Controlled biological and biomimetic systems for landmine detection

Maki Habib

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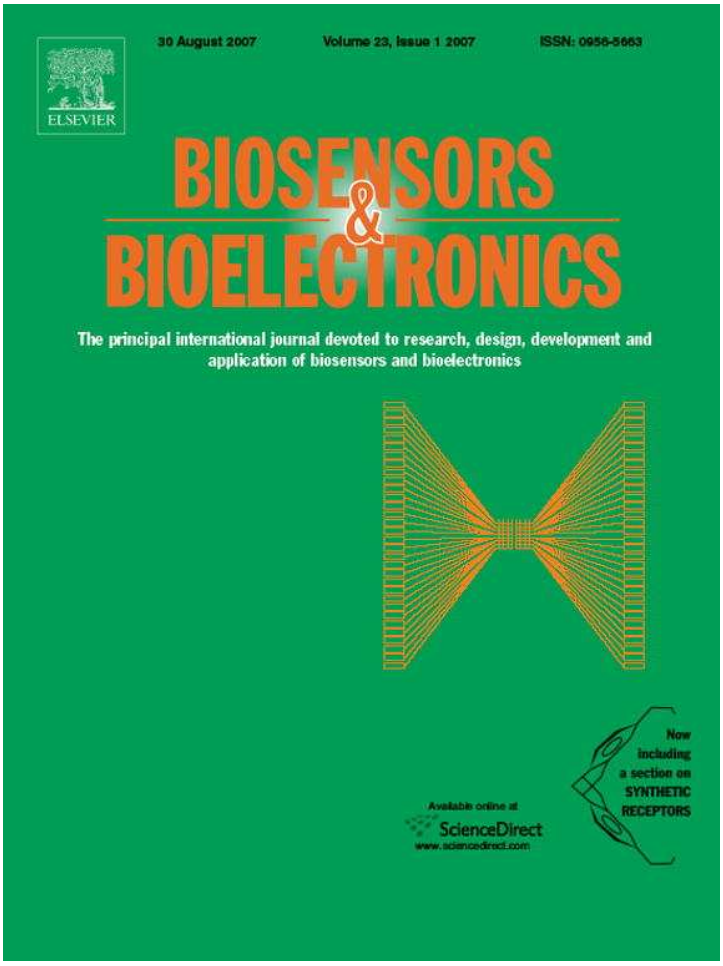
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Review

Controlled biological and biomimetic systems for landmine detection

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Abstract

Humanitarian demining requires to accurately detect, locate and deactivate every single landmine and other buried mine-like objects as safely and as quickly as possible, and in the most non-invasive manner. The quality of landmine detection affects directly the efficiency and safety of this process. Most of the available methods to detect explosives and landmines are limited by their sensitivity and/or operational complexities. All landmines leak with time small amounts of their explosives that can be found on surrounding ground and plant life. Hence, explosive signatures represent the robust primary indicator of landmines. Accordingly, developing innovative technologies and efficient techniques to identify in real-time explosives residue in mined areas represents an attractive and promising approach. Biological and biologically inspired detection technology has the potential to compete with or be used in conjunction with other artificial technology to complement performance strengths. Biological systems are sensitive to many different scents concurrently, a property that has proven difficult to replicate artificially. Understanding biological systems presents unique opportunities for developing new capabilities through direct use of trained bio-systems, integration of living and non-living components, or inspiring new design by mimicking biological capabilities. It is expected that controlled bio-systems, biotechnology and microbial techniques will contribute to the advancement of mine detection and other application domains. This paper provides directions, evaluation and analysis on the progress of controlled biological and biomimetic systems for landmine detection. It introduces and discusses different approaches developed, underlining their relative advantages and limitations, and highlighting trends, safety and ecology concern, and possible future directions.

Keywords: Mine detection; Humanitarian demining; Biomimetic; Biosensors; Landmines; Genetically engineered; Bio-systems

Contents

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1. Introduction

Landmines (antipersonnel (AP) and antitank (AT) mines) are prominent weapons, and they are harmful and effective, yet cheap, easy to make and lay. It is so difficult and dangerous to find and destroy them due to their unknown positions. Landmines subject people life to a continuous danger, deny access to the land and its resources, affect development and rebuilding process, etc. Besides this, the medical, social, economic, and environmental consequences are immense (O'Malley, 1993; Blagden, 1993; Habib, 2002). It is estimated that 800 persons are killed and 1200 maimed each month by landmines around the world (ICRC, 1998), while survivors face terrible physical, psychological and socio-economic difficulties.

The canonical approach to humanitarian demining aims to have efficient tools that can accurately detect, locate and deactivate/remove every landmine, unexplored ordnance (UXO), and other buried mine-like objects as fast and as safe as possible while keeping cost to a minimum. The efficient fulfillment of such a task represents vital prerequisites for any region to recover from landmines and associated battlefield debris by making land safer and allows people to use it without fear. Such a process involves a high risk and a great deal of effort and time, which results in high clearance cost per surface unit.

The development of new demining technologies is difficult because of the tremendous diversity of terrains and climatic conditions, and due to the wide variety of landmines. These mines range from very simple devices to high technology, and modern landmines are fabricated from sophisticated non-metallic materials and incorporated advanced electronics.

Many methods and techniques have been developed to detect explosives and landmines (Habib, 2001). However, the performance of the available mine detection technologies are limited by sensitivity and/or operational complexities due to type of terrain and soil composition, vegetation, mine size and composition, climatic variables, burial depth, grazing angle, and ground clutter, such as, shrapnel and stray metal fragments that produce great number of false positive signals and slow down detection rates to unacceptable levels. It is almost impossible with the current technology to assure the detection of every single mine that has been laid within an area. It is estimated that the current rate of mine clearance is about 10–20 times lower than the rate of ongoing continuous laying of mines, i.e., for every mine cleared, 10–20 mines are laid. Current mine detection technologies and techniques are labor intensive, time consuming, very dangerous, expensive, and low technology step within the demining process. Demining is costly and searching an area that is free of mines is adding extra high cost. Hence, the first essential objective should be to identify what areas are mined by having sensing technology that can facilitate surveying and reducing suspected mined-area.

The solution to this problem is very difficult because, given the nature of landmines and the requirements of humanitarian demining, any device or technology dedicated for humanitarian demining must be at least 99.6% reliable to assure safety of the operators and the people whom will use the land (Blagden, 1993; Habib, 2002), and a 100% to a certain depth according to International Mine Action Standards (IMAS). Hence, it becomes urgent to develop detection (individual mine, and area mine detection), identification and removal technologies and techniques to increase demining efficiency by several orders of magnitude to achieve a substantial reduction to the threat of AP mines within a reasonable timeframe and at an affordable cost (Habib, 2007). New technology, techniques, tools have emerged in the last decade that improve the ability to look beneath the ground surface in pursuit of hazards. The emerged technology and techniques have the ability to identify dense objects that might be UXO, but there is no good methodology for distinguishing between inert metal fragments, dummy rounds, and potentially energetic remnants. Accordingly, new innovative approaches are needed to achieve the stated requirements and accelerate the process of demining.

It has been recognized that all landmines leak with time small amount of their explosives that can be found in and on the surrounding ground and plant life. Hence, explosive signatures may represent the robust primary indicator of a landmine and UXOs. Accordingly, developing efficient techniques and tools to identify explosives residue in mined areas may yield attractive and promising results. Bio-inspired sensor technologies have identified as a potential solution to the problem of detecting pico-gram quantities of either explosive vapor or particles. It must be noted from experience that mines do not seem to release significant trinitrotoluene (TNT) vapor or other explosive materials after more than 18 months of burial. Under certain circumstances and in order to improve the probability of detection rate, it might be necessary to induce the evaporation of molecules of explosives using microwaves. Such a procedure has yet to be investigated, but seems to be a valid research topic.

Animals and other species have senses more acute than those of humans. Biological systems offer excellent examples of highly sensitive, versatile, and robust sensors. Even relatively simple organisms can boast sophisticated abilities to sense their environments and move about in them. Sharks and hawks are examples of animals with strong senses. A shark's sense of smell allows it to detect one drop of blood in an area of water equivalent to the size of an Olympic swimming pool. Birds of prey have visual abilities that are extremely well-tuned, allowing them to spot small animals very far away. Insects have been used for many years to collect environmental information, such as the presence of pollutants or trace materials on plants. Research on controlled biological and biomimetic systems has been under consideration by different research groups around the world and

for different purposes and applications. Many research questions remain and further research are still needed.

Four categories of research directions can be recognized in relation to the biological and bio-inspired approaches for the detection of landmines, explosives and other chemical residues. The main research directions can be categorized as follow:

(a) Bio-hybrid systems. This category focuses on the possibility to integrate a suitable technology with a bio-system to boost its natural abilities.
(b) Bio-systems. The research within the category aims to understand and conclude how existing bio-systems can be trained and used efficiently as a stand detection tools.
(c) Genetically engineered bio-systems. This may include animals, insects, bacteria and plants.
(d) Biomimetic systems. Exploring technologies that exploit natural abilities of bio-systems and biological organisms to get new understanding and inspiration that lead to build new systems and hardware. Researchers are studying wide range of bio-systems and are trying to mimic (not necessary duplicating 100%) certain natural capabilities particularly where the performance of bio-systems exceeds the available artificial systems and technologies.

The first category is focusing on studying and understanding the full range of species combined with creative thinking, design and innovative technology in association with possible and relevant applications. Bio-hybrid systems aim to boost the natural capabilities of selected biological systems to support certain applications, and solutions that are more than just learning from nature. Application examples of such a category may include:

- Biological molecules and cells can be merged with approved microelectronics and micro-systems technology. In such a case, the technology interacts with the bio-system as a whole or with certain component of it, and performs or monitors an assigned task accordingly.
  a. A certain species of beetle that lays its eggs in freshly burned wood has a unique sensory organ under its wings that can discern a forest fire's infrared signature (Rudolph, 1999) from about 60 km away. Understanding how the beetle's sensor works opens the possibility to use them in detecting forest fires, using suitable bio-interface design, before any humans may see it, and also to replicate the sensor for other applications. Biologically, the beetles use a combination of sensors, some to look for smoke and other to detect the infrared emission of the forest fire. A small radio tag can be integrated with the beetle through bio-interface to help tracking them and receive relevant alarms when smoke is detected (see Fig. 1).
  b. Possibility to monitor a patient's blood flow, blood pressure and temperature with tiny, implanted devices.
  c. A brain chip might be used to replace damaged part of the brain, such as, replacing the hippocampus, where the storage of memories is coordinated, in patients affected by strokes, epilepsy or Alzheimer's disease.

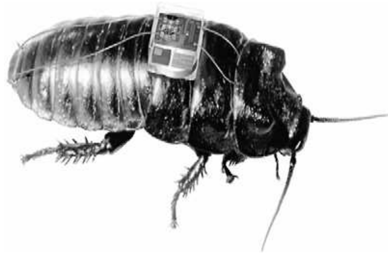

Fig. 1. A beetle carried a sensor with RF tag (Rudolph, 1999).

- External integration of microelectronics and micro-systems technology, i.e., the bio-system is just a passive carrier of the technology. Bees were under study for consideration as flying mine detectors by tracing their daily journey through an integrated RF tag. The main purpose is to check if there is any particle of TNT residue attached into their body during the journey.

The second category extends the studies to examine whether an organism, without the aid of technology, simply takes advantage of existing bio-systems, can be trained and used in direct support of certain task, such as locating mines, explosives, drugs, etc. Application examples of such a category may include,

- Social land animals, such as dogs, bees, rats, pigs, etc. are highly trainable and respond well to positive reinforcement and rewards. Dogs have long been used to sniff out landmines in postwar regions. But, now other competitions are arising from rats and possibly pigs as the efforts highlight the quest to find low-cost alternatives to safely detect underground explosives. The bees' have impressive ability to be trained and locate the residue of TNT.
- In the sea, trained bottlenose dolphins were helping to locate potentially lethal and obstructive anti-ship mines. Dolphins demonstrate an unsurpassed ability to detect and identify submerged and buried objects into the seabed and can distinguish them from clutter, such as coral rock, and man-made debris through the use of its biological sonar. The dolphin sonar system is tolerant to noise and reverberant environment (Helweg et al., 2000). Hence, Dolphins have been employed to assist with sea mine countermeasure efforts. Sea-lions lack sonar, but they do possess superb directional underwater hearing and the ability to see in near-darkness. Sea-lions are used by the US navy to retrieve submerged objects, and have also been trained to locate divers, attach restraint devices or markers, and make a speedy getaway.

The third category focuses on the development of biotechnology and genetically modified microbial techniques to help in environmental cleaning, waste management, detection of (bio-agents, explosives and landmines), etc. Application examples of such a category may include,

- Genetically engineered soil bacteria aims to cause the bacteria to fluoresce under laser light when in contact with certain explosives residue.
- Genetically engineered plants for different purposes, such as:

a. Transform toxic mercury to a much weaker, virtually harmless, form known as elemental mercury.
b. Detect chemical or explosive residue in land.

In the fourth category, biomimetic is based on biological inspiration and attempts to produce engineered systems that possess characteristics, and resemble or function like living systems (Vogel, 1996), i.e., new technologies can be developed from nature. Biomimetic systems can be designed by extraction of the biological principles that govern them, which is possible only by a synergy of the basic and applied sciences. Biomimetics has been utilized as a mechanism for technological advancement in an attempt to facilitate the realization of the novel features in nature. There is a growing awareness among scientists and engineers that biological systems can be a valuable source of inspiration for man-made materials and systems by mimicking novel aspects of biological systems. Scientists mimic everything from worm brains to fish jaws to create better technologies. Application examples of such a category may include:

- Researchers were able to replicate beetle's sensors and the US air force is trying to integrate it with their fighter to help identify bombing targets from far distances (Roach, 2003).
- Los Alamos researchers have developed miniaturized biosensors that can detect bio-agents and markers for disease by mimicking cellular membranes and depositing these membranes onto optical chips. By copying nature's functions using nanoscaled materials, the useful properties of sensors can be optimized permitting entirely new approaches, for example, the early detection of disease.
- Hair-cells are used for many biological functions including invertebrate hearing and equilibrium, insect flow sensing, fish lateral line flow sensing, and insect priopreceptors.
- Fish rely on a row of specialized sensory organs along the sides of their bodies, called the lateral line to provide guidance for synchronized swimming, predator and obstacle avoidance, and prey detection and tracking. A research team at the University of Illinois at Urbana-Champaign has built an artificial lateral line that could be integrated with a submarine or underwater robot aiming to extend their ability to detect and track moving underwater targets, and avoid collisions with moving or stationary objects. The artificial lateral line consists of an integrated linear array of micro fabricated flow sensors, with the sizes of individual sensors and spacing between them matching those of their biological counterpart.
- Nature has evolved a very efficient sensing system for a small worm, about 50 μm in diameter, much smaller than the diameter of a human hair, and with only 302 neurons altogether. The worms are very good at finding mates, finding food, avoiding predators and finding a good home. Research group at Georgia Tech, is studying how sensory and memory related genes are expressed and regulated in tiny micro-sized worms by observing the worms' behavior on an equally micro-sized chip. Such efforts aim to create brain-inspired sensors and gain new insight into how memories are formed in the human brain.
- Behavior-based robotics uses biology as the best model for understanding intelligence. Understanding high-mobility, robustness, learning, adaptation, etc. of biological system helps to develop robots that can make difficult missions possible, such as search-and-rescue, detection, sampling, and removal of biohazards and mines.
- Create better control of legged robots and human prostheses using biological inspiration. Such an approach requires understanding on how the nervous system communicates with joints and muscles for movement and balance. Then, design systems that closely replicate the natural fluid movement of animals and humans. The target goal is to help build robots with better mobility and prosthetics with natural movement more similar to a real limb.
- Biologists and engineers are cooperating to develop a new class of microrobotics, spawning a paperclip-sized mechanical flying insect that will weigh one-tenth of a gram and will measure 2.54 cm (1 in.) from wing tip to wing tip. The result will be applied in search and rescue missions, mine detection and even planetary exploration.

The remaining part of the paper is organized to discuss the importance and provide directions, evaluation and analysis on the progress of controlled biological and Biomimetic systems for landmine detection. It presents and discusses different approaches developed, such as bio-systems, hybrid bio-systems, genetically engineered bio-systems, and Biomimetic systems while underlining their relative advantages and limitations, and highlighting trends, safety and ecology concern, and possible future directions.

2. Dogs

Dogs can be trained and clearly learn to find the scent of any explosive filler and case material or container. The odor discrimination super skills of dogs considerably exceed the abilities of laboratory equipment that are used to investigate those skills, and hence limiting the ability of researchers to study the capabilities of dogs for detection of mines. Currently, there is no technology that can detect as wide a range of explosive devices as a dog does. Dogs are considered so far the best detectors of explosives. Their sensitivity to the substances associated with landmines is estimated to be a factor of 10,000 higher than a man-made detector (Sieber, 1995). The availability of odor to dogs varies in complex ways with the environment in which the mine occurs. Influences include soil types, soil moisture, activity of micro-organisms, and climatic variables. Specially trained dogs are used to detect the characteristic smell of explosive residue emanating from mines regardless of their composition or how long they have been implanted.

The selection of a suitable dog for mine detection starts within the first few weeks of birth to assess their potential and the dog will work closely with his handler every day to enhance their capabilities. The partnership between the selected dogs and their handlers is essential for successful performance. The training period will take between 18 and 24 months. Trained dogs are able to discriminate extremely faint signals (target explosive vapor odors) against a very noisy background. Fig. 2A and B show a dog with its handler and a dog in a search mode respectively.

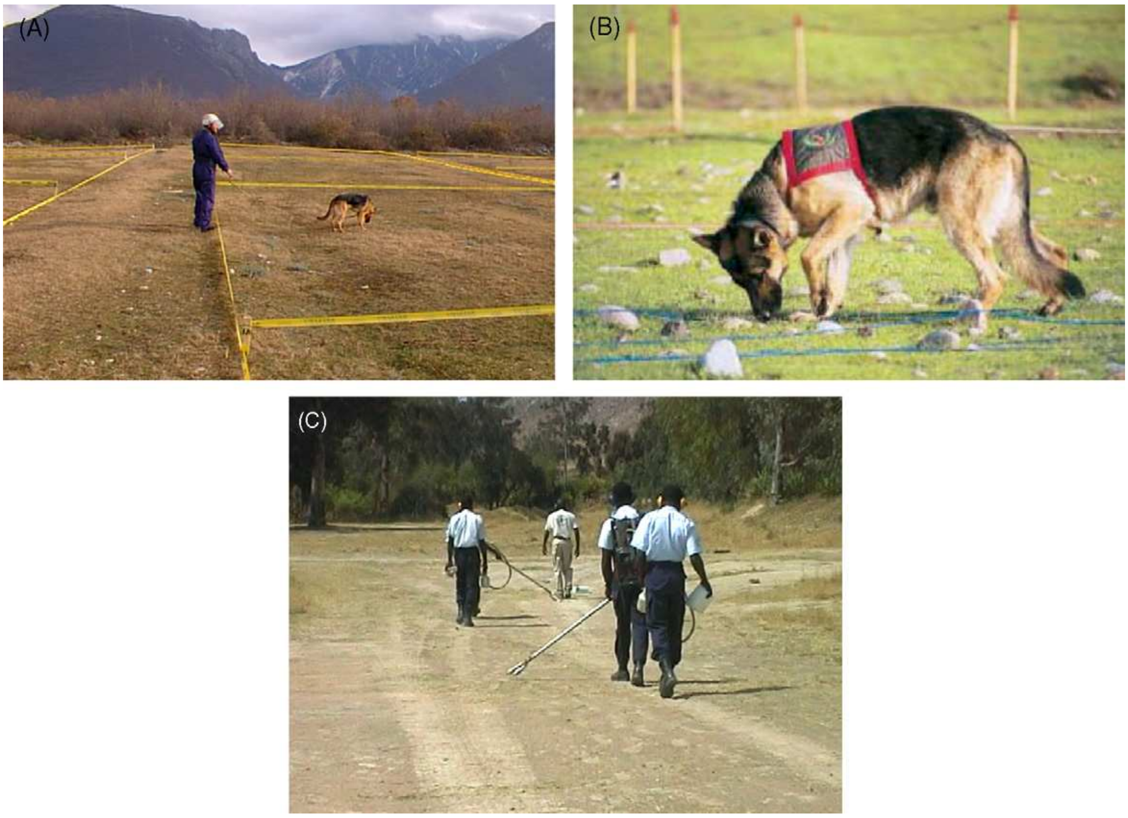

Fig. 2. Mine detection dogs in use. (A) A dog with its handler (DRG-UWA, 2000). (B) A dog in search mode (Wyk and Le Roux, 2003). (C) Scent sampling team in Angola (Fjellanger, 2003).

Currently, dogs have been able to learn to discriminate up to 10 different odors with no indication of a decline in odor discrimination performance when this number was learned. The limits to the number of odors that can be learned have not been fully explored (Williams et al., 1998; Göth et al., 2003). Researchers investigated the period in which dogs can retain their discrimination skill without refresher training. There was very little loss of learned discrimination skills after delay periods ranging from 14 to 120 days (Johnston et al., 2000) while a timely refresher practice is recommