of obsolete buildings and mainly weaponry. Warfare activities are one of the primary threats for soil and water resources, as they affect physical, chemical and biological properties of the ecosystem (Certini et al. 2013; Pereira et al. 2020). The production, testing and improper disposal of explosives along with unexploded

ordnance led to large areas that are contaminated with explosives (Pichtel 2012; Celin et al. 2020). 2,4,6-trinitrotoluene (TNT) was the most used explosive in both World War I and World War II, resulting in the introduction of nitroaromatic compounds in soil at former production sites (Weiss et al. 2004). Besides, at least 2% and up to 42% of TNT remain as residuals on the soil surface after a high- and low-order detonation of a TNT-filled shell which increases the number of affected areas, especially on former testing ranges (Kaldreis et al. 2011).

Biotic and abiotic transformation as well as binding processes influence the fate of TNT in soil (Thiele et al. 2002). Furthermore, TNT is slightly water soluble (Juhász and Naidu 2007). Thus, rain water can transport it and its degradation products into groundwater, rivers and sediments. From the aqueous phase it may form crystalline solids or be adsorbed by ground particles (Habineza et al. 2017). Additionally, natural circumstances as pH, humidity, soil composition, the microbial community and vegetation can influence the spread of explosives in the ground (Singh

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et al. 2008; Limane et al. 2011; Via and Zinnert 2016; Celin et al. 2020). TNT and its derivatives exhibit several harmful effects. The exposure of workers at production facilities to high levels of TNT resulted in disorders of blood and liver, affected the immune system and led to allergic reaction and cataracts (Bolt et al. 2006; Sabbioni et al. 2007). Studies in animals gave reason to classify TNT as possible human carcinogen (Bolt et al. 2006). Increased mortality and reduced reproduction were also shown for soil organisms which can cause a loss of natural biome (Schäfer and Achazi 1999; Pichtel 2012; Nawała et al. 2020). Compared to the cyclic nitramines hexogen (RDX) and octogen (HMX), that were used frequently for military purposes as well, TNT exhibits the highest toxicity (Chatterjee et al. 2017). Its metabolites (e.g. 2-ADNT, 4-ADNT) may be even more harmful (Maser and Strehse 2020). Consequently, heavily TNT-contaminated sites pose serious health risks and environmental monitoring methods as well as remediation strategies are needed (Wasilewski and Gębicki 2021). Low contaminated ranges on the other hand may be naturally detoxified through biotic and abiotic degradation or irreversible binding processes and do not need complex remediation measures (Bečanová et al. 2009).

The biggest challenge for the environmental monitoring of contaminated sites is the requirement to analyze wide areas to track the distribution of the toxic compounds and their degradation products, leading to a large number of samples (Caygill et al. 2012). Routinely applied analytical procedures to identify the extent of contamination in environmental samples usually involve liquid (LC) or gas chromatography (GC) eventually in combination with mass spectrometry (MS). Further classical instruments for the detection of explosives include ion mobility spectrometry (IMS), Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR) or other spectroscopic methods using spectrophotometry, fluorescence or (chemi)luminescence (Caygill et al. 2012; Klapac et al. 2020). Although those methods show a high sensitivity, the analysis is also associated with high costs, time issues and call for specialist training of the user (Caygill et al. 2012; Wasilewski and Gębicki 2021). Hence, improvements of existing analytical techniques or new high-throughput detection methods are required to enable a faster, less expensive and simpler detection of explosives to give a comprehensive picture of the contamination in large areas (Caygill et al. 2012; Panagos et al. 2013).

Current trends in explosive detection techniques explore the reduction of size and costs of conventional methods and introduce different sensor techniques (Caygill et al. 2012; Wasilewski and Gębicki 2021). Furthermore, the use of field portable instruments in environmental site assessment has emerged to evaluate the spatial and temporal distribution of contaminants (Gałuszka et al. 2015). Portable

X-ray fluorescence (XRF) spectrometers or portable IMS is often applied for the detection of environmental pollutants. Whereas XRF instruments are mainly used for the detection of heavy metal contaminations, portable IMS is applied for the on-site analysis of warfare agents, explosives and toxic industrial compounds in environmental samples (Gałuszka et al. 2015). Previously, a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) based quantification of nitroaromatic explosives in contaminated soil samples was presented (Kober et al. 2019), that comprises a fast sample preparation, simple operation and quick analysis time. Other future prospects of environmental monitoring involve smartphones that may be coupled to detection devices or assist the data collection on-site (Wasilewski and Gębicki 2021).

Several advances have been made in the analysis of explosives in environmental samples in the last years. However, up to date there is no comprehensive system for the high-throughput sample collection, analysis and evaluation of large areas contaminated with explosives. Hence, the aims of this work were the development of an analysis platform comprising a smartphone assisted collection of sample information on-site, a fast sample preparation and simple MALDI-TOF MS quantification of TNT and its degradation products and finally a visualization of the explosives spread using two and three dimensional heatmaps as well as analysis of correlation between soil characteristics and explosives' concentrations. The system was tested on a former production site for TNT and artillery shells in Brandenburg (Germany). The exact location is not given to protect the area from unauthorized localization.

