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Using Encapsulated Fluorescent Bioprobes to Detect Explosive Materials in Soil

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tent daily supervision by senior researchers made it difficult to ensure that procedures were consistently implemented correctly by laboratory assistants and trainers. Finally, in part because procuring required equipment and supplies was slow and difficult, detailed chemical analysis of samples proved extremely challenging and failed to provide useful information for preparing training samples.

Despite these struggles, much has been learned from the Morogoro research. This knowledge has valuable applications in the training and testing of animals in a range of odor-detection roles (including direct detection of ERW), and efforts, supported by GICHD, are underway to publish some of those findings.

The Future for REST

Developing an operational REST system for ERW detection is an extremely complex interdisciplinary undertaking that poses significant challenges for engineers, analytical chemists and behavioral scientists. In the end it may be impossible to overcome those challenges and develop a workable system. It is, however, premature to assume that this is the case. In our opinion, the best way forward is to focus generally on the variables that affect odor detection by animals while endeavoring to develop a variety of useful operational Remote Scent Tracing applications, including ERW detection. Doing so affords opportunity for obtaining funds outside humanitarian demining and enlisting the services of experts in a range of industries. APOPO presently is taking this tack with its R&D.

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Using Encapsulated Fluorescent Bioprobes to Detect Explosive Materials in Soil

This article examines the methods involved in using fluorescent bioprobes to detect explosive devices within soil. By genetically modifying plants through the injection of certain chemicals, visible responses indicate the presence and placement of explosive material, aiding demining agents in the process of mapping and removing various landmines or other explosive remnants of war. The adoption of these tools proves useful for stand-off detection of low TNT concentrations in the laboratory and controlled microcosm studies.

by Clint B. Smith [U.S. Army ERDC] and Joel S. Tabb [Agave BioSystems]

Most of the current methods for analyzing explosive contaminants involve chemical extraction of explosives from collected soil samples. The complexity of these techniques typically requires that the samples be moved off-site. In addition to requiring extensive handling, expensive equipment and highly skilled workers, these methods involve transferring soil samples to a laboratory and using extraction techniques according to U.S. Environmental Protection Agency Method 8330.[1]

Over the past decade, novel efforts for detecting landmines in field environments included using genetically modified plants, which have been one of the focuses for biosensors. The idea involves plants that have been genetically modified to consume trace explosive materials and aid in landmine detection via a fluorescence or visual response when interrogated with an external light source. Plant leaves glow a brighter green when consuming the trace explosive material. These genomic analyses of plants may one day provide a range of bio- and nanotechnologies for development to look for trinitrotoluene (TNT)-based materials using fluorescent or bright tags such as green fluorescent protein.[2] These plant alterations will need to withstand the natural constraints of the environmental conditions, i.e., changes in soil pH. In addition to using plants as biosensors, genetically modified microorganisms have been investigated for their potential to detect various chemicals, namely TNT.[3,4,5,6] While GFP may serve as a useful bioreporter in the laboratory setting, recent reports suggest that this reporter may not be suitable for soil-contaminant detection. Smith et al. demonstrated that expressed GFP produced high fluorescence levels at pH 7.0, but at more acidic or alkaline pH levels, such as those likely encountered in potentially contaminated soil, fluorescence output was diminished, rendering the "ON switch" unreadable for a potential end user or operator.[7]

With support from the United States Army Engineer Research and Development Center, via the U.S. Army Small Business Technology Transfer program, Agave BioSystems is developing a novel fluorescent system capable of detecting explosive materials present in surface soils. The research initiative involves the proof of concept and experimentation on TNT detection in select soils using solution-based bioprobe slurries.

