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Preconcentration Techniques for Trace Explosive Sensing

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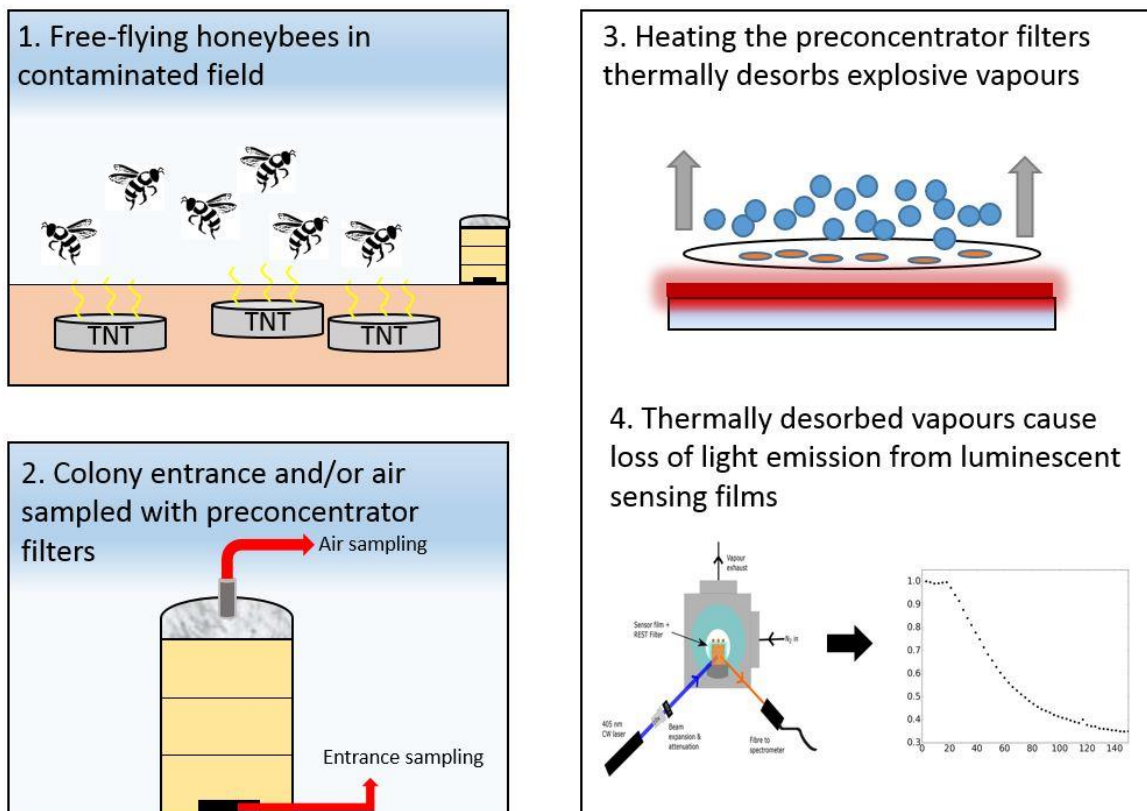
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Graphical Abstract



Abstract

Trace sensing of explosive vapours is a method in humanitarian demining and Improved Explosives Device (IED) detection that has received increasing attention recently, since accurate, fast, and reliable chemical detection is highly important for threat identification. However, trace molecule sampling in the field can be extremely difficult due to factors including weather, locale, and very low vapour pressure of the explosive. Preconcentration of target molecules onto a substrate can provide a method to collect higher amounts of analyte for analysis. We used the commercial fluoropolymer Aflas as a preconcentrator material to sorb explosive molecules to the surface, allowing subsequent detection of the explosives via the luminescence quenching response from the organic polymer Super

Yellow. The preconcentration effect of Aflas was confirmed and characterised with 2,4-DNT, prior to field sampling being conducted at a test minefield in Croatia by placing preconcentration strips in the entrance of the hives, where honeybees have collected explosive materials during free-flying. In this work we show for the first time a method for confirmation of landmines combining honeybee colonies containing a preconcentration material and subsequent monitoring of luminescence quenching.

Highlights

- Trace detection of explosive vapours is challenging in real-world environments.
- By “preconcentrating” trace vapours onto solid substrates, higher amounts can be collected for detection.
- Our approach combines fluoropolymer preconcentrators, free-flying honeybees for sampling, and luminescent thin films as the sensing mechanism.
- The inexpensive, commercially-available fluoropolymer Aflas has shown good preconcentration abilities for 2,4-DNT which is typically found in landmines.
- Laboratory results and initial field results indicate this method is a very promising tool for the detection of trace explosive vapours in contaminated land.

Keywords: Nitroaromatic; *Apis mellifera carnica*; REST sampling; Luminescence quenching; fluoropolymer; honeybee

1. Introduction

Chemical sensing of nitroaromatic compounds can be extremely challenging in the field, particularly if the target analyte is in trace amounts in the environment. An important

scenario is the detection of buried landmines, which remain active and consequently a high public risk even after decades. While animals can be used for trace vapour mine detection, alternative techniques that are fast-responding and highly sensitive with low probability for false detections would be a significant addition to a demining toolkit. Current methods to detect explosives on minefields include sniffer dogs and Ion Mass Spectrometry (IMS), which have drawbacks primarily in terms of training, upkeep, cost and behaviour in the former (Porritt et al., 2015), or cost, lack of selectivity, and reportedly high level of false positives in the latter (Caygill et al., 2012; Giannoukos et al., 2016).

Remote Explosive Trace Sampling (REST) is a method used in pre-clearance technical surveys of suspected minefields to ascertain the presence of explosives, and again at the end of the process for Quality Assurance (QA) (Beyene, 2010; Fjellanger, 2004; Lugo et al., 2017). This process involves sampling the air above a potential mine into a PVC or polyethylene net, tagging the sample and sending off-site to specialist centres for trained sniffer dogs to identify, before sending the information back to the field. However, this process can take up to several weeks between initial sampling and receiving the result, and requires an operative to walk on potentially contaminated land. There is therefore a strong interest in alternative methods both to collect samples across mine-suspected areas without entering the area, and to detect promptly and sensitively any sampled explosive residues.