Materials and methods

Reagents/chemicals

All used solvents (acetonitrile, water) were HPLC or MS grade and purchased from Carl Roth (Karlsruhe, Germany). Further chemicals were HPLC or analytical grade (p.a.). 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) were provided by our cooperating company (terracon GmbH). 1,5-diaminonaphthalene (DAN), mefenamic acid (MEA), chlorogenic acid (CGA) and the standard explosives 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 1,3,5-trinitrobenzene (TNB), 1,3-dinitrobenzene (DNB), 2-nitrotoluene (NT) and 5-chloro-2,4-dinitrotoluene (CDNT) were obtained from Sigma Aldrich (St Louis, MO, USA). Citric acid was acquired from Merck (Darmstadt, Germany). A standard stock solution was prepared as a mixture of all nitroaromatic explosives with a concentration of 50 ng/μL.

Soil sample collection and preparation

Soil sample collection was carried out on a former, highly polluted production site for TNT and artillery shells, accompanied by our cooperating soil sampling company (BLM Geotest GmbH). 105 samples were taken from three different depths (30 cm, 60 cm, 90 cm) at 35 different spots that were evenly distributed within an area of approximately 400 m² and labeled for the subsequent analysis. About 5 g soil was collected for each sample and the location determined with the global positioning system (GPS) receiver of the smartphone as well as a highly accurate Global Navigation Satellite System (GNSS) receiver with a high-performance antenna. A self-programmed Android application named “Localyzer” was used to collect soil sample characteristics on-site. Features as soil type (humus, sand, loam or soil mixtures), landscape (vegetation), humidity and depth were recorded. Furthermore, map services based on OpenStreetMap were integrated for a visualization of already sampled spots (Haklay and Weber 2008).

In the laboratory a simplified extraction procedure was performed. Briefly, 2 g of soil of each sample were mixed with 4 mL of acetonitrile and shaken for one hour (Intelli-Mixer RM-2; ELMI Ltd., Riga, Latvia). Following this, 1 mL of the solution was transferred into a reaction tube and centrifuged at 10,000g for 5 min. The supernatant was stored at 4 °C until further use.

MALDI-TOF MS analysis of soil samples

Soil sample extracts were analyzed using MALDI-TOF MS mass spectrometry, which was shown to give comparable results to conventional LC–MS/MS analysis (Kober et al. 2019). Explosive standard mixtures and soil sample extracts were mixed with the matrix (DAN 20 mg/mL in ACN/water 80/20) and the internal standard (CDNT 25 ng/μL). A total volume of 1 μL of each mixture was applied onto the target in triplicates and air-dried. Calibration was carried out within a concentration range of 0–50 ng/μL. Soil extracts were used directly or diluted with ACN prior to mixture with the matrix and deposition onto the target.

Mass calibration of the MALDI-TOF mass spectrometer was achieved with a mixture of matrix, CDNT, mefenamic acid, citric acid and chlorogenic acid. After deposition of the solutions and mass calibration of the mass spectrometer all spots on the target were measured automatically and spectra exported as .mzXML files.

Experiments were carried out on an Axima Confidence mass spectrometer (Shimadzu Biotech, Manchester, UK) operating in the negative, high-resolution reflectron mode. The maximal laser repetition rate was 50 Hz and the laser firing power filter set to 20. For each spectrum 250 profiles in a mass range of 120–300 Da were recorded, accumulated

and finally averaged. Peak processing was performed using the Launchpad™ v. 2.9 software (Shimadzu Biotech).

Data processing and visualization

The on-site collected sample information was stored in a database on the smartphone and later exported into a text file with a message-digest algorithm 5 (MD5) hash. The text file was copied to a PC, the integrity was checked with an MD5 hash and the information was written into a database. The database was used to transfer the sample information to the MALDI-TOF mass spectrometer and to assign all sample information to the deposited extracts correctly. The generated mass spectra were saved as .mzXML files on a server and the database complemented with the file locations. The exported mass spectra were analyzed using the statistical computing language R within RStudio (<https://www.r-project.org/>; <https://www.rstudio.com/>). Prior to data evaluation, MALDI-TOF mass spectra were aligned and a total ion current (TIC) calibration applied. Specific explosives' signals of radical anions $[M]^{•-}$ and fragment ions $[M-O]^{-}$ in the calibration spectra were identified and normalized to the ion abundance of the internal standard (m/z 216). The normalized ion abundances were plotted against the concentrations to create calibration curves for the signal of each explosive. A classical linear least square regression (CLS) was performed to calculate the concentration of explosives in the samples. The final concentration of each explosive was determined after subtraction of the humidity, having regard to the dilution factor and as average of the ion abundances of the radical and fragment anions. All features (name, GPS data, depth, vegetation, soil type and explosives' concentrations) of the samples were summarized in a table and analyzed in detail (Table S2 in supplementary information).

Interpretation of data was simplified through the use of RStudio and implementation of an interactive R Shiny application. The main features of the application are the import of MALDI-TOF MS spectra, the creation of calibration curves and the calculation of explosives' concentrations in samples as well as the download of results (figures, tables). Furthermore, samples can be selected and the contamination visualized with a color-coded map and a 3D-model. The 2D interpolation of the surface scattered data (x,y) was achieved with the Akima algorithm (Akima 1978). To visualize the contamination in the ground an additional interpolation in z-direction was implemented using the function “intern” of Octave (<https://www.gnu.org/software/octave/>). For that purpose, the surface-interpolation data of R was gridded and sent to Octave. The resulting matrix was returned to R and visualized in a 3D model.