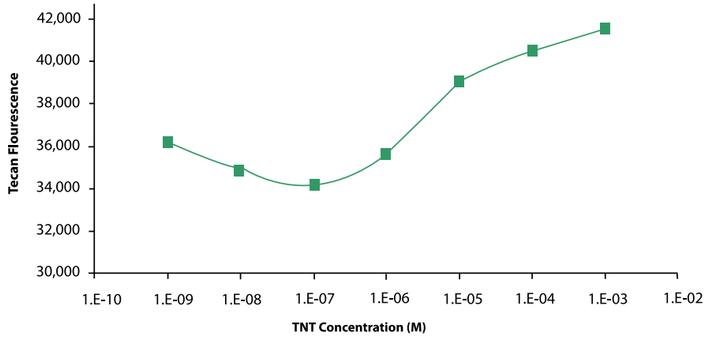

Figure 1: Measurement of silica microspheres comprised of fluorescent antibody-based bioprobes at a concentration range of 0.2 ppm TNT in solution to 100 ppm TNT in soil samples. Y-axis clarification: Tecan fluorescence is a measure of relative fluorescence. *Graphic courtesy of Joel Tabb/CISR.*

The bioprobes, or "dust" material, use fluorescent-labeled biological components called antibodies (known as the "ON switch") and fluorescent quencher analogs (known as the "OFF switch") to detect the presence of specific explosive residues like TNT. To provide environmental stability to the dust material's functional part, the bioprobe was encapsulated to protect and preserve the ON/OFF switch's functionality. When free TNT is present in the soil, the soil containing the TNT turns the dust ON, causing an increase in fluorescence and a brighter soil area when illuminated, indicating that a landmine is present beneath the soil's surface. Using the "dust" material, TNT concentrations from low levels (0.02 ppm) to higher levels (200 ppm) were readily detected at room temperature by spiking soil samples with TNT within our laboratory experimentation microcosm. Future efforts will focus on scale-up of materials for attempting experiments at larger ranges and keeping the bioprobe at the soil's surface to adapt for stand-off detection in field conditions and testing in various soil types and conditions (wet/dry, hot/cold, low pH/high pH, low salinity/high salinity). This research focuses on the technical clearance stages and non-daylight exercises to

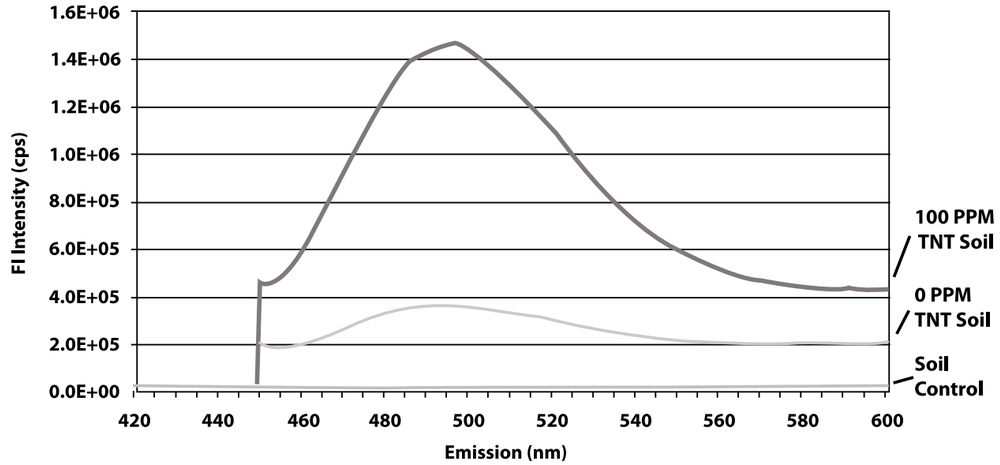

Figure 2: Soil-based detection of TNT using fluorescent bioprobe. Samples were excited with a HORBIA Jobin Yvon Spectrofluorometer with the fiber optic attachment positioned nadir or 90-degree geometry at 355 nm Excitation. The 495 nm fluorescence emission is viewed in this data plot. *Graphic courtesy of Clint Smith/CISR.*

maximize output from the fluorescence emissions obtained from the bioprobes' interaction with the explosive materials.