Luminescent organic semiconductor films have been attracting a high level of attention in recent years as explosive vapour sensing films due to their very high sensitivity to nitroaromatic vapours through an electron transfer process from the polymer to nitroaromatic molecule giving a light-reducing quenching effect (Ali et al., 2016; Bolse et al., 2017; Gillanders et al., 2018; Narayanan et al., 2008; Shoaee et al., 2016; Thomas et al.,

2007; Toal and Trogler, 2006; Wang et al., 2011b; Yang and Swager, 1998). Instrumentation based on exploiting these materials has been developed for humanitarian demining applications (Gillanders, 2017; Wang et al., 2011a) in recent years. The commercial organic polymer shown in Figure 1 (a) from Merck, Super Yellow, exhibits high sensitivity to nitroaromatic compounds including 2,4-DNT, a common component in landmine manufacture and a degradation product of TNT. However, for use in the field, where uncontrollable environmental factors can disperse the trace vapour plume, a method to pre-concentrate the target analytes prior to exposure to the sensor would offer an advantage.

Certain polymers have properties that enable them to accumulate explosive vapours to the surface, based on the hydrogen bond acidity between polymer and target vapour (Abraham, 1993; Houser et al., 2001; McGill et al., 1994). These have been successfully developed for highly-sensitive Surface Acoustic Wave sensors for hazardous targets including nerve agents and radionuclides (Grate, 2008), and for HPLC columns applied to environmental monitoring (Egorov et al., 2006; Grate et al., 2007). These polymers effectively act as a “magnet” for the explosives, whereby the nitroaromatic compound adsorbs to the surface, and the application of heat leads to thermal desorption (Camara et al., 2014; Martin et al., 2007; Serrano et al., 2013; Tiwary et al., 2016; Voiculescu et al., 2006), as shown in Figure 2. The specially designed materials studied in the literature for this purpose are usually expensive (approx. \$600 for 100 mg at time of writing) which inhibits their ability to be prepared in large batches for humanitarian demining efforts. A commercial, amorphous random copolymer of tetrafluoroethylene (~56 wt.%), vinylidene fluoride (~27 wt.%), and propylene (~17 wt.%), sold as Aflas and illustrated in Figure 1 (b), has similar structural properties to materials in the literature, and has previously been used as host matrix for oxygen-sensitive

dyes (Gillanders et al., 2004a; Gillanders et al., 2004b), and is known to have good sorption kinetics for organic vapours (Wang et al., 1999).

A method to collect these explosives without human activity on the mine-suspected area would decrease the danger in field sampling. Honeybees foraging for explosives residues potentially have the advantage of being able to access terrain that may be difficult for humans or larger animals such as dogs, to safely survey. Honeybees have previously been used in biosensing applications for targets including radionuclides, pollutants, and explosives (Barisic D et al., 2002; Girotti et al., 2013; Rodacy et al., 2002; Zarić et al., 2018). In such an approach, the honeybees' body hair can electrostatically attract trace particles of explosives as they free-fly around a contaminated area; after returning to the hive, the inner environment of the colony may then become rich in explosive molecules for subsequent detection.

In this work we show for the first time an approach that combines colonies of honeybees to collect material across an area of landmine contaminated land, with a REST sampling technique to establish the presence of trace explosives in the field. We show that the fluoropolymer Aflas can act as an effective preconcentrator material for the accumulation of nitroaromatic molecules, which can later be released thermally and detected via luminescence quenching in a semiconducting polymer film. By placing a cartridge of tubes holding the adsorbent material in the entrance of a hive, bees returning from foraging may deposit explosive particles on the surface of the preconcentrator, which can subsequently

be collected and analysed. We compare this with an alternative approach in which air from inside the hive is pumped through a preconcentrator-coated filter for subsequent analysis.

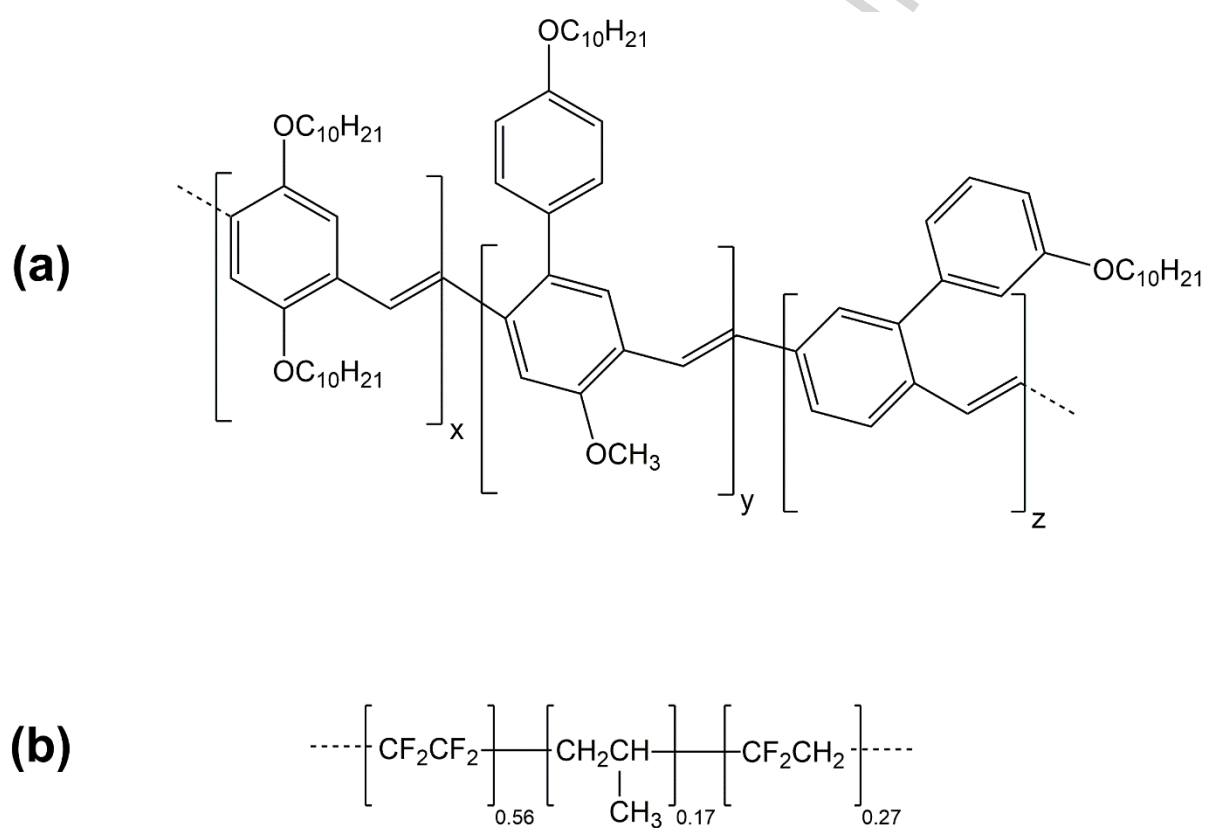


Figure 1. Chemical structures of the polymers (a) Merck Super Yellow and (b) Aflas.