The following R packages were imported for the data analysis and visualization: abind, akima, DBI, DT, FactoMineR, ggplot2, Hmisc, htmltools, leaflet, MALDIquant,



MALDIquantForeign, scatterplot3d, shiny, shinyFiles, sp, raster, Rcpp, RcppOctave, registry, reshape2, rgeos, RMySQL (<https://cran.r-project.org/>) and webshot (<https://github.com/>).

Results and discussion

The developed system provides a comprehensive analysis platform from sample collection to analysis and evaluation of the results (Fig. 1). The sample collection is supported by the Android application “Localyzer”, that can not only determine the current GPS coordinates for each sample but also store further information that are added on-site.

In the laboratory, the information of the sample collection is transferred to the database and combined with the analysis results that were obtained after extraction of explosives from the soil samples and the MALDI-TOF MS measurements. Finally, the R Shiny application visualizes all results and allows the quantification of explosives in the soil samples, heatmap illustrations of the explosives’ spread and creation of boxplots to correlate sample characteristics and the contamination.

Soil data collection and documentation

Soil sample collection and documentation of soil properties were simplified with the “Localyzer” application - programmed with the goals of user-friendliness and intuitive

operation (added as Localyzer.zip in supplementary information). It contains functions for the registration and login of multiple users (Fig. 2a) with the first registered user being appointed as administrator and solely authorized to export and delete the database entries. The starting page gives an overview of samples already collected and the possibility to add further samples (Fig. 2b). Furthermore, the menu contains functions for logout, visualization of locations on a map as well as map and database tools. If a sample is added, the properties soil type, vegetation, humidity, landscape, pH, collecting depth, weight and further information (e.g. presence of metal ions) can be documented (Fig. 2c). The position data can be acquired with the integrated GPS chip of the phone or added manually. All database entries are visible on a map (© OpenStreetMap) for better orientation and planning of further sample collections (Fig. 2d). Map areas may be downloaded before the collection for offline usage (Fig. 2e). Finally, the database can be exported and transferred to a PC.

The pilot use of the application on a former TNT production site revealed the easy and fast handling for the documentation of the 105 soil samples. At the same time, while one person was collecting the soil sample, the second person added all sample information and GPS data in the “Localyzer” application. The collection of accurate position coordinates on-site is a prerequisite for the creation of spatial contaminant distribution maps. For this purpose, GPS is used frequently which consists of 28 satellites in orbit allowing positioning everywhere on earth and easy availability

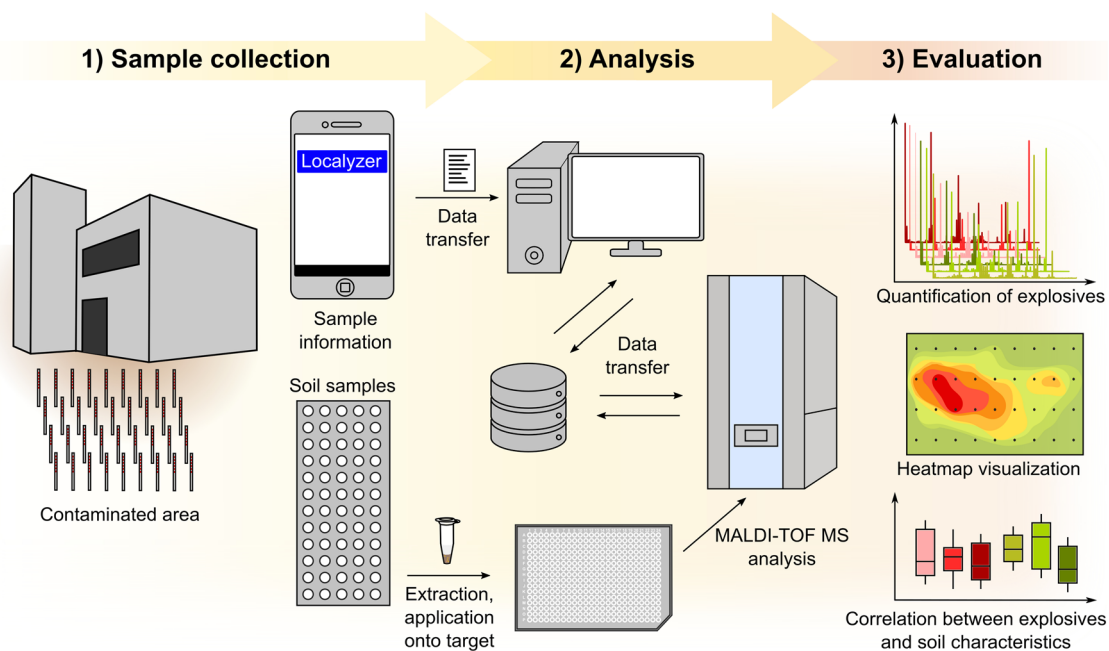


Fig. 1 Illustration of the sample collection on contaminated sites, laboratory analysis using MALDI-TOF MS and automated visualization of the results