Methods

BRITE MINE, or Bioprobe Immuno-encapsulated Microspheres with Nanopore-based Energetics, is a hybrid organic and inorganic approach to detecting explosive agents within the environment. The advantages of this design are that the materials are not associated with recombinant DNA or genetic alterations, and are based on an environmentally stable silica (sand) construct. The silica microspheres (Millipore, USA) are nontoxic and inactive with the environment after use. Tested for TNT, the bioprobe detector has shown high sensitivity and high specificity. This biologically-based detection probe has disadvantages involving the necessary stabilization of antibodies in the environment. In other words, this technology requires proper storage prior to application and does have a shelf life; however, that is not uncommon with organic-based approaches. Expanding the shelf life and stability of this approach, the inorganic silica-based microspheres containing nano-size pores are used for antibody encasement and protection. In addition, there are potential signal-to-noise problems; part of the investigation continues in later stages of the research with use of fluorescence detector amplification or enhancement techniques illustrating that the "ON switch" works properly. Finally, as these detectors are scaled up and produced, materials will face certain challenges over a wide area of applications. Results from use of the laser-imaging system from 1-m stand-off distances have suggested that an optimization of the fluorescent "ON switch" would need to be used with a green-colored emission of the fluorescent tag when visualized by the operator. By using Dansyl-X (Sigma-Aldrich, USA) rather than the Blue/Green Alexa Fluor-488 nm (Invitrogen, USA), the tag could potentially amplify the distances at which greater stand-off distances could be seen.

To visualize landmine or TNT detection in contaminated soil samples, using an airborne imaging system is necessary to sufficiently detect and measure fluorescence from the dispersed bioprobes on soil surfaces contaminated with landmines.

Bioprobe Soil Testing

Preliminary experiments demonstrated that the fluorescent bioprobes could readily detect low (0.2 ppm) to high (100 ppm) TNT levels dissolved in 99.9% spectral grade acetone (Sigma-Aldrich, USA) in soil samples at room temperature using a Tecan spectrofluorometer (See Figure 1 on previous page). To further characterize the TNT detection in soil, additional experiments were conducted using standard topsoil from a local garden-supply center as a test matrix.

For these studies, topsoil was placed into 100-mm black Petri dishes or plastic tubs. To keep the soil moist and the bioprobes from being drawn into the soil matrix during TNT detection, samples were mixed with 10% (weight volume) water prior to being placed in containers. Tap water was used for mixing with the soils. Soil samples were then compressed into the containers using 15–30 lbs of weight for 15 min. This compression flattened the soil surface, making photography and bioprobe detection easier and more consistent.

To reduce the amount of Alexa Fluor-488 nm-labeled bioprobes used in each experiment and to increase consistency between sprayed samples, additional soil tests were conducted using 1-kg (2.2-lb) plastic tubs. In these experiments, soil samples with known TNT concentrations were placed into the tubs and compacted with 13.6 kg (30 lbs) of weight for 15 min. Alexa Fluor-488 nm bioprobes were incubated with BHQ10-TNB [Tri-Nitrobenzene] quencher first. This mixture was then sprayed onto the soil surface for TNT detection with a 15-min minimum incubation time. BHQ10 quencher was acquired from Bioresearch Technologies, USA and then synthesized with TNB to produce the BHQ10-TNB complex after a 1-hour incubation which was completed in the laboratory. Figure 2 (above) and Figure 3 (next page) show the experiment's results. Figure 2 shows spectral plots of the 1-kg plastic tubs 15 min after the bioprobe application.

