Biomonitoring for wide area surveying in landmine detection using honeybees and optical sensing

Ross N Gillanders[*1], James ME Glackin[1], Zdenka Babić[2], Mario Muštra[3], Mitar Simić[2], Nikola Kezić[4], Graham A Turnbull[1], Janja Filipi*[*5]

[1] Organic Semiconductor Centre, SUPA, School of Physics & Astronomy, University of St Andrews, Fife KY16 9SS, Scotland
[2] Faculty of Electrical Engineering, University of Banja Luka, Patre 5, 78000 Banja Luka, Bosnia and Herzegovina
[3] University of Zagreb, Faculty of Transport and Traffic Sciences, Vukelićeva 4, HR-10000 Zagreb, Croatia
[4] University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000, Zagreb, Croatia
[5] Department of Ecology, Agronomy and Aquaculture, University of Zadar, Trg kneza Višeslava 9, 23000 Zadar, Croatia

*Corresponding authors: rg89@st-andrews.ac.uk (R.N. Gillanders); jfilipi@unizd.hr (J. Filipi)

Highlights

- Collection and detection of trace explosives is challenging in real-world environments.
- Honeybees are used to passively collect explosives on body hair via free-flying.
- Returning to the colony, the bees deposit explosives onto a sorbing preconcentrator for subsequent analysis by optical sensor.
- Several potential distractant chemicals are tested and found not to give false positives.
- A model has been developed to estimate the amount of explosive returned to the colony by the foraging bees.

Keywords

Nitroaromatic; *Apis mellifera carnica*; REST sampling; Luminescence quenching; Environmental modelling; Honeybee

Abstract

Humanitarian demining is a worldwide effort and the range of climates and environments prevent any one detection method being suitable for all sites, so more tools are required for safe and efficient

explosives sensing. Landmines emit a chemical flux over time, and honeybees can collect the trace residues of explosives (as particles or as vapour) on their body hairs. This capability was exploited using a passive method allowing the honeybees to freely forage in a mined area, where trace explosives present in the environment stuck to the honeybee body, which were subsequently transferred onto an adsorbent material for analysis by a fluorescent polymer sensor. Potential false positive sources were investigated, namely common bee pheromones, the anti-varroa pesticide Amitraz, and the environment around a clean apiary, and no significant response was found to any from the sensor. The mined site gave a substantial response in the optical sensor films, with quenching efficiencies of up to 38%. A model was adapted to estimate the mass of explosives returned to the colony, which may be useful for estimating the number of mines in a given area.

1. Introduction

There are still over 100 million landmines buried globally from current and historic conflicts, and the costs to remove them are many orders of magnitude higher than the cost to manufacture and lay them. Mined areas, particularly in developing nations, prevent communication and trade, and place an enormous burden on local communities in terms of the non-discriminatory nature of those killed or maimed by detonation. Humanitarian demining efforts are expensive, time-consuming, and dangerous, and finding individual mines presents different challenges across different climates and terrains. Tools for landmine detection vary in complexity, from mechanical prodders to ground-penetrating radar. Sniffer dogs are often deployed for trace explosive vapour detection, though have some drawbacks in terms of the costs of upkeep, animal behaviour, and time allowed on-site per day. Remote Explosive Scent Tracing (REST) is a method that has been used for site surveying, where a plastic mesh filter is placed in the inlet of an air pump which is then used to sample air and dust across an area[1,2]. The mesh is then stored in a sealed container and sent off-site to be assessed by sniffer dogs for the presence of explosives in the suspected area, which is subsequently communicated back to the deminer. This method has drawbacks in terms of cost, slow timescale, and relying on dogs to confirm the presence of explosives in a sample.

Organic semiconductors provide an alternative approach to trace explosives detection, and have been studied and applied as highly sensitive trace explosive sensors in recent years[3-6]. The advantage of these light-emitting materials is that the electron-rich polymer can readily donate photo-excited electrons to electron-deficient molecules, including many explosive species such as TNT. When the explosive species is in contact with the polymer this electron transfer leads to a rapid decrease in light emission, known as fluorescence quenching, which can be monitored via spectrometer or photodiode[7] to indicate the presence of explosives.

The challenge of using these materials in the field chiefly concerns the collection of trace amounts of explosives in a real-world environment, e.g. windy and unpredictable weather dispersing the particles/vapour plumes. The use of preconcentrator materials is a promising method to address this challenge, where trace levels of explosives are accumulated over time on a substrate surface to aggregate a substantially higher sample mass. Preconcentration is a method commonly used in analytical and environmental chemistry to sorb target analytes for analysis[8-13], where typically a sorbent material with an affinity to the target molecule will subsequently desorb with the application of Joule heating. In environmental analysis the preconcentrator may be placed at a single point in a waterway, for instance, or by using a swab for ion mass spectroscopy as commonly seen in airport security. However, for surveying an area with suspected landmines, a practical method for sample collection must be used to avoid human contact on the ground while quickly and efficiently sampling the area.

Honeybees have been used as environmental sentinels for various pollutants[14-18], since their electrostatic body hair can attract molecules during foraging activity[19]. The concept for explosives collection has been proven in a previous work by some authors of this study[20], where a difference in quenching response between honeybee-collected samples from a clean site, and that from a contaminated site, was evident, though with substantial potential for improvement.

In this study we link two state-of-the-art technologies – organic semiconductor sensing films and honeybees as biomonitoring elements – to establish an improved methodology for landmine detection as a tool for global demining.

2. Materials and Methods

2.1 Honeybees used

Honeybees were selected from a commercial apiary near Gorica, Zadar County (44°02'14.2"N 15°24'10.3"E), since this site is known not to be near any mined areas. The local subspecies of Carniolan bee (*Apis mellifera carnica*), in standard LR hives with two supers, a bottom board, a top board and roof were used. Colonies were prepared for the trial by ensuring sufficient pollen storage to prevent bees from pollen-foraging outside the targeted area, and empty combs were placed to provide enough space for collection over the duration of the trial. Colonies were in good health conditions, evidenced by strong activity and a lack of clinical signs of diseases. Bees were counted according to the modified Liebefeld method[21] with the populations ranging from around 23,000 up to 30,000 bees. Colonies were carefully selected from the apiary in order not to use contaminated colonies. Three colonies were selected and labelled N1, N2 and N4, containing 28,890, 30,105, and 22,815 bees respectively.