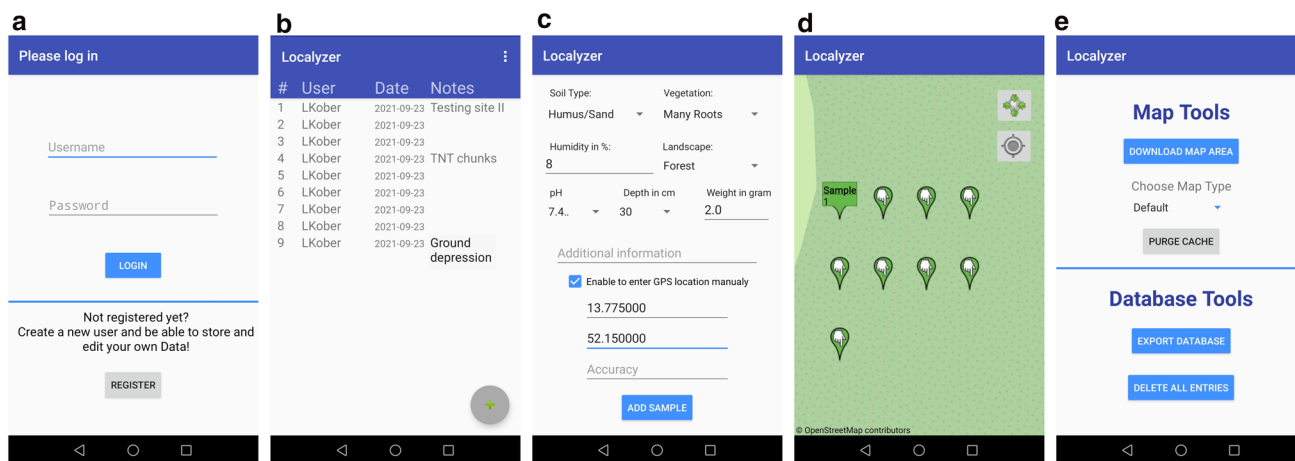


Fig. 2 Android application "Localizer" to record GPS data and soil sample properties. **a** login screen; **b** overview of samples; **c** sample properties; **d** map of collected samples; **e** map and database tools

because of integration in every smartphone (Grewal et al. 2001; Korpilo et al. 2017). Smartphone GPS tracking offers several benefits as cost effectiveness, high accessibility and user-friendliness (Korpilo et al. 2017). However, a comparison of the smartphone integrated GPS chip and the GNSS receiver showed the low accuracy of the smartphone GPS measures. In open field the accuracy was between three and four meters, while it increased up to six or exceptionally up to 17 m near houses or in dense forests. Contrarily, the GNSS device had an accuracy of under one meter. The low accuracy of the smartphone GPS chip is known and was shown before (Mok et al. 2012; Bauer 2013). Hence, a GNSS receiver is preferred if a highly accurate localization is needed or in areas with limited smartphone GPS accuracy, respectively.

MALDI-TOF MS analysis and quantification

The sample information of the Android application was transferred to a database and combined with the results of the MALDI-TOF MS analysis of the soil sample extracts. Prior to the MALDI-TOF MS analysis of the collected soil samples, mixtures of explosive standards within the concentration range of 0 to 50 ng/ μ L were analyzed. Spectra of the explosive standards showed explosive specific radical ($[M]^{\bullet-}$) and fragment ions ($[M-O]^-$) of TNT (m/z 227, m/z 211), ADNT (m/z 197, m/z 181), DNT (m/z 182, m/z 166), DNB (m/z 168, m/z 152), whereas only the radical anions of TNB (m/z 213) and NT (m/z 137) were detected (Fig. 3a). The CLS regression was carried out only for TNT, ADNT and DNT radical and fragment ions, because the other explosives were not found in the analyzed soil samples. For that purpose, the ion abundances of the explosive related ions were normalized to the ion abundance of the internal standard (CDNT $[M]^{\bullet-}$, m/z 216) and plotted against

the concentration (Fig. 3b). Detailed regression information can be found in electronic supplementary material S1. Some explosive related signals in the calibration exhibited an increased standard deviation which may be a result of an improper mixture of standard solutions and matrix leading to an increased heterogeneity of the analyte-matrix surface (Su et al. 2005). However, the CLS regression resulted in a linear correlation between concentrations and ion abundances of selected m/z and was therefore suited to calculate explosives' concentrations in soil sample extracts.

The soil samples were mainly contaminated with TNT, whereas mostly only low contaminations with ADNT and DNT were found. TNB, DNB or NT were not detected. Light and heavy contaminations can be already distinguished when looking at the MALDI-TOF MS spectra of the samples, e. g. by showing low (sample 98) or high (sample 72) ion abundances of the TNT related ions m/z 211 and m/z 227 (Fig. 3c). Highly contaminated soil sample extracts often had a yellow or orange color, indicating a necessary dilution of the extract before the MALDI-TOF MS analysis. Nevertheless, some calculated sample concentrations were outside the linear range and a further dilution had to be analyzed subsequently for a correct quantification. Due to the speed of the method the results of the second run were obtained within half an hour.

Mass spectrometry techniques without a prior separation method as chromatography always lack some point of identification, e. g. the distinction of isomers (Klapec et al. 2020). On the other hand, the MALDI-TOF MS offers several advantages, as it is fast, cheap and tolerant towards sample impurities. In combination with a shortened extraction time, which was previously argued to show only a small loss of sensitivity (Kober et al. 2018), the results of a large sample collection were obtained within a few hours. Furthermore, it combines the benefits of an on-site and off-site analysis.