Several points related to the plots shown in Figure 2 need addressing. First, in the absence of TNT (soil control), fluorescence intensity is near 0 photon counts per second due to the lack of TNT and activated bioprobes. Second, without activated components and TNT measuring 0 ppm, the addition of bioprobes had an increase in the fluorescence spectral signature from residual Alexafluor-488 nm that was unquenched. This minimal fluorescent

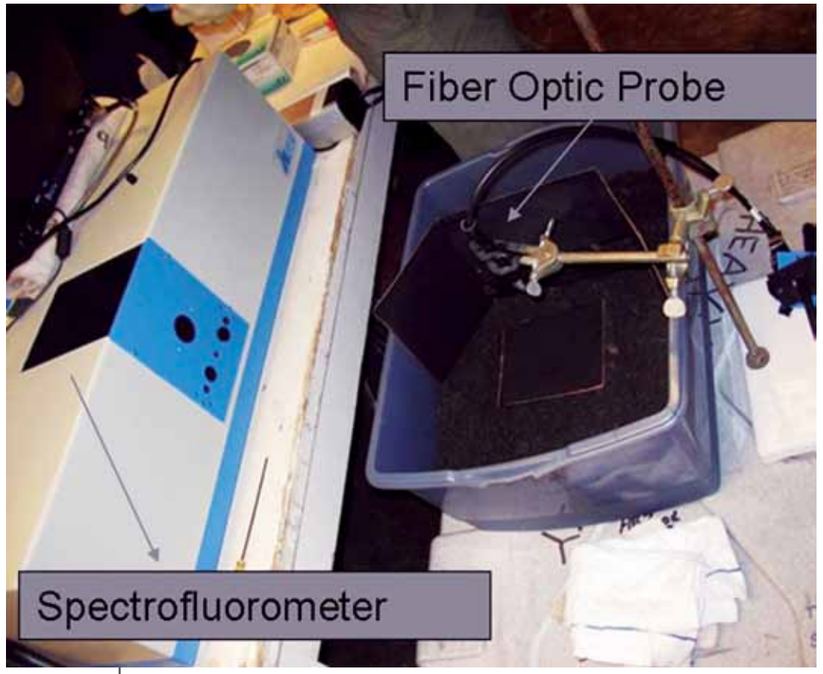

Figure 3: Experimental setup with spectrofluorometer (HORIBA Jobin Yvon) measurements with fiber-optic attachment. 1 kg sample soils (not shown) were placed in this larger tub (shown) to obtain spectral measurements of non-active and active bioprobes with and without TNT. *Graphic courtesy of Clint Smith.*

yield can be reduced with further experimental technique that shuts down the fluorescence spectral signals; this is related to the bioprobe manufacturing process. Finally, the increases in bioprobe fluorescence were readily apparent 15 min after application. Bioprobes were comprised of the fully activated components in the presence of 100 ppm TNT. The maximum time at which the highest fluorescence was observed was about 20 min. The fluorescent magnitude of the fully activated bioprobes is greater than the bioprobes background fluorescence without full activation or the added quencher.

The results shown in Figures 2 and 3 demonstrate that the fluorescent bioprobes were capable of detecting TNT presence in soil samples. Currently, the shelf life of the bioprobe is at least one year stored at 4 C in a phosphate buffered saline solution. The detection system used for these experiments is a tripled Nd:YAG [Neodymium-doped Yttrium Aluminium Garnet] laser-induced fluorescence imaging system (Big Sky Laser Technologies, USA) used to excite (355 nm) the bioprobes at a 1-m detection distance. As such, this project's objectives were achievable within the controlled soil conditions. Even with a greater magnitude order, this increase of fluorescent emission may not be enough for making a positive identification when investigated at stand-off distances greater than 100 m. Future efforts will focus on the investigation of the utility of retro-reflecting particles. These particle types would aid in amplifying the detection mechanism for better TNT signal output, thereby providing a better detection visual for the end user.

Conclusions

The results presented above demonstrate that novel inorganic and organic approaches can work for the stand-off detection of low TNT concentrations in the laboratory and controlled microcosm studies. Incorporating bioprobes into future minefield exercises may come about from these results; however, more research must be completed to enable the technology to be operationally field-ready. Future endeavors need to focus on the scale-up of materials for attempting experiments at larger ranges, the adoption of long stand-off detection, and development of materials or material modifications to aid in enhancement. Furthermore, research efforts such as this one may lead to more novel studies involving explosives-detection applications for identification of post-conflict landmine proliferation.

see endnotes page 83

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