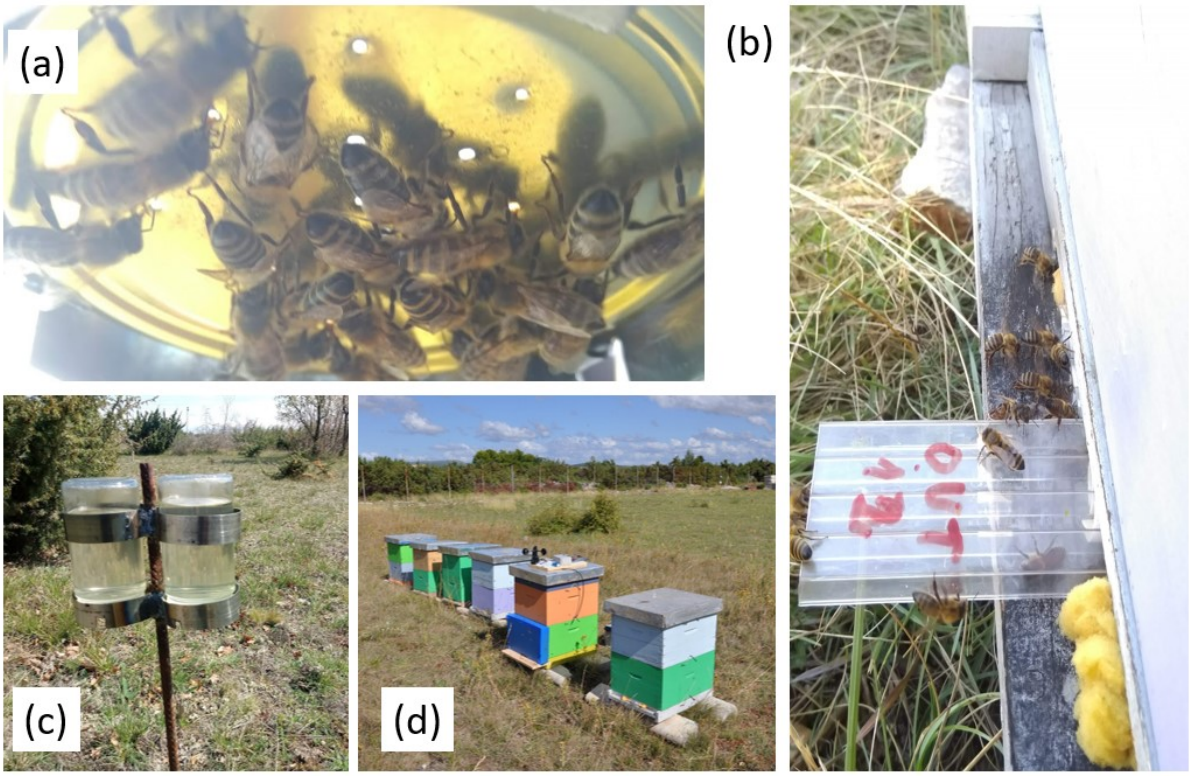


Figure 1 – (a) Honeybees on the underside of the feeder; (b) Bees entering and exiting via the preconcentration cartridges at the colony entrance; (c) Full feeders placed at distance from colonies; (d) Sampling colonies on the test minefield at Benkovac.

In order to keep bees in the area of interest, upside down feeder jars were used (Figure 1a-1d); feeders were deployed in an upside-down configuration to prevent bees from clustering and becoming covered with sugar solution. A sucrose feeder solution of 1 kg sugar dissolved in 1 l tap water was used to both acclimatise and constrain the honeybees in the area of interest. A 720 ml glass jar was filled with the sugar solution and the metal screw-top lid was punctured with 1.5 mm holes to allow drip-feeding. Feeders were initially placed close to the colony entrances to draw the honeybees to the sucrose solution (Figure 1b), and subsequently moved further away to promote flying over the test site (Figure 1c), and bees were observed to readily feed from the sucrose solution (Figure 1d). Fifteen feeders were placed around the test site in order to equally distribute bees across the test minefield.

2.2 Sensor materials & instrumentation

The methodology for preconcentrator preparation and sensor fabrication is outlined in detail in a previous work[20]. Briefly, to make fluorescent sensor films, first glass substrates (Agar Scientific) were cleaned in an ultrasonic bath for 5 minutes in toluene, acetone and propan-2-ol, and dried in a dry nitrogen stream prior to being plasma ashed for 3 minutes in a 100% oxygen plasma (Plasma Technology MiniFlecto). Merck Super Yellow polymer was dissolved in toluene at a concentration of 6.5 $mgml^{-1}$ and sensor films were deposited by spin coating the polymer at 2000 rpm onto the 1 cm^2 clean substrates. Film thicknesses, measured with a Veeco Dektak 150 surface profilometer, were found to be in the region of 100 nm.

The response of the sensors to a number of potential distractant chemicals were assessed as follows: pheromones ethyl stearate, methyl oleate, ethyl oleate, and methyl linoleate were obtained from Merck Sigma Aldrich, and were selected for being four of the most common pheromones found in bee colonies[22]. The pheromones were dissolved in acetonitrile to give typical masses present in colonies in a 20 µl drop for exposure to the sensor films, while reference measurements were made with exposure

to the same volume of clean acetonitrile. Measurements were made in triplicate. Commercially-available Apivar (Veto-pharma) Amitraz in plastic strips (500 mg Amitraz per 15 g strip) was sourced from a local beekeeper in St Andrews. The strips were cut into approximately 1 g pieces for a final mass of 33 mg Amitraz per piece.

Immediately after fabrication, the Photoluminescence Quantum Yield (PLQY) of the sensor films was measured as a reference, then the PLQY was measured again after drop-casting the pheromone solutions and leaving to dry. PLQY measurements of the sensors were performed in an integrating sphere[23] using a Hamamatsu Photonics C9920-02 measurement system with an excitation wavelength of 440 nm.