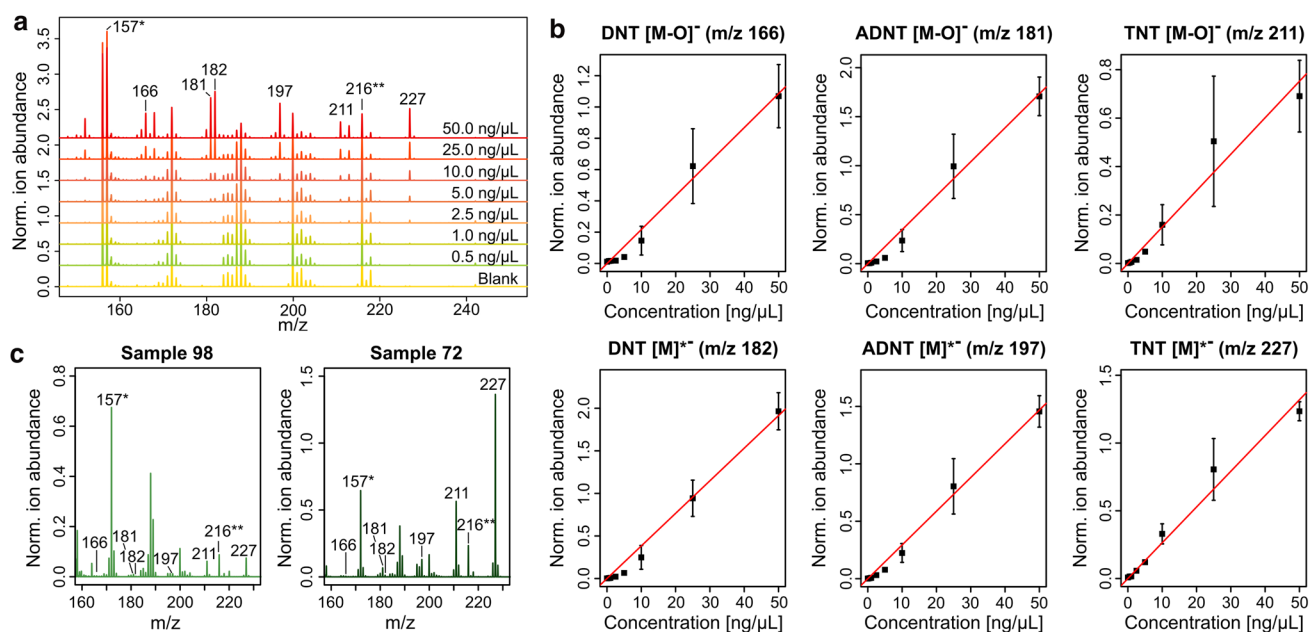


Fig. 3 MALDI-TOF MS quantification of explosives **a** averaged spectra of calibration mixtures; **b** regression lines for DNT, ADNT and TNT anions; **c** spectra of selected soil samples showing low (sample 98) and high (sample 72) TNT contaminations

Normally, an off-site analysis is associated with higher costs and a poor understanding of the pollution distribution, whereas the on-site analysis using field portable instruments enables the analysis of higher sample numbers and results in a higher information value of the data set (Gałuszka et al. 2015). However, field portable instruments may be affected by environmental factors and experienced field analysts are required (Gałuszka et al. 2015). The MALDI-TOF MS method is not only suited for the high-throughput analysis of samples, but is conducted in a controlled laboratory environment and can be easily performed and evaluated in combination with the presented system. Thus, the MALDI-TOF MS-based detection of explosives is of particular interest for the investigation of large areas to identify contamination hotspots. Individual sample spots might be sampled subsequently for in depth analysis or quality assurance (Caygill et al. 2012; Gałuszka et al. 2015).

Visualization of the explosives' spread

The final concentration of the explosives in soil samples in mg/kg were calculated with the averaged concentrations of radical and fragment ions in the spectra (ng/μL), the dilution factor, the used amount of soil and solvent in the extraction as well as the measured humidity, and recorded in the database. The samples were contaminated with TNT, ADNT and DNT which are among the most frequently found explosive related compounds in former military areas (Kalderis et al. 2011). The concentration range was very broad. Eight samples were above the industrial soil screening level (SSL) for

TNT of 96 mg/kg, which was defined by the EPA (<https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables>, May 2021). The suggested residential SSL of 21 mg/kg was exceeded by 32 soil samples.

To visualize the spread of explosives in the testing area color coded maps were built. Two-dimensional heatmaps show the contamination with TNT, ADNT and DNT in the different collection depths (Fig. 4). The highest detected concentration of TNT was 16,321 mg/kg soil and found in the north-west of the range next to a former production facility for grenades. Another seven samples exhibited high

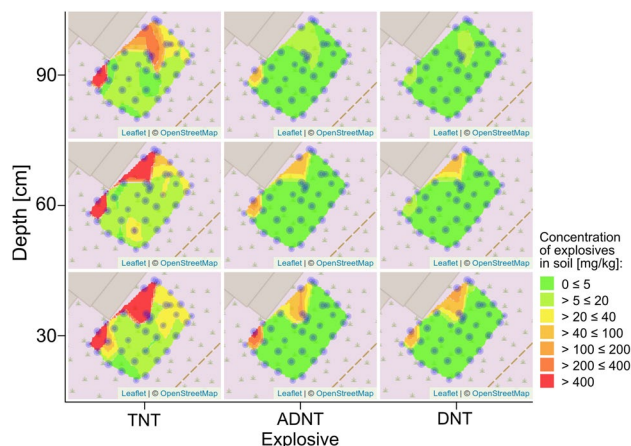


Fig. 4 Heatmap of the contamination with TNT, ADNT and DNT in the three different collection depths. Sample spots are indicated in blue

TNT concentrations between 323 and 4,466 mg/kg soil. The highest explosives' concentrations were found in the upper soil layer (30 cm), whereas they shrink in deeper soil layers. Additionally, the concentrations of TNT, ADNT and DNT were elevated next to the building and decrease with larger distance from the building. However, in the north of the test range and within the deepest soil layer (90 cm) a distinct contaminant plume is visible that spreads into the center of the area.

The high contamination next to the production facility is probably related to the spill of TNT through improper handling and disposal techniques (Pennington and Brannon 2002). The presence of DNT and ADNT can be ascribed to impurities during manufacture of TNT as well as biotic and abiotic transformation processes in soil (Pichtel 2012; Gumuscu et al. 2015). The formation of the contaminant plume might be a result from rainwater transport over several decades that transported TNT into deeper soil layers and the center of the investigated area. Anaerobic and aerobic reduction reactions in the different soil layers assist in the formation of TNT derivatives as ADNT and DNT that leach continuously into the surrounding soil and pose similar threats to the environment (Kalderis et al. 2011; Chatterjee et al. 2017). However, high contaminations can be mostly found within a 20 m radius of the building and within the upper soil layer, showing a slow spread and degradation. This correlates with previous findings and can be explained by the stability and slow dissolution rate of solid explosives (Pennington and Brannon 2002). Additionally, types of residues from explosives and their distribution within a contaminated area depend highly on environmental factors like topography, soil condition and composition (Chatterjee et al. 2017).

To enable a three-dimensional visualization of the contamination in the sampling area, a further linear interpolation between the collection depths was implemented. The body can be adjusted regarding to collection depth and angle of view (north-west, NW or south-east, SE). GPS data (longitude, latitude) can be selected to view a specific area (Fig. 5). However, the GPS data of the testing area were removed in Fig. 5 to protect the investigated area from unauthorized localization. The 3D-model illustrates the decrease of TNT contamination in deeper soil levels from the hotspots on the surface (Fig. 5a). The identification of pollution hotspots can help to predict the risk of remobilization of pollutants (Elznicová et al. 2019). Heatmaps simplify the identification as high contaminations become visible with a glance.

The primary migration process for nonvolatile explosives is dissolution into water (Lynch et al. 2002). Thus, rainwater has probably attributed to the slow transport of TNT into the soil and surroundings and also initiated adsorption or transformation processes (Juhász and Naidu 2007). In the south-west of the testing site a subarea with a very high contamination of over 400 mg TNT/kg soil within all three soil

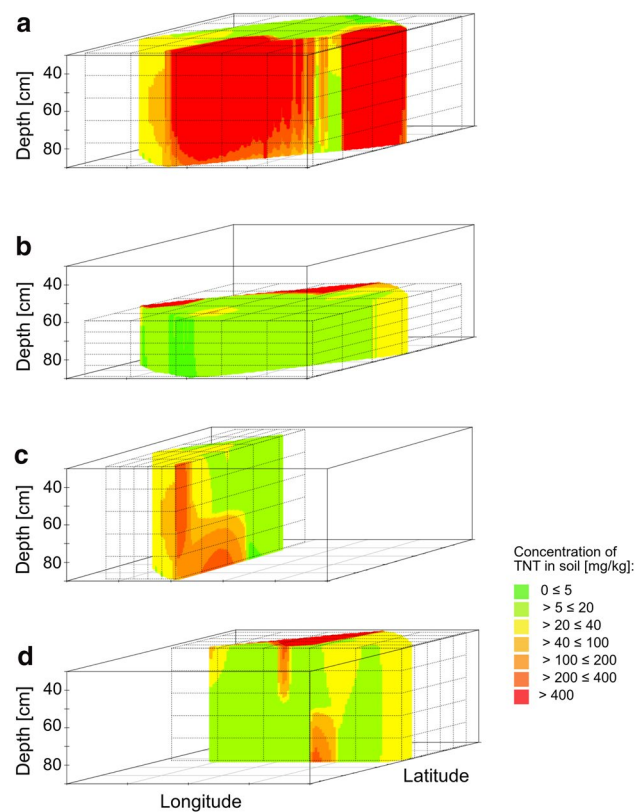


Fig. 5 3D models of selected parts of the sampling area that visualize the TNT contamination from different angles of view. **a** whole area (from NW); **b** 60–90 cm depth (from SE); **c** eastern part (from NW); **d** northern part (from SE)

levels was found (Fig. 5a). A detailed analysis of the area south-west of the building in even deeper soil layers might be of interest to see how far the contamination spreads there. However, some spots in the middle or deep soil layer of the testing range were found which exhibited a high contamination, whereas the contamination in the upper soil layer was lower. Those spots can be visualized by selecting specific depths and adjustment of the latitude and longitude, respectively (Fig. 5b–d). They might be a result of small TNT chunks that were buried in deeper soil layers by accident or on purpose and show a slow dissolution into the surrounding soil. Conspicuousities like this would not be detectable with the standard grab sampling method, where a large amount of soil is collected for each sampling spot, mixed and used for the sample analysis. Hence, the described method can support the identification of hotspots and give important insights into the paths of distribution of nitroaromatic explosives in soil.