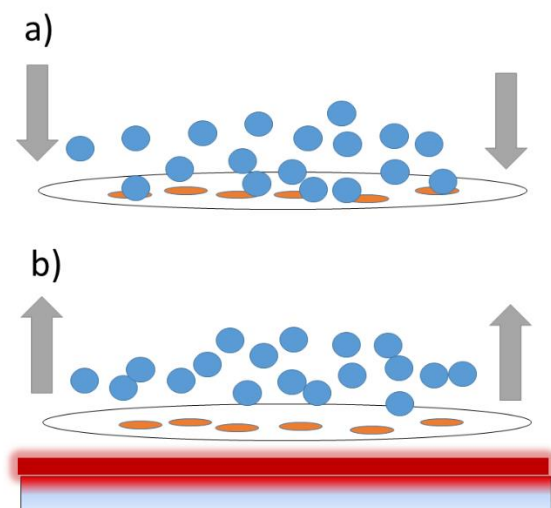


Figure 2 –Schematic concept of preconcentrating filter. (a) Air containing the explosive vapour (shown as blue circles) is drawn through the filter where the molecules of the explosive adsorb to the polymer surface. (b) Subsequent heating of the preconcentrator desorbs the explosive molecules for detection by a change in the light emission from a separate luminescent polymer film.

2. Material and Methods

2.1 Polymer sensor and REST filter preparation

Films based on Merck Super Yellow were prepared by spin coating the polymer at 2000 rpm from toluene solution of a concentration of 6.5 mg/ml onto 1 cm² cover glasses (Agar Scientific). Prior to spin coating, the substrates were cleaned ultrasonically for 5 minutes in toluene, acetone and propan-2-ol, and dried in a dry nitrogen stream prior to being plasma ashed in a 100% oxygen plasma (Plasma Technology MiniFlecto) for 3 minutes. Film

thicknesses, measured with a Veeco Dektak 150 surface profilometer, were found to be in the region of 100 nm.

Aflas[®] was purchased from AGC Chemicals Europe Ltd, and was prepared by dissolving in Tetrahydrofuran (Sigma Aldrich) at a concentration of 155 mg/ml. For characterisation and air sampling, 10 μ l of the solution was then drop-cast with a micropipette in a polka-dot grid pattern of 3x3 spots, with a mass per spot of 1-3 mg, either on silicon wafers (Approx.. 2.5 x 2.5 cm) or standard Whatman filter paper of diameter 4.5 cm, and left to dry prior to use. An example image of the Aflas-spotted filter is shown in Figure 3. It can be seen that the uncoated surface of the paper allows for unobstructed air-flow from the air sampler.

For in-situ placement of the preconcentration material in the hive entrance and exit, sheets of Whatman filter paper were blade-coated with Aflas solution as above and cut into strips as seen in Figure 3. The strips were placed against strips of acetate sheets and rolled into tubes with an approximate diameter of 1 cm.

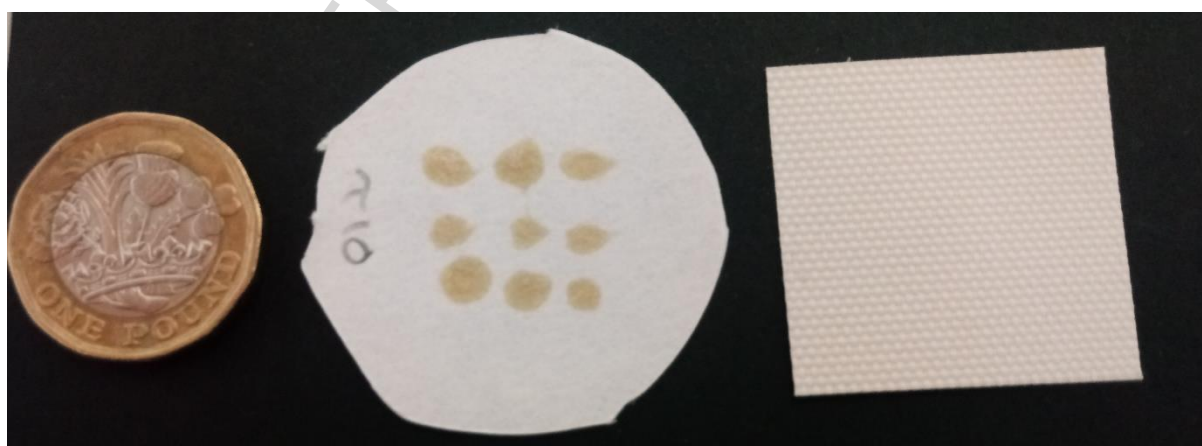


Figure 3 – Photograph of a coin for scale (left) with an Aflas-spotted Filter paper (middle) and an Aflas blade-coated substrate (right)

2.2 Limit of Detection of Super Yellow sensors

To determine the limit of detection of the super yellow film to nitroaromatic molecules, and obtain a measure of the typical level of photoluminescence quenching due to a known mass of explosives, the efficiency of photoluminescence was measured for various concentrations of 2,4 DNT loaded into the polymer film. The Photoluminescence Quantum Yield (PLQY) of the Super Yellow films was measured with an integrating sphere (Greenham et al., 1995) in a Hamamatsu Photonics C9920-02 system with an excitation wavelength of 405 nm. The PLQY of the film was first measured in its pristine as-spin-coated state. The films were then contaminated with 2,4-DNT by drop-casting a solution of acetonitrile containing 2,4-DNT and allowing the solvent to evaporate. The PLQY of the contaminated films, containing masses of 2,4-DNT between 6 pg and 60 mg, were measured and compared with the initial values. The error associated with these measurements is 1% of the measured value.