To fabricate preconcentrators for minefield tests, the fluoropolymer Aflas was purchased from AGC Chemicals Europe Ltd, and dissolved in Tetrahydrofuran (Sigma Aldrich) at a concentration of 155 $mgml^{-1}$. For in-situ placement of the preconcentration material in the hive entrance and exit, sheets of poster canvas were blade-coated with the Aflas solution and cut into squares of approximately 3 cm^2. The squares were rolled into tubes with an approximate diameter of 1 cm and inserted in Standard Lexan plates (1 × 1 cm tube) cut into 10 cm lengths and used as a cartridge with 4 channels, two of which were inserted into the entrance of the hives: one for bees entering the colony and one for bees leaving. This procedure was performed for both the clean site and the test site experiments. At the end of the placement period the preconcentrators were removed from the colony, inserted into glass vials, sealed with Parafilm then placed in airtight sealed bags prior to shipping and testing in St Andrews.

To test the preconcentrators for explosive residue, the canvas square was attached to a heating element placed 1 cm from the fluorescent film sensor in a homemade cell. The sensor was excited with a 405 nm continuous-wave 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 preconcentrator sample to 100°C. The loss in light emission from the sensor film was measured as a function of the initial emission. After measurements had been completed the chamber was flushed with clean nitrogen to clear the chamber of any residual explosive vapour contamination. Amitraz strips were tested against Super Yellow films by using the 1 g piece in place of the canvas preconcentrator

and heating in cycles of 100 seconds heating, 350 seconds cooling, and 100 seconds reheating. The experiment was performed in this way to allow for Amitraz embedded deep in the plastic strip to diffuse from the strip surface.

2.3 Field conditions

Field samples were taken in early April 2019. On the first day, control samples were collected from the clean apiary site, before the colonies were moved to the explosive-contaminated test site in Benkovac, Croatia (Figure 2a). This test site was designed for the testing and validation of mine detectors, with an area of 10,000 m^2 and 1,000 deactivated mines buried in a series of test lanes. Where previously[16] the flight of the bees were aligned with the feeders along the test lanes (Figure 2b), in this test we oriented their flight to be across multiple test lanes (Figure 2c).

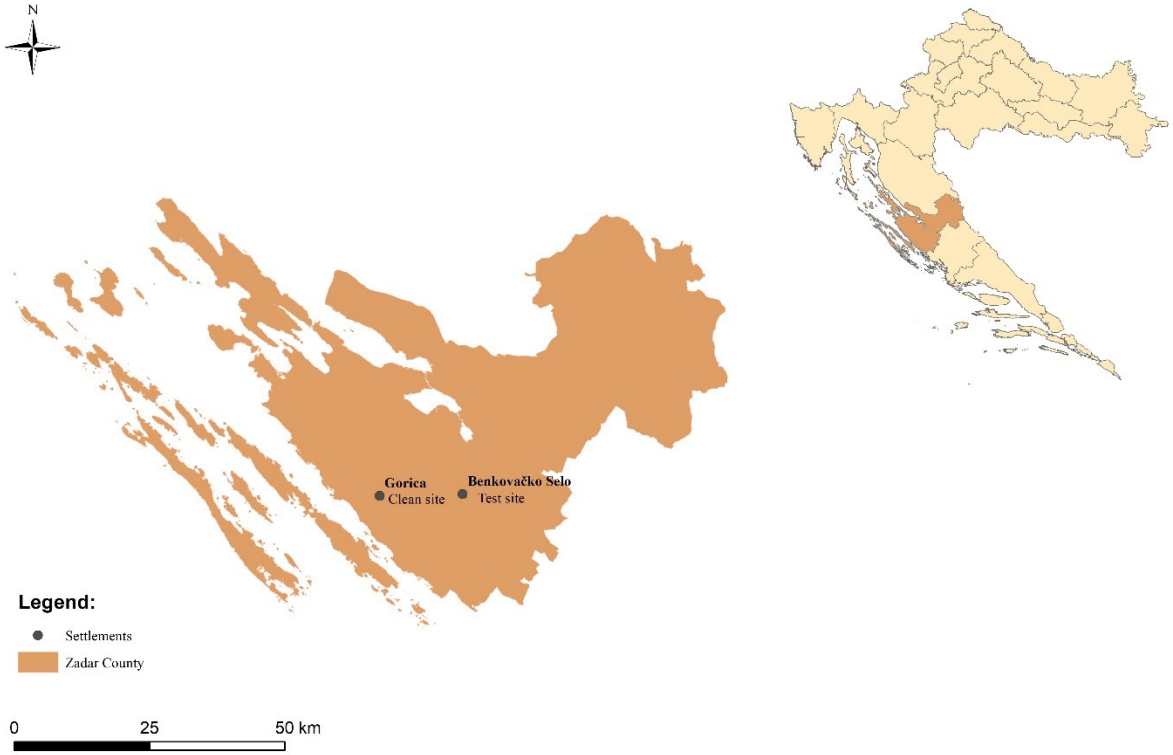
In their search for food bees fly up to 3 km, but have been found to forage in areas more than 10 km away from the colony[24,25]. Most of the foraging flights bees perform, however, are in close range of the colony. The number of foraging flights can be influenced by the distance of the food source; environmental conditions like temperature, wind velocity, and rain; and internal colony conditions, for example colony strength, size of brood, or diseases. The average number of foraging trips is 10 per day[26], while a water collector may make as many as 100 trips per day[27]. In situations when artificial food (sugar syrup) is offered, bees in the vicinity can make up to 100 foraging trips per day[28].

Weather affects bee behaviour, particularly foraging. Table 1 shows that the initial days on-site were comparatively cold which dissuaded bee activity. Meteorological measurements for both the clean site and the Benkovac site recorded temperature and wind speed from 0900 until 1800. Light to heavy rainfall in Benkovac was recorded on the first day of sampling.

Day of April	Temp [°C]	Min	Max	Wind speed [m s^{-1}]	Min	Max
	Average			Average		

8 (Clean site)	**12.6**	11.0	13.5	**5.6**	4.8	6.6
11 (Day 1)	**13.5**	12.1	15.7	**3.5**	2.3	5.1
12 (Day 2)	**10.7**	9.5	11.4	**8.4**	7.5	9.2
13 (Day 3)	**13.3**	9.9	15.6	**6.7**	5.0	9.2
14 (Day 4)	**14.2**	11.0	16.1	**5.1**	3.6	8.3
15 (Day 5)	**17.0**	14.9	18.6	**10.0**	8.0	11.5

Table 1 – Weather conditions for days on-site.



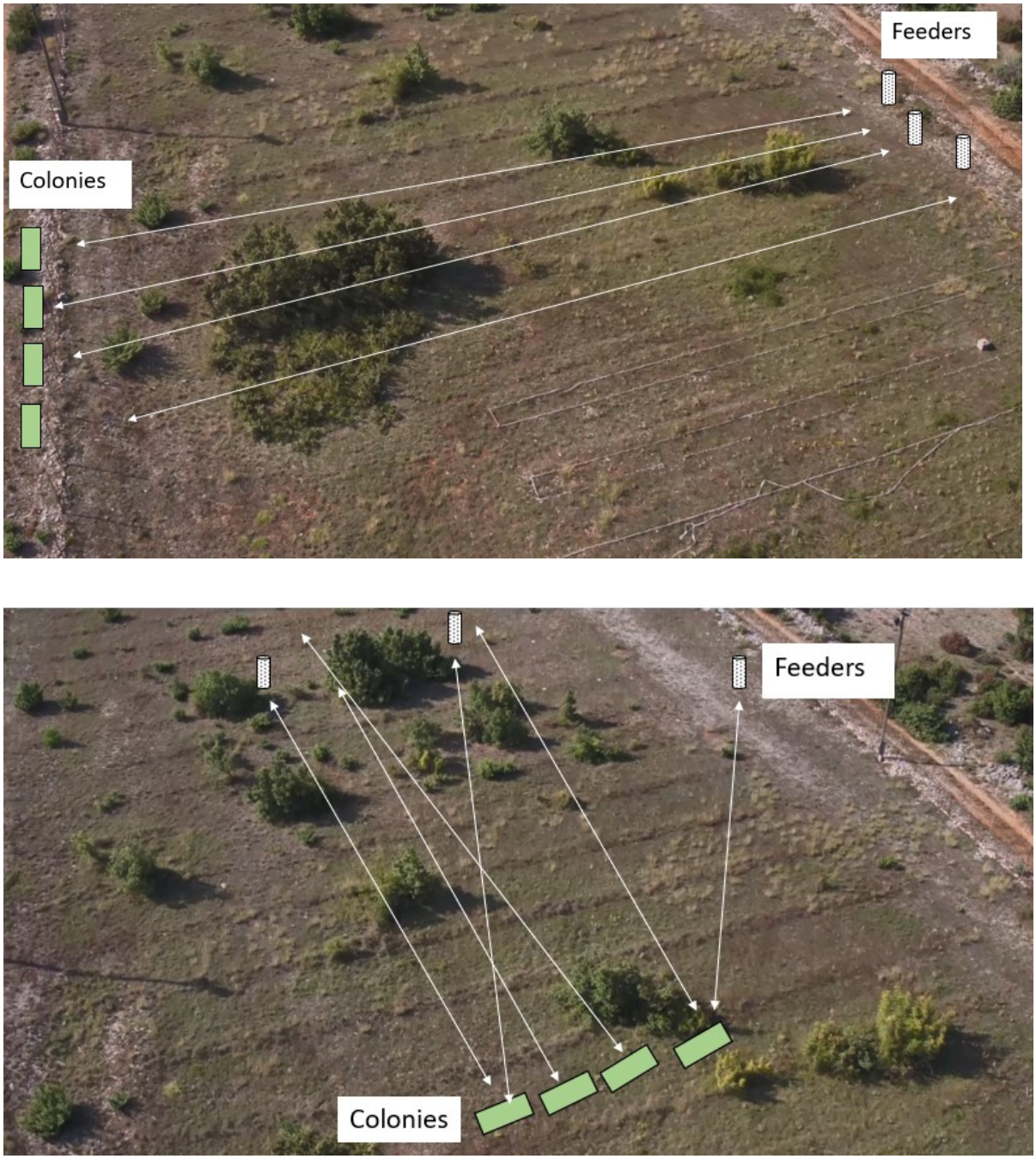


Figure 2 – (a) Sampling site map in Zadar County, Croatia; (b) Colony placement along lanes in previous studies; (c) Colony placement in the current study across lanes.

3. Results & Discussion

3.1 Control samples

The presence of distractants in the atmosphere from agricultural chemicals, naturally occurring compounds, or commercial ingredients can potentially give rise to false positives by causing quenching of sensor emission. To assess and characterise the sensors against distractants, three control tests were conducted: sampling at a clean site known not to be within 5 km of any mine fields; testing the effect of the common anti-varroa pesticide Amitraz on the sensor films; and by testing four common pheromones produced inside the colony.

Exocrine glands in the honeybee produce pheromones which induce different behavioural and physiological responses in other bees in the colony. Pheromones regulate activities in the colony including foraging activity, brood rearing, defence, swarming, orientation, and homeostasis[29,30]. Pheromones are produced by queens, workers, drones, and the brood. Brood pheromones influence the physiology and behaviour of the nurse bees, and also release pollen foraging behaviour[31-33]. Ethyl oleate delays the onset of foraging and is present at high concentrations on the cuticle of the foragers. It is also found as a component of queen and brood pheromones[34]. Given the presence and abundance of brood pheromones and ethyl oleate in the colony, they are reasonable target pheromones to investigate as potential false positive analytes.

The PLQY of Super Yellow sensor films was measured before and after exposure to various bee pheromones and the results are shown in Table 2. The quantity of pheromone deposited on the sensor film corresponded approximately to the total amount present in a typical beehive. Following deposition of the solutions of pheromones, the PLQY of the films were observed to decrease by approximately 10%. The PLQY drops observed for the pheromones are comparable to the pheromone-free reference measurements, where a drop in PLQY can be attributed to photo-degradation of the polymer sensor over a similar time in ambient conditions. We also note that only a fraction of the total pheromones present in the hive would be expected to accumulate in the preconcentrator, and so it is likely that any reductions in sensor PLQY in these tests would be much greater than that arising from desorbed pheromones in preconcentrator field tests. This indicates that the variations seen in measurements of samples collected from clean apiaries is unlikely due to pheromones produced by the bees and brood.