Influence of environmental characteristics

To get further insight into the correlation between soil properties and detected explosives' concentrations boxplots were created (Fig. 6). Collection depth and soil type were

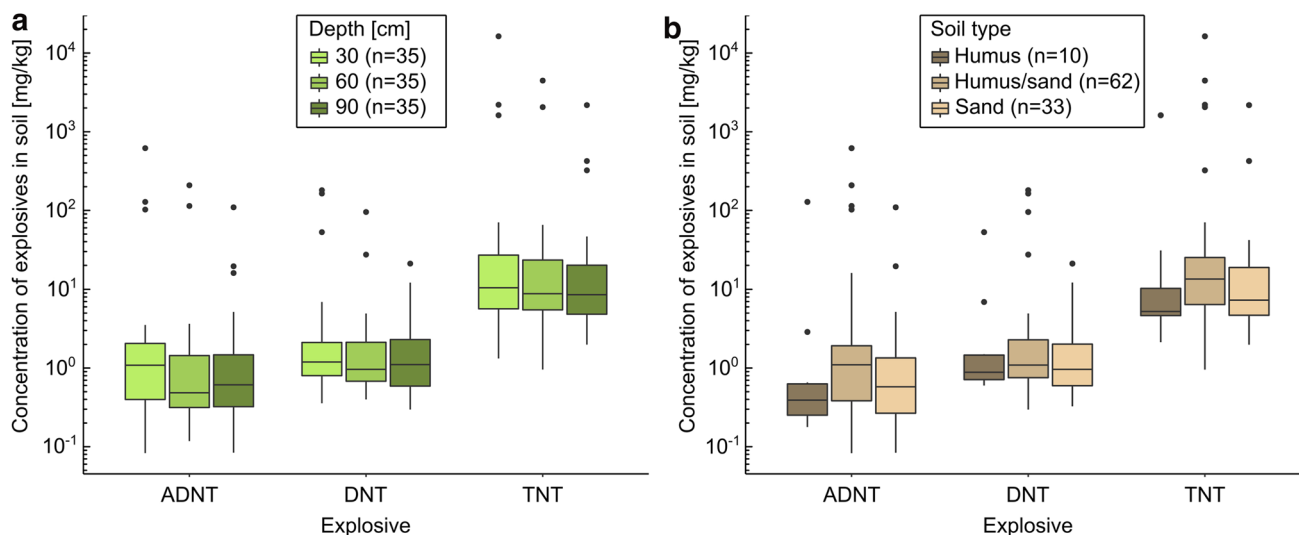


Fig. 6 Correlation of sample properties and detected ADNT, DNT and TNT concentrations in the testing area. **a** different collection depths; **b** different soil types

analyzed in detail. The surface layer (30 cm) exhibited the highest contamination whereas the deeper layers showed slightly lower values (Fig. 6a). TNT concentrations were at least one magnitude higher than ADNT or DNT concentrations which were both on similar levels. Highly contaminated samples can be seen as single dots above the boxplots. A similar pattern of those outliers can be seen above ADNT and TNT boxplots, which shows that high TNT and ADNT contamination in the samples were correlated. ADNT and DNT are decomposition products of TNT. Hence, higher amounts of TNT result in more biotic and abiotic reduction reactions and the transformation into DNT and ADNT (Juhász and Naidu 2007).

Apart from the collection depth the soil type influenced the detected contamination with explosives. Only few samples ($n=10$) were characterized as pure humus and localized in the upper soil layers (30 and 60 cm), whereas in the deepest layer no humus was found at all. About one third ($n=33$) was assigned as sand and the majority of samples described as humus/sand mixtures. Those mixtures exhibited a wide span in explosives' contamination (Fig. 6b). The highest concentrations of explosives were found in sand and humus sand mixtures. With the exception of one humus sample which was highly contaminated ($c_{\text{TNT}} = 1,618$ mg/kg, $c_{\text{ADNT}} = 128$ mg/kg, $c_{\text{DNT}} = 53$ mg/kg) the detected explosives' concentrations in humus samples were considerably lower. It is supposed, that TNT and its transformation products in particular adsorb or even bind covalently to soil

organic matter (Juhász and Naidu 2007; Singh et al. 2008; Bečanová et al. 2009). Wang and coworkers described an oxidative coupling of TNT and 4-ADNT with humic monomers through nucleophilic addition and condensation reactions as means of soil remediation (Wang et al. 2002). In presence of an oxidative biocatalyst and the humic monomer catechol up to 80% TNT were transformed through oxidative coupling and integrated into the humic material (Wang et al. 2002). Thus, the lower contamination of pure humus samples in the investigated area may be related to an increased binding potential and a reduced bioavailability of explosives (Singh et al. 2008). Contrarily, explosives show a higher mobility in sandy environments and may enter the groundwater, which can impact human health and aquatic ecosystems (Ampleman et al. 2004). Hydrogeology and the composition of soil significantly influence the distribution of explosives in soil and the risk of exposure for humans and wildlife (Ampleman et al. 2004, Juhász et al. 2007). Hence, the detailed characterization of a sampling site is inevitable to determine the bioavailability of explosives and conduct ecological risk assessments (Juhász et al. 2007).