2.3 Preconcentration capability of Aflas

To confirm the preconcentrating action of Aflas, the polymer was drop cast onto silicon wafers which were placed in a custom-built sealed chamber and exposed to 2,4-DNT vapours generated by flowing nitrogen vapour at a flow rate of 10 Lmin⁻¹ over 1 g of 2,4-DNT powder sealed in a glass tube and held at room temperature at a relative humidity of 40-50%. After exposure to the DNT vapour stream, the samples were placed against a heating element in a sealed chamber of inner dimensions 53 x 53 x 53 mm, along with the super yellow polymer sensor film as shown in Figure 4. Silicon was used as a substrate for

these measurements due to its good thermal conductivity which allowed the Aflas spots to be heated effectively. To minimise sensor degradation via photo-oxidation in ambient conditions, the chamber was flushed with clean nitrogen and sealed at the start of each measurement. The sensor was placed 1 cm from the preconcentrator, excited with a 405 nm CW laser diode laser (Photonic Solutions) and its photoluminescence measured over 300 s with a fibre coupled CCD spectrometer taking measurements every 3 s. The photoluminescence at room temperature was measured for 30 s, then the heater turned on for approximately 100 s to heat the sample to 100°C. After measurements had been completed the chamber was flushed again with clean nitrogen to clear the chamber of any residual explosive vapours.

To confirm the preconcentration action in a simulated minefield environment, the Aflas polka-dotted onto Whatman filter paper discs were placed over a simulated landmine containing 1g of DNT in a metal container buried in soil at a depth of 2 cm as described in (Gillanders, 2017). The relative humidity around the simulated landmine was increased to 70-80% before sampling with a water mist as this has been shown to maximise the amount of explosive vapour emitted by a landmine (Bach and Mclean, 2003). The paper filter was placed into a home-made holder attached to an air sampling pump (JS Holdings HF812e). The filter was held above the simulated landmine, as shown in Figure 5 and air sampled at a rate of 60 Lmin⁻¹ for 10 minutes. These samples were tested in the same way described for the silicon samples.

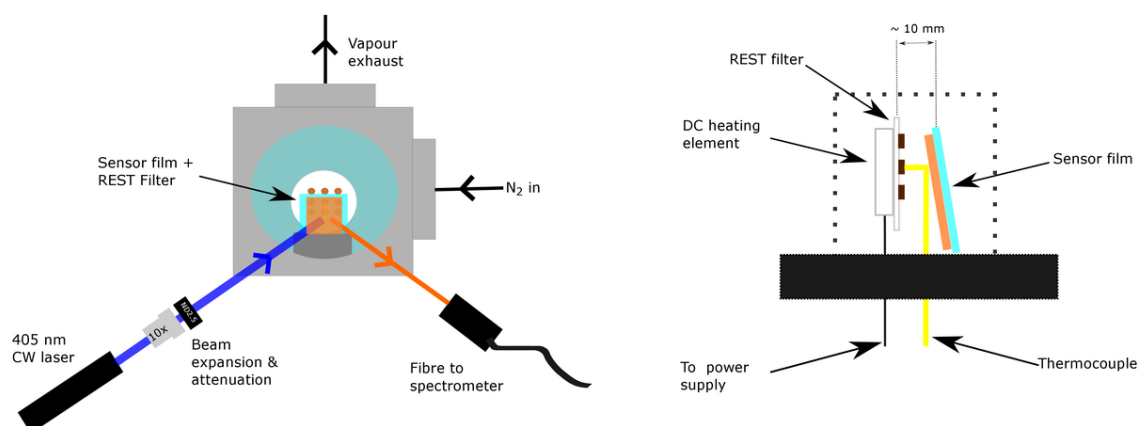


Figure 4 – Experimental set-up for detecting the release of vapours from a heated preconcentrator film.

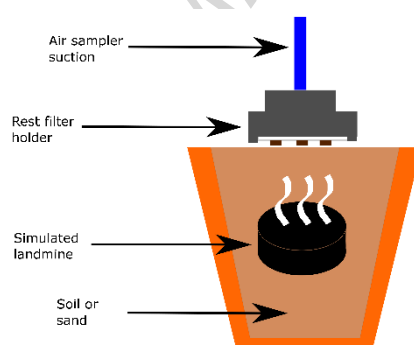


Figure 5 – Schematic diagram of the approach to collect vapour and particle samples of explosives above a simulated landmine.

2.4 Preconcentrator sampling of trace explosives in the field using honeybee colonies

Honey bee (*Apis mellifera carnica*) colonies were obtained from a local beekeeper in Zadar County, Croatia. Three colonies were selected from an area that is not contaminated with land mines (Vrsi). The population of the bees in each colony was counted according to the

modified Liebefeld method (Delaplane et al., 2013). For the field trial colonies were selected with similar populations of bees, on average 20,000 per colony. We used standard LR hives with two supers, a bottom board with entrance, a top board and roof. For the air sampling the top board and roof were removed to place the cupola on top (figure 8).

The three colonies were located initially on a clean site to collect explosive-free control samples, before being moved to an explosive-contaminated site to allow sampling of explosive material in the preconcentrators. Once the colonies were in-situ the bees were allowed to free-fly, with a sucrose solution of 500 g sugar dissolved in 1.5 L tap water used to keep the bees within the test area of interest. The control sampling was performed near Vrsi ("clean site", Figure 6) and the contaminated colony sampling was conducted on Benkovac test site ("test site", Figure 6), in September 2017 and mid-April 2018. The test site was designed for the testing and validation of mine detectors and mine-clearance vehicles, with an area of 10,000 m² and 1,000 deactivated mines buried in a series of test lanes.

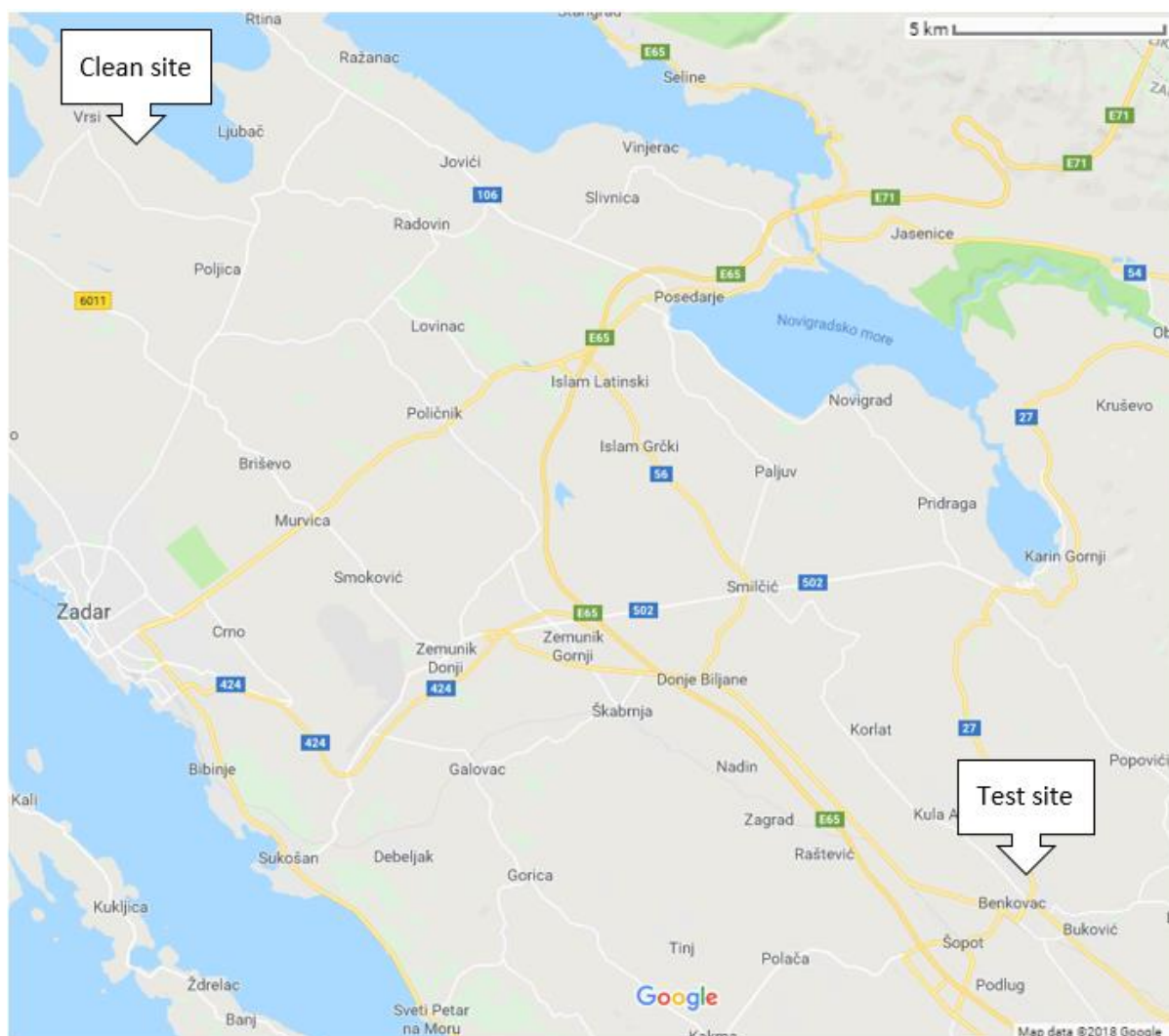


Figure 6 – Sampling site map in Zadar County, Croatia. Map data ©2018 Google.

The main approach tested to collect material gathered by the colonies was to locate preconcentrators at the entrance to the hives for the bees to mechanically deposit material onto the Aflas film. Blade-coated filter papers were cut into strips of approximately 3 cm x 10 cm (Figure 7a), placed against a strip of acetate of the same dimensions and rolled into tubes prior to insertion in plastic cartridges of 4 x 1 cm². Standard Lexan plates (1 x 1 cm tube) were cut into 10 cm lengths and used as a cartridge (Figure 7b), which was then inserted into the entrance of the hives (Figure 7c). To separate bees entering the colony from the bees leaving, two cartridges were used. For the bees entering the colony, the

cartridge is protruding from the colony, while for the exit the cartridge was extending into the colony (figure 7d). Samples were left for periods of one, three days and seven days prior to collection. At the end of the placement period the papers were removed from the colony, inserted into glass vials, sealed with Parafilm then placed in airtight sealed bags and stored in the dark, for subsequent testing as described in section 2.3.

(a)



(b)



(c)



(d)



AC

Figure 7 – (a) A substrate coated with Aflas polymer; (b) Substrates rolled up and inserted into the adapted entrance tubes; (c) The adapted entrance on the colony; (d) Entrance (left) and Exit (right) cartridges in the bottom board.

The air from within the bee hives was also sampled after collection of the entrance cartridges, to compare with the samples of explosive materials deposited on the entrance and exit cartridges. Uncoated papers were also inserted into the cartridges to confirm preconcentration efficacy of the Aflas in the field. To sample the hive air, the filters were inserted into a home-made nozzle and attached to the colony via a specialised cupola (Figure 8) on the hive, with air being sampled for 10 minutes with a vacuum pump at a flowrate of 60 Lmin^{-1} . The filters were then immediately placed in glass vial, sealed with Parafilm, and sealed in a resealable bag.



Figure 8 – Air sampling via specialised cupola on top of the bee hive.

3. Results and Discussion

3.1 Limit of Detection of Super Yellow with 2,4-DNT

Figure 9 shows the Limit of Detection via change in the PLQY of neat Super Yellow films with the addition of 2,4-DNT dissolved in acetonitrile. A loss in PLQY efficiency between a pristine film, with an initial PLQY of 40%, and a contaminated film indicates an increase in the non-radiative recombination rate, due to photoluminescence quenching by the analyte. At sub-ng levels the application of the 2,4-DNT solution can be seen to increase the PLQY slightly; this may be attributable to some swelling of the polymer caused by the solvent reducing non-radiative interactions between the polymer chains and thereby outweighing any quenching effect from trace amounts of explosives. Above ng levels however, a large loss of PLQY can be observed. The results suggest that below 10 ng of explosive material per cm^2 on the concentrator is required to quench luminescence at detectable levels.

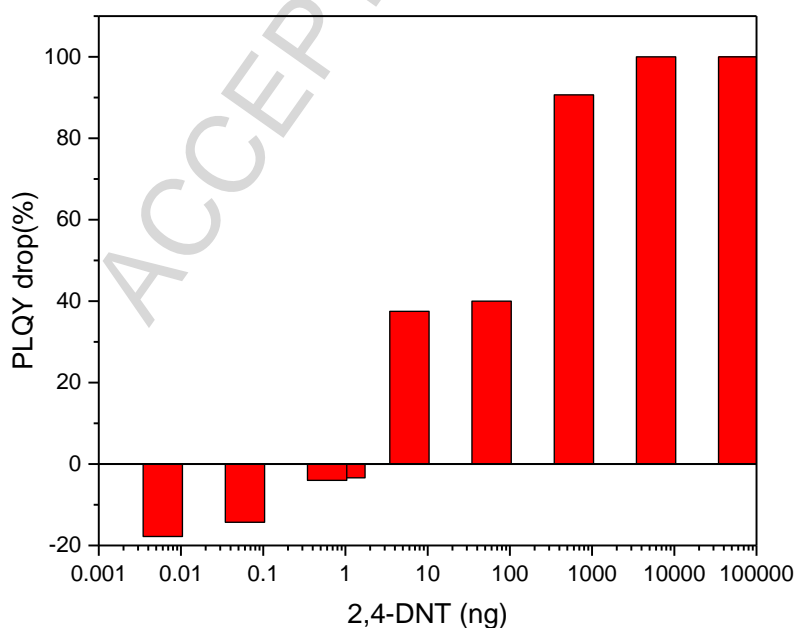


Figure 9 - Reduction in PLQY of Super Yellow films exposed to different masses of 2,4-DNT in acetonitrile solution.

3.2 Efficacy of Aflas as a preconcentration material

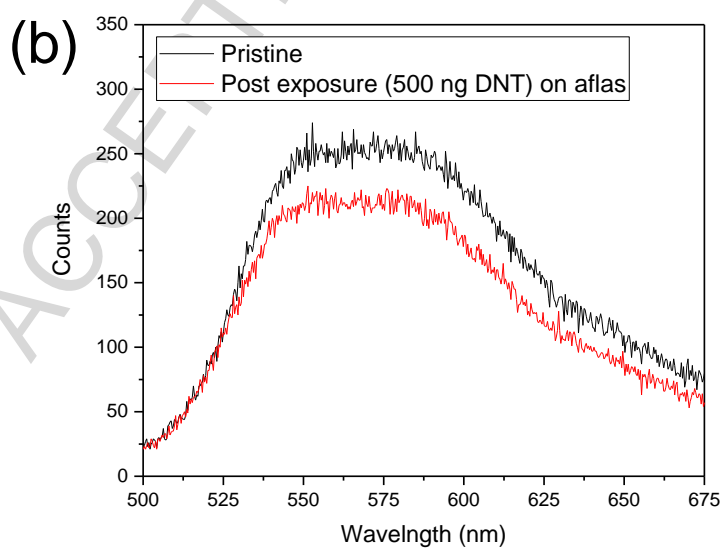
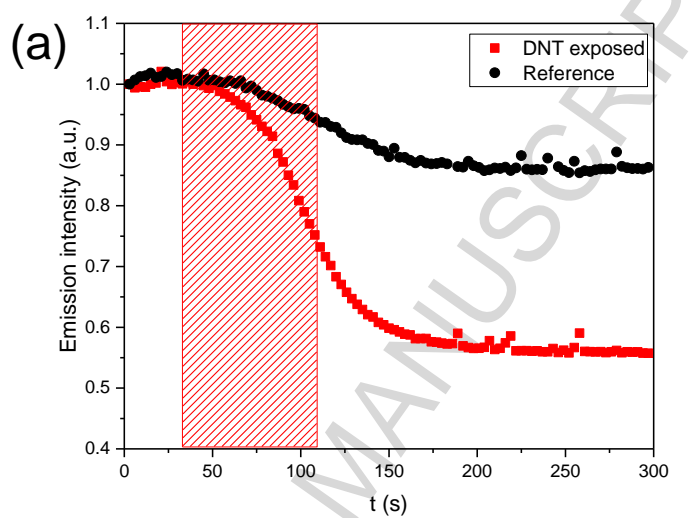


Figure 10 – (a) Photoluminescence emission from the Super Yellow sensor film during the thermal release of 2,4-DNT vapours from silicon test samples. An Aflas coated silicon substrate exposed to 2,4-DNT vapour (red line) is compared with a reference uncoated silicon substrate also previously exposed to 2,4-DNT (black line). The red box indicates the substrate heating time of 90 s. (b) Emission spectrum from a Super Yellow sensor before and after exposure to a heated preconcentrator doped with 500 ng of 2,4-DNT.

The Aflas polymer is shown in Figure 10a to be a suitable adsorber for nitroaromatic vapours. A clean, uncoated silicon reference substrate was exposed to 2,4-DNT vapour and then heated in the sealed chamber shown in figure 4, while the photoluminescence emission from a Super Yellow film was measured. A silicon substrate coated with Aflas was likewise exposed to 2,4-DNT, heated, and the emission from Super Yellow monitored. The uncoated reference substrate can be seen to cause a decrease in Super Yellow sensor emission intensity of around 12%, which may in part be due to the elevated temperature causing some thermal and/or photo-oxidative degradation of the Super Yellow sensing film. However, the Aflas-coated substrate causes a much greater quenching response of approximately 45%, showing that explosive vapours have been adsorbed to the Aflas surface prior to successful thermal desorption. Figure 10b shows the decrease in fluorescence of Super Yellow after exposure to an Aflas substrate doped with 500 ng 2,4-DNT.

The process was repeated in a simulated real-world environment by sampling air into filter paper substrates from above a buried simulated landmine. An uncoated filter paper, and an Aflas-spotted filter paper were each exposed to the air above the simulated landmine. They

were subsequently heated in the chamber to measure the quenching response of vapours released onto a Super Yellow sensor film. As shown in Figure 11, the unexposed Aflas-spotted paper filter causes approximately 10% quenching of the Super Yellow photoluminescence, attributable to degradation of the sensing polymer. The exposed uncoated filter paper gives a quenching response of around 27%, which suggests the rough, fibrous structure of the paper is able to trap some explosive molecules and desorb via heating. Plain paper has been recently shown to successfully adsorb explosive particles for photoacoustic sensing (Sharma et al., 2017). However, a clear quenching response of over 70% is shown by the Aflas-spotted filter paper exposed to 2,4-DNT vapour. In addition to the Aflas polymer sorbing vapours of the 2,4-DNT, the spots on the filter were found to pick up particles of dust and dirt from the sampling area more effectively than paper alone, further increasing the effectiveness of the filter.

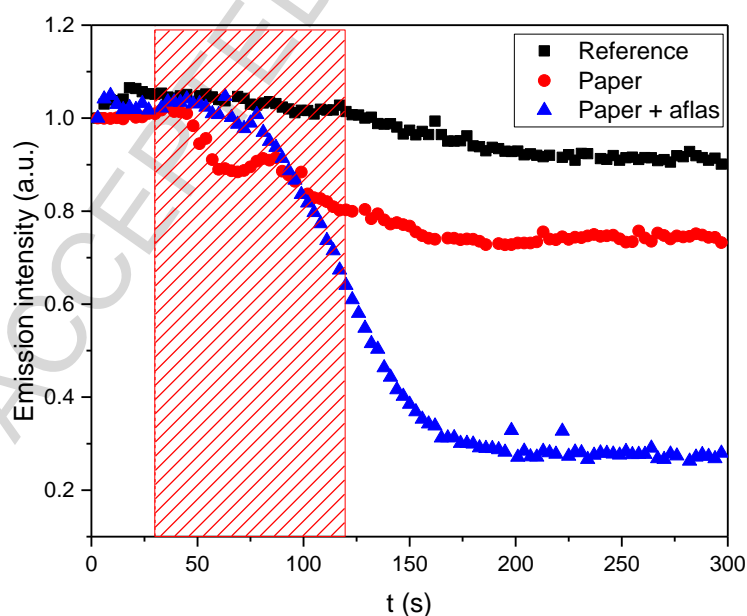


Figure 11 – Photoluminescence emission from a Super Yellow sensor film during the thermal release of 2,4-DNT vapours from filter paper samples previously exposed to DNT vapours from a simulated landmine. Data show the responses to an uncontaminated Aflas-spotted filter paper (Reference, black line), uncoated filter paper (Paper, red line), and an Aflas-spotted filter paper (blue line). The red box indicates the substrate heating time of 90 s.

3.3 Preconcentrator sampling of trace explosives in the field using honeybee colonies

Following the initial validation tests of the preconcentrators, samples collected from the test mine field were subsequently analysed. Preconcentrators taken from the entrance and exit cartridges in the hives were heated to release sorbed molecules and the photoluminescence from Super Yellow films was measured as described in section 2.3. The sensing results of a single hive entrance and exit sampling in September 2017 are shown in Figure 12, while averaged results from three hives (two hives on the Clean Site) in April 2018 are shown in Figure 13. The horizontal black line is the benchmark average loss of luminescence from three separate control measurements (of an unused Aflas filter) due to photo-oxidation of the Super yellow film in atmospheric conditions. It can be seen in Figure 12 that the highest quenching response is from the preconcentrator samples collected at the hive entrance after one day on-site. Weather data from the sampling period shown in Figure 12 show less favourable conditions in terms of temperature and wind speed on the third and seventh days thereby reducing bee activity. For instance, the temperature on the first day had a minimum of 16°C while the other two sampling days had minimums of 12°C and 9.8°C respectively, which may have reduced bee activity. There was also heavy rainfall of 123.1 mm between collecting the three-day and seven-day samples in the Benkovac region at that

time, which would have reduced foraging and may have inhibited explosive vapour release at ground level.

The spread of responses over the trial periods may be attributed to several factors chiefly including the weather (e.g. during strong winds the bees will remain inside the hive) or potentially bees finding a reliable pollen (protein) food source after 24-48 hours on-site.

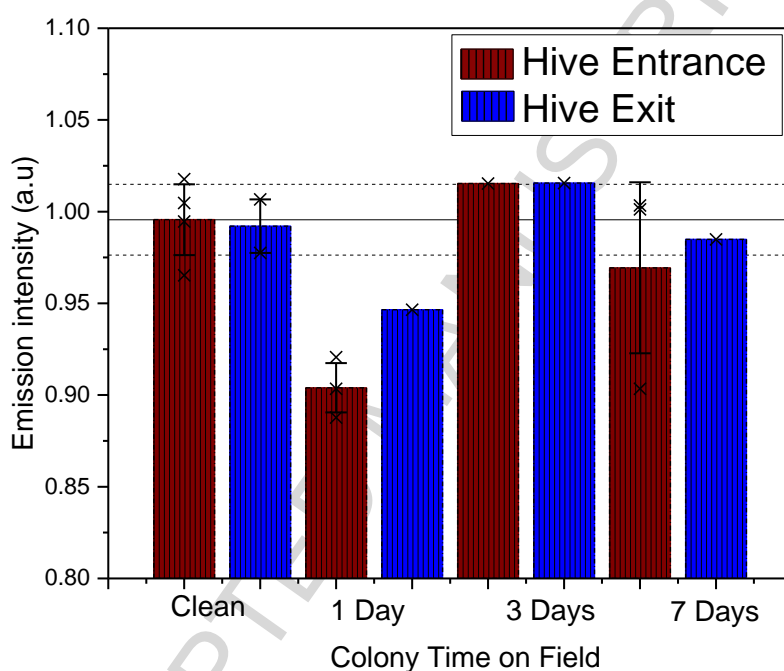


Figure 12 – Response to vapours released after 90 seconds' heating of the preconcentrator strips from a single hive entrance in September 2017; sampled both before placement at, and following 1, 3 and 7 days located on, the test minefield. Bars show the average fraction of the initial photoluminescence intensity remaining after exposure to molecules released from the heated preconcentrator, while crosses show individual measurements. Each bar is an average of three measurements, while the dotted horizontal lines show the standard

deviation from the averaged reference. Red bars show results for preconcentrators at the hive entrance while blue bars show results for preconcentrators at the exit.

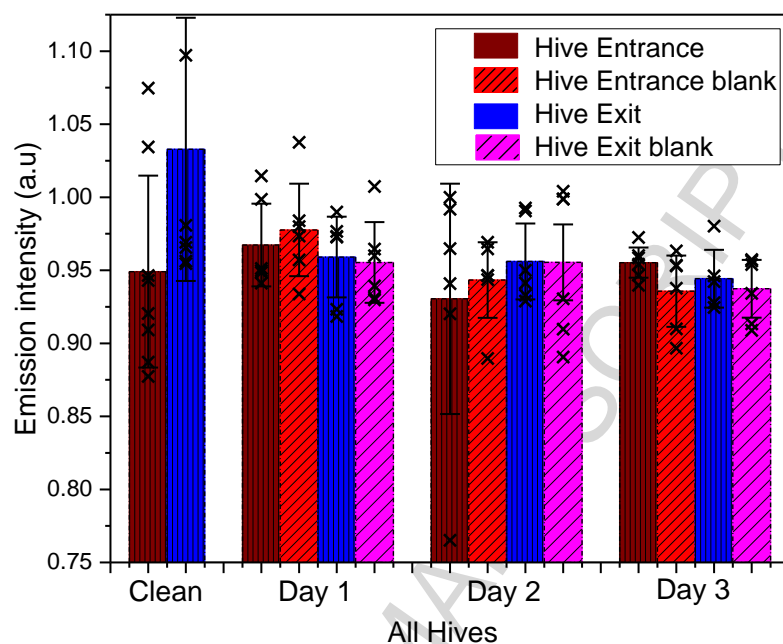


Figure 13 – Response to vapours released from the preconcentrator strips after 90 seconds heating - results averaged from three hives on the minefield over three days in April 2018. The bars show the average fraction of the initial photoluminescence intensity remaining after exposure to molecules released from the heated preconcentrator, while crosses show individual measurements. The horizontal line shows the average response from a blank measurement. “Hive entrance” refers to Aflas-coated papers in the hive entrance, “Hive Entrance Blank” refers to uncoated papers in the hive entrance; “Hive Exit” refers to Aflas-coated papers in the hive exit, and “Hive Exit Blank” refers to uncoated papers in the hive exit.

Figure 14 shows the response of the Super Yellow sensor to vapours released from preconcentrator filters loaded by air-pumping from within a hive. The figure shows indicative measurements taken over a 3 day period for a hive located on the test minefield. The samples taken from within the colonies show similar levels of luminescence quenching from the air-sampled preconcentrator as those from the entrance preconcentrators. This indicates that some quantities of explosive molecules may accumulate in the colony even after adsorbing to those in the entrance cartridges, and there is potentially enough explosive material collected inside the colony to be air-sampled and detected.

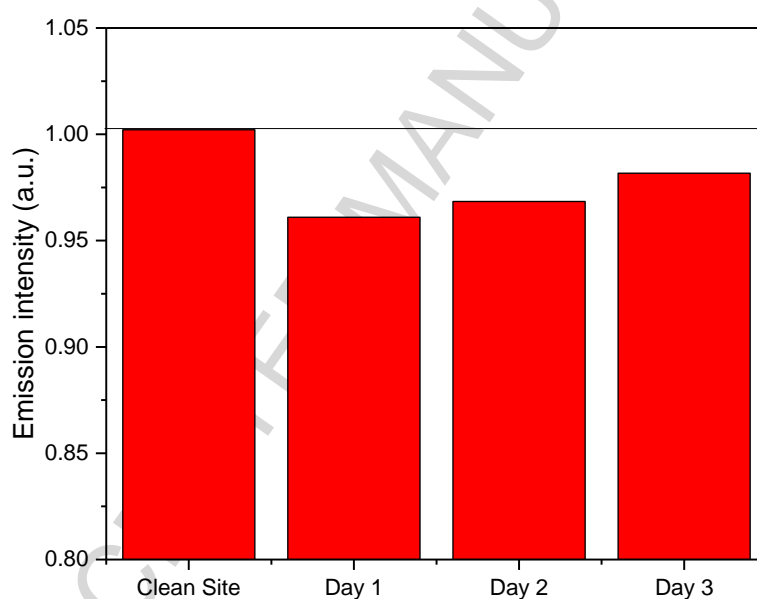


Figure 14 – Response to vapours released from the preconcentrator air filters after 100 seconds', sampled from within a hive, following 1, 2 and 3 days located on the test minefield. Bars show the fraction of the initial photoluminescence intensity remaining after exposure to molecules released from the heated preconcentrator.

4. Conclusions

An inexpensive, commercially-available preconcentrator polymer was used to efficiently adsorb explosive materials, which could subsequently be released thermally for detection using a photoluminescence quenching sensor. Preconcentration was tested in the field with honeybee colonies used as a biological sampling approach to gather residues of buried explosives over an area. The honeybees were allowed to free-fly around a test site with known landmine presence, to allow explosive molecules to be picked up electrostatically by the bees' body hair. On return to the hive, the bees passed through a tube lined with the preconcentration polymer to accumulate gathered residues. The preconcentrator was subsequently heated in a sealed chamber and the released vapours were detected via luminescence quenching of the conjugated polymer Super Yellow. These results indicate a preconcentrator at the hive entrance has potential for collecting gathered explosive materials. This technique was shown for the first time, to the best of our knowledge, and could provide a pathway to real-time identification of explosives in the field. While the results are still at an early stage, we expect that improvements in the sampling methods could provide a robust, inexpensive system to aid humanitarian demining efforts worldwide.

Acknowledgements

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Appendix A: Supporting Information

Data supporting this research can be found at <https://doi.org/10.17630/1b2fa689-d709-4276-8161-ea7918c17716>.

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