Pheromone	Amount [ng]	PLQY pristine [%]	PLQY post [%]	Fraction of PL remaining
Reference	0.0	43.4	33.8	0.77
Ethyl stearate	9.1	43.4	33.5	0.77
Methyl oleate	29.3	44.5	34.0	0.76
Ethyl oleate	19.5	45.4	36.3	0.80
Methyl linoleate	20.0	44.5	35.5	0.79

Table 2: Drop in PLQY observed when exposing Super Yellow films to pheromones. The PLQY figures are an average of three repeat measurements, and the error associated with these measurements is ±1%.

The effect of Amitraz heated in the sensor chamber and exposed to a Super Yellow film over time was also investigated. The control measurement was a sensor film in the chamber with no Amitraz strip. The control sensor film was observed to undergo some degradation via thermal and photo-oxidation, losing around 12% of its emission, whereas the Amitraz-1 sample gained 2% and Amitraz-2 lost 5%. Slight gains in fluorescence can be attributed to a swelling of the Super Yellow film by desorption of residual solvent in the Amitraz strips. However, no significant quenching behaviour was observed, indicating that Amitraz is not a significant distractant molecule for the sensors.

Finally, samples taken from the clean site were analysed as shown in Figure 3a. We note that two of the twelve samples were damaged in transit and were unable to be analysed. The clean site samples can be seen to be broadly consistent in response, with a small characteristic linear degradation under ambient conditions; one sample however, gave a typical quenching response which may indicate a false positive due to the presence of a distractant, e.g. a pesticide or similar compound in the environment. None the less, the broad agreement between samples as illustrated in Figure 3b indicates a general lack of distractants in the field and the suitability of this site for control measurements.

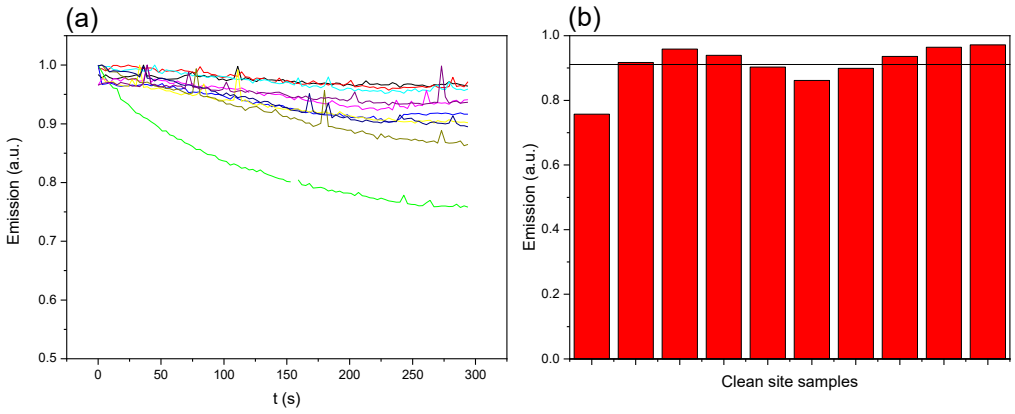


Figure 3 – Quenching response from samples taken from the clean apiary near Gorica, April 2019. 3a shows the quenching response over time of Merck Super Yellow to heated preconcentrators, and 3b shows the remaining emission after exposure. The horizontal black bar shows the average loss in emission of the control samples (from a normalised initial fluorescence signal of 1 a.u.).

3.2 On-site trials

Fluorescence quenching in response to explosive materials were observed in previous trials to cause a decrease in emission of between 2-5% [20], whereas drops of up to 40% were observed in the April 2019 trials as seen in Figure 4. This is partly attributed to orientating the bee colonies across the lanes on Benkovac where in previous trials they were aligned along the lanes, as illustrated in Figures 2b and 2c. By aligning across the lanes, the higher mine density allows higher accumulation of trace explosives on the honeybees, and by using the feeders to gradually draw the bees further from the colony by approximately 150 m (from 50 m to 200 m), the total foraging area is increased.

As described in section 2.3, the weather conditions for the first 4 days on-site were not conducive to bee flight and the observed results were inconclusive. This indicates that this method is suited to operate within a range of ambient temperatures and rainfall; this is however also true for other demining methods, and all demining technologies have operational limitations[35]. For instance, metal detection

works in all climates but does not easily detect plastic mines, while ground-penetrating radar has limited depth range in very wet soil.

The more favourable conditions on Day 5 allowed full foraging activities across the colonies.

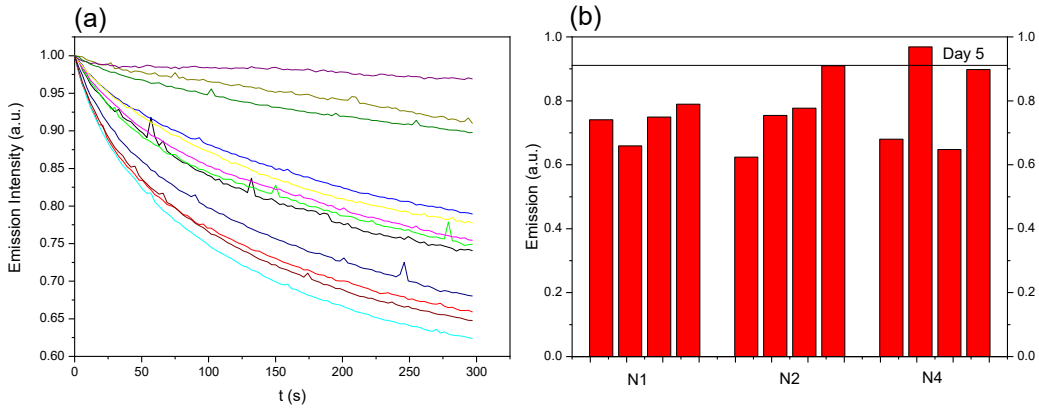


Figure 4 – Quenching response from samples taken from the test site near Benkovac, April 2019. 4a shows the quenching response over time of Merck Super Yellow to desorbed explosives, and 4b shows the remaining emission after exposure. The horizontal black bar shows the average loss in emission of the control samples (from a normalised initial fluorescence signal of 1 a.u.).

The alignment across the lanes (Figure 2c), with a more randomised placement of feeders, encouraged the bees to forage more widely above explosive vapour plumes in the search for the sugar solution food source. Then on return to the colony more material was deposited on the preconcentrator for subsequent analysis by fluorescent sensor. In contrast to the clean site samples (Figure 3), the samples from the test site on Day 5 (Figure 4) show a wide spread of responses, which corresponds to each preconcentrator having a different load of explosive material. Previous work from an author in this group[36] has shown that 0.5 µg of 2,4-DNT deposited on a preconcentrator strip causes a drop in luminescent emission of Super Yellow of approximately 12%. This suggests that there may be quantities of around 1 µg or more of explosives collected per preconcentrator. The spread in response from each preconcentrator and

sensor film can be due to a combination of factors, including the number of bees passing through each channel and the amount of explosive carried (and deposited) per bee.

3.3 Estimation of total explosive mass per colony

An estimation of the amount of explosive carried per bee can be calculated using environmental factors originally used for pesticide load on bees[37]. In that work, three main routes to pesticide exposure are identified: dermal, ingestion, and inhalation. In the context of the present work, dermal contact is the key route since explosives on the body of the bee taken up via foraging behaviours in the general environment are most likely to be deposited onto the preconcentrator. Warnke[38] and Greggers et al.[39] described the accumulation of electrostatic charge on honeybee bodies during flight, which plays a crucial role in pollination[19,40] via transfer of pollen through dermal contact. Van der Steen and colleagues[41,42] described the same mechanism for biomonitoring of pesticides, heavy metals and plant pathogens in certain environments.

A useful method for estimating the mass of explosives transferred from the field by foragers back into the colony can be made by adapting the model found in the work by Crenna et al.[37] for pesticide contamination. First, we consider the following rate equation to estimate the change in concentration of explosives (C_E) on site with time:

$$\frac{dC_E}{dt} = k_E N_{\text{mines}} - C_E \frac{\ln(2)}{t_{1/2}} \quad (1)$$

Where k_E is the mass of explosives released per mine per day, N_{mines} are the number of mines per m^2 and $t_{1/2}$ is the half-life of explosives due to degradation or dispersal out of the area. In steady-state conditions it follows that

$$C_E = \frac{1}{\ln(2)} \sum k_{Ej} N_{\text{mines}j} t_{1/2j} \quad (2)$$

Where the subscript *j* represents each different type of explosive present (e.g. DNT, DNB, and TNT) in the mines. C_E can then be used in Equation 3 to estimate the total amount of explosive in a bee colony:

$$M_{\mathrm{E}} = \frac{N_{\mathrm{f}}\, Q_{\mathrm{f}}^{\mathrm{d}}\, f_{\mathrm{A}}\, C_{\mathrm{E}}}{C_{\mathrm{P}}} \qquad (3)$$

Where M_{E} is the total mass of explosive brought back to the hive, N_{f} is the number of foragers per colony, $Q_{\mathrm{f}}^{\mathrm{d}}$ is the quantity of pollen brought back per bee per day, f_{A} is the fraction of bee body surface in contact with explosive residues, and C_{P} the concentration (kg m^{-2}) of pollen on site.

The flux from some of the most common anti-personnel and anti-tank mines has been characterised by previous researchers[43,44], with PMA-1, PMA-2, TMM-1, and TMA-5 mines emitting chiefly 2,4-DNT, DNB and TNT at different rates in ambient conditions. For instance, a PMA-1 mine has a flux of 1880, 4700, and 230 ng day^{-1} for DNT, DNB and TNT respectively, and the half-life of each explosive is again respectively 25 days, 10 days, and 1 day.

The precise distribution of landmines in the test site is not known due to its function as a blind test site for demining sensor validation; however, the total number (1,000) is known, as is the general proportion of anti-personnel mines and anti-tank mines. The amount of pollen per m^2 available on-site is estimated to be 0.12 g m^{-2}, which is a reasonable value for this terrain and time of year[45]; $Q_{\mathrm{f}}^{\mathrm{d}}$ = 7.8 mg bee^{-1} day^{-1} and f_{A} is 30%.[46]

An example estimation using these figures, for a random distribution of 1,000 mines and forager strengths of 15,000, 16,000, and 11,000 bees per hive, is shown in Table 3:

Mine	**No. mines**	**DNT [mg day^{-1}]**	**DNB [mg day^{-1}]**	**TNT [mg day^{-1}]**
PMA-1	530	1.88	4.70	0.23
PMA-2	260	0.49	0.57	0.05
TMM-1	140	2.31	0.40	0.86
TMA-4	70	28.80	6.50	1.60
	Total [mg]	3.46	3.15	0.37
	C_E [mg m^{-2}]	0.017		
	Hive N1	**Hive N2**	**Hive N4**	
No. foragers	15,000	16,000	11,000	
Total explosives in hive [mg]	**5.3**	**5.7**	**3.9**	

Table 3 – Example estimation of the total explosives in three hives

The model shows between around 3.9 mg and 5.7 mg per colony may be estimated corresponding to the colony strengths during this trial. Since there are four preconcentrators per hive, this crude estimate would suggest as much as 1 mg of explosives may pass through each preconcentrator tube per day, of which a fraction would be collected as the bees enter the hive.

This quantity of explosives is plausibly sufficient for subsequent detection by the organic semiconductor sensors used in this work, since the limit of detection is 55 ng cm^{-2}. Previous results characterising sensor response from known amounts of explosive[36,47] indicate that 0.5 μg of DNT on an Aflas preconcentrator gives a 15% drop in luminescence from a Super Yellow film. Figure 5 shows between 5 – 38% quenching, indicating a lower mass of collected explosive at 5 ng and an upper mass of well over 1 μg per field sample. The model also estimates that an individual bee might carry 35 ng of explosives per trip.

An alternative approach to estimate C_E is by considering the mass of explosives-contaminated dust that might be collected by foraging honeybees originating from the amount of explosive leached from landmines into the soil[43]. For instance, in the case of a PMA-1 mine, measurements of explosives contamination in the soil above landmines suggest as much as 10 μg of TNT and 100 μg of DNT can be present per kg of soil. If one considers a landmine buried 0.1 m deep that contaminates an area of 0.1 m^2 with leached explosives, of which the top 1 mm depth is a mobile dust layer, C_E can be estimated to be of the order of 2.2 ng cm^{-2} for a soil density of 2000 kg m^{-2}. This reduces the total amount of explosive per colony by a factor of 100 from the estimate above, and may represent a lower limit for explosive collection by bees in an area with limited food sources.

This model may be a useful tool for estimating the amount of free explosive on a site available for detection and, following further detailed validation, may even be useful to estimate the number of mines on a given site from a quantifiable mass of accumulated explosive. Used in conjunction with local

knowledge, other demining tools, and laying maps, a more accurate picture of a minefield may be obtained, leading towards more targeted and safer demining.

4. Conclusions

A passive method for sampling explosives in the field has been developed that aspires towards an effective procedure for surveying a suspected mined area as a complementary tool for humanitarian demining. In the passive methodology the bees were constrained in the contaminated location with artificial food and brought explosive materials back to the colony to adsorb to a preconcentrator in the entrance, which was subsequently tested using organic semiconductor sensors showing a significant response against control samples. An uncontaminated site, a commonly used pesticide, and typical bee pheromones were tested and found not to give false positives, while samples from the test site gave strong quenching behaviour with up to 40% loss of light emission. The drawback to this method is the dependency on the weather, where low temperatures can prevent bees from foraging. However, the results indicate that this method has potentially high impact for humanitarian demining, and a model developed in conjunction with the method may be a useful means for estimating the total explosive load of a given area, and estimates an upper limit of around 1 mg cm^{-2} collected material per preconcentrator strip per day.

Acknowledgements

We acknowledge Iain Campbell and Gordon Clauss for scientific discussions and contributions in earlier field work which helped inform the design of the current study. We thank Zlatko Elveđi for supplying the bees and Prof David Evans for supplying Amitraz strips. This project has received funding from NATO Science for Peace & Security under grant agreement MYP G5355, and the Engineering and Physical Sciences Research Council under EP/K503940/1.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Data supporting this research can be found at https://doi.org/10.17630/bf7a117d-9e9f-419f-855c-add2b1e10449[48].

References

1 Lugo, J. H., Zoppi, M. & Molfino, R. Design and Kinematic Modeling of a Screw-Propelled Mobile Robot to Perform Remote Explosive Scent Tracing Filter Sampling in Forest during Humanitarian Demining. *Advances in Cooperative Robotics*, 699-715 (2017).

2 Fjellanger, R. *Remote explosive scent tracing - a method for detection of explosive and chemical substances*. Vol. 167 (2004).

3 Rose, A., Zhu, Z. G., Madigan, C. F., Swager, T. M. & Bulovic, V. Sensitivity gains in chemosensing by lasing action in organic polymers. *Nature* **434**, 876-879, doi:10.1038/nature03438 (2005).

4 Thomas, S. W., III, Joly, G. D. & Swager, T. M. Chemical sensors based on amplifying fluorescent conjugated polymers. *Chemical Reviews* **107**, 1339-1386, doi:10.1021/cr0501339 (2007).

5 Sohn, H., Sailor, M. J., Magde, D. & Trogler, W. C. Detection of nitroaromatic explosives based on photoluminescent polymers containing metalloles. *Journal of the American Chemical Society* **125**, 3821-3830, doi:10.1021/ja021214e (2003).

6 Toal, S. J. & Trogler, W. C. Polymer sensors for nitroaromatic explosives detection. *Journal of Materials Chemistry* **16**, 2871-2883, doi:10.1039/b517953j (2006).

7 Gillanders, R. N., Samuel, I.D.W., Turnbull, G.A. A Low-Cost, Portable Optical Explosive-vapour Sensor. *Sensors and Actuators B-Chemical* **245**, 334-340 (2017).

8 Camara, M. *et al.* in *28th European Conference on Solid-State Transducers* Vol. 87 *Procedia Engineering* (eds G. Sberveglieri & V. Ferrari) 84-87 (2014).

9 Cizek, K. *et al.* Integrated explosive preconcentrator and electrochemical detection system for 2,4,6-trinitrotoluene (TNT) vapor. *Analytica Chimica Acta* **661**, 117-121, doi:10.1016/j.aca.2009.12.008 (2010).

10 Egorov, O. B., O'Hara, M. J. & Grate, J. W. Equilibration-based preconcentrating minicolumn sensors for trace level monitoring of radionuclides and metal ions in water without consumable reagents. *Analytical Chemistry* **78**, 5480-5490, doi:10.1021/ac060355m (2006).

11 Grate, J. W. *et al.* in *2007 Ieee Sensors, Vols 1-3 IEEE Sensors* 1357-1360 (2007).

12 Houser, E. J. *et al.* Rational materials design of sorbent coatings for explosives: applications with chemical sensors. *Talanta* **54**, 469-485, doi:10.1016/s0039-9140(00)00545-2 (2001).

13 Voiculescu, I. *et al.* Micropreconcentrator for enhanced trace detection of explosives and chemical agents. *Ieee Sensors Journal* **6**, 1094-1104, doi:10.1109/jsen.2006.881431 (2006).

14 Barisic D, Bromenshenk JJ, Kezic, N. & Vertacnik, A. in *Honey Bees: Estimating the Environmental Impact of Chemicals* (eds J Devillers & M Pham-Delegue) 160-185 (Taylor & Francis, 2002).
15 Bromenshenk, J. J. *et al.* Bees as Biosensors: Chemosensory Ability, Honey Bee Monitoring Systems, and Emergent Sensor Technologies Derived from the Pollinator Syndrome. *Biosensors-Basel* **5**, 678-711, doi:10.3390/bios5040678 (2015).
16 Girotti, S., Ghini, S., Maiolini, E., Bolelli, L. & Ferri, E. N. Trace analysis of pollutants by use of honeybees, immunoassays, and chemiluminescence detection. *Analytical and Bioanalytical Chemistry* **405**, 555-571, doi:10.1007/s00216-012-6443-3 (2013).
17 Rodacy, P. J., Bender, S. F. A., Bromenshenk, J. J., Henderson, C. B. & Bender, G. in *Detection and Remediation Technologies for Mines and Minelike Targets Vii, Pts 1 and 2* Vol. 4742 *Proceedings of the Society of Photo-Optical Instrumentation Engineers (Spie)* (eds J. T. Broach, R. S. Harmon, & G. J. Dobeck) 474-481 (2002).
18 Zarić, N. M. *et al.* Honeybees as sentinels of lead pollution: Spatio-temporal variations and source appointment using stable isotopes and Kohonen self-organizing maps. *Science of The Total Environment* **642**, 56-62, doi:https://doi.org/10.1016/j.scitotenv.2018.06.040 (2018).
19 Vaknin, Y., Gan-Mor, S., Bechar, A., Ronen, B. & Eisikowitch, D. The role of electrostatic forces in pollination. *Plant Systematics and Evolution* **222**, 133-142, doi:10.1007/bf00984099 (2000).
20 Gillanders, R. N. *et al.* Preconcentration techniques for trace explosive sensing. *Science of The Total Environment* **658**, 650-658, doi:https://doi.org/10.1016/j.scitotenv.2018.12.160 (2019).
21 Delaplane, K. S., van der Steen, J. & Guzman-Novoa, E. Standard methods for estimating strength parameters of Apis mellifera colonies. *Journal of Apicultural Research* **52**, doi:10.3896/ibra.1.52.1.03 (2013).
22 Le Conte, Y., Sreng, L. & Trouiller, J. The recognition of larvae by worker honeybees. *Naturwissenschaften* **81**, 462-465, doi:10.1007/s001140050110 (1994).
23 Greenham, N. C. *et al.* Measurement of absolute photoluminescence quantum efficiencies in conjugated polymers. *Chemical Physics Letters* **241**, 89-96, doi:10.1016/0009-2614(95)00584-q (1995).
24 Visscher, P. K., Crailsheim, K. & Sherman, G. How do honey bees (Apis mellifera) fuel their water foraging flights? *Journal of Insect Physiology* **42**, 1089-1094, doi:10.1016/s0022-1910(96)00058-3 (1996).
25 Eckert, J. E. The flight range of the honeybee. *Journal of Agricultural Research* **47**, 257-285 (1933).
26 Winston, M. L. *The biology of the honey bee*. (Harvard University Press, 1991).
27 *Apiculture Bulletin* (ed Plant & Animal Health Branch) (British Columbia Ministry of Agriculture, 2015).
28 Butler, C., Jeffree, E. & Kalmus, H. The behaviour of a population of honeybees on an artificial and on a natural crop. *Journal of Experimental Biology* **20**, 65-73 (1943).
29 Bortolotti, L. & Costa, C. in *Neurobiology of Chemical Communication* (ed C. Mucignat-Caretta) (CRC Press/Taylor & Francis, 2014).
30 Conte, Y. L. & Hefetz, A. Primer pheromones in social hymenoptera. *J Annu. Rev. Entomol.* **53**, 523-542 (2008).
31 Apidologie, T. P. J. Cued in: honey bee pheromones as information flow and collective decision-making. **35**, 217-226 (2004).
32 Le Conte, Y., Mohammedi, A. & Robinson, G. E. Primer effects of a brood pheromone on honeybee behavioural development. *Proceedings of the Royal Society B-Biological Sciences* **268**, 163-168, doi:10.1098/rspb.2000.1345 (2001).

33 Ma, R., Villar, G., Grozinger, C. M. & Rangel, J. Larval pheromones act as colony-wide regulators of collective foraging behavior in honeybees. *Behavioral Ecology* **29**, 1132-1141, doi:10.1093/beheco/ary090 (2018).
34 Muenz, T. S., Maisonnasse, A., Plettner, E., Le Conte, Y. & Rossler, W. Sensory reception of the primer pheromone ethyl oleate. *Naturwissenschaften* **99**, 421-425, doi:10.1007/s00114-012-0909-1 (2012).
35 Habib, M. K. *Humanitarian Demining: Difficulties, Needs and the Prospect of Technology*. (2008).
36 Glackin, J. M. E. *Explosive vapour sensing with organic and hybrid semiconductors* PhD thesis, University of St Andrews, (2020).
37 Crenna, E., Jolliet, O., Collina, E., Sala, S. & Fantke, P. Characterizing honey bee exposure and effects from pesticides for chemical prioritization and life cycle assessment. *Environment International* **138**, doi:10.1016/j.envint.2020.105642 (2020).
38 Warnke, U. Effects of Electric Charges on Honeybees. *Bee World* **57**, 50-56, doi:10.1080/0005772X.1976.11097592 (1976).
39 Greggers, U. *et al.* Reception and learning of electric fields in bees. *Proceedings of the Royal Society B-Biological Sciences* **280**, doi:10.1098/rspb.2013.0528 (2013).
40 Clarke, D., Morley, E. & Robert, D. The bee, the flower, and the electric field: electric ecology and aerial electroreception. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology* **203**, 737-748, doi:10.1007/s00359-017-1176-6 (2017).
41 Gutiérrez, M. *et al.* Assessing heavy metal pollution by biomonitoring honeybee nectar in Cordoba (Spain). *Environmental Science and Pollution Research* **27**, 10436-10448, doi:10.1007/s11356-019-07485-w (2020).
42 Van der Steen, J. J. M. *Beehold, the colony of the honeybees (Apis mellifera L) as a bio-sampler for pollutants and plant pathogens*, Wageningen UR, (2016).
43 Jenkins, T. F. *et al.* Chemical signatures of TNT-filled land mines. *Talanta* **54**, 501-513, doi:https://doi.org/10.1016/S0039-9140(00)00547-6 (2001).
44 Leggett, D. C. *Release of explosive-related vapors from land mines*. (US Army Corps of Engineers, Engineer Research and Development Center, 2001).
45 Hicks, D. M. *et al.* Food for Pollinators: Quantifying the Nectar and Pollen Resources of Urban Flower Meadows. *Plos One* **11**, doi:10.1371/journal.pone.0158117 (2016).
46 Poquet, Y. *et al.* A Pragmatic Approach to Assess the Exposure of the Honey Bee (Apis mellifera) When Subjected to Pesticide Spray. *Plos One* **9**, doi:10.1371/journal.pone.0113728 (2014).
47 Glackin, J. M. *et al.* Explosives detection by swabbing for improvised explosive devices. (2020).
48 Gillanders, R. N. *et al.* (University of St Andrews Research Portal, 2021).