The vegetation in the collection area had no significant effect on the distribution of the detected explosives (Fig. S1 in supplementary information). The analysis of larger areas or more diverse landscapes may show a pronounced impact of the explosive contamination on vegetation. It was shown that explosives enter plant roots, are distributed throughout the plant and have various impacts on plants regarding

morphology, physiology, growth and germination (Via and Zinnert 2016). Even more effects on community composition and species diversity may be observed (Via and Zinnert 2016). A high-throughput analysis and on-site collection of detailed sample characteristics can therefore be a valuable tool to analyze plant community responses in contaminated areas. Moreover, plants are an important bio-component for natural remediation (phytoremediation) and have a huge potential for decontaminating munitions at contaminated sites (Chatterjee et al. 2017). It was shown, that TNT was transformed in tree tissues and approximately 80% was non-extractable bound in roots, stems, wood, and leaves or in needles (Schoenmuth and Pestemer 2004). Hence, slightly contaminated areas may be naturally detoxified without the need for complex clean-up strategies. On the other hand, heavily contaminated sites require detoxification using chemical, physical, thermal remediation or biodegradation and bioremediation methods (Kalderis et al. 2011). However, the optimal remediation strategy depends highly on specific characteristics of the contaminated site and must be monitored regularly to evaluate the progress of remediation (Kalderis et al. 2011; Gałuszka et al. 2015).

Military activities lead not only to the introduction of nitroaromatic explosives, but are often accompanied by other chemical pollutants, e. g. oil components or heavy metals (Certini et al. 2013). Weapon residues in former battlefields or testing ranges have been found to release different metals to the soil environment, as lead, chromium, antimony, arsenic, cadmium, copper, mercury, nickel, and zinc (Certini et al. 2013). Consequently, the accumulation of heavy metals can be observed frequently in contaminated soil (Ampleman et al. 2004). A subsequent development of the Android application “Localyzer” might be useful, if heavy metal ions shall be correlated to the explosive contamination in soil, and measured data entered on-site using for example XRF instruments.

The number of analytical methods for the detection of explosives comprises IMS, chromatography, mass spectrometry and different sensor technologies (Singh 2007; Mäkinen et al. 2011). The high-throughput MALDI-TOF MS quantification of nitroaromatic explosives in soil is a new approach and exhibits several advantages regarding costs, sample preparation and time of analysis. Thus, it opens perspectives for the fast high-throughput screening of contaminated areas. Different applications in literature can be found for the analysis of environmental samples and the combination of analytical and GPS data, i. e. assessing water, soil and air quality concerning heavy metals, radiation and carbon dioxide, respectively (Paridaens 2005; Diawara et al. 2006;

Schreiner et al. 2006; Antunes and Albuquerque 2013; Wei et al. 2014; Suh et al. 2016). However, the spatial and spatiotemporal studies often focus on the detection of heavy metal contaminations using portable instruments as XRF (González-Corrochano et al. 2014; Elznicová et al. 2019). The Defence Research and Development Canada-Valcartier published a report, where heavy metals and explosives’ concentrations on a former weapon range were investigated and evaluated (Ampleman et al. 2004). The thorough characterization of the area included the collection of GPS data and analysis of energetic materials for 180 samples. However, the time consuming HPLC was used for the measurements and there is no attempt to visualize the contamination or correlate it to natural circumstances. In contrast to that, the introduced system combines the documentation of sample characteristics on-site, a fast MALDI-TOF MS analysis, a 2D and 3D visualization of the explosives’ spread in soil and the evaluation of sample characteristics. Thus, this is the first attempt for a comprehensive system to address the distribution of explosives in soil. Applications in environmental research include site characterization, emission monitoring and control, identification of pollution sources, and evaluation of the remediation progress (Gałuszka et al. 2015). Furthermore, it might be used not only for the spatial and spatiotemporal analysis of contaminated soils, but also to monitor sediments, seawater or sea biota in oceans and coastal regions where warfare materials were dumped (Maser and Strehse 2020) or to analyze sewage and natural waters impacted by explosive production facilities (Patokin et al. 2020).

Conclusion

The US army has estimated that over 1.2 million tons of American soil are contaminated with explosives and similar magnitudes of contamination can be found all over the world (Panagos et al. 2013; Via and Zinnert 2016). Hence, comprehensive screening tools are of great interest to characterize those areas and visualize the associated risk potential (Celin et al. 2020). Therefore, we established a method for a fast analysis and risk evaluation of explosives’ contaminated areas. Starting with the sample collection, we recorded GPS data and soil characteristics using a smartphone. After the fast MALDI-TOF MS analysis of all samples, explosives’ concentrations are automatically linked to the data of the sample collection, enabling the visualization of contamination with two and three dimensional heatmaps as well as the analysis of correlation between contamination and soil



characteristics. The application of the system for a large-scale sample collection could precisely describe a distinct soil contamination next to a former production facility for TNT and grenades. The analysis of collection depths and soil types showed a slow migration of contaminants into deeper soil levels and lower concentrations in humus. Thus, our method can give important insights into source and future spread of contamination. Furthermore, the system can be adapted easily for the screening of other environmental compartments and contaminants.

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Authors' contributions SLK developed the experimental design, implemented functions for the presentation and analysis of MALDI-TOF MS spectra, analyzed the experimental data and drafted the manuscript. PS coauthored the manuscript, performed the experiments and implemented the Android application and further functions for data analysis. HH supervised the study and revised the manuscript. MF is supervisor and corresponding author of the manuscript. All co-authors helped to refine the manuscript and agreed on the final version.

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Availability of data and materials Original analysis results for all samples and the Android application Localyzer.apk are provided as electronic supplementary material.

Code availability Original code is available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing financial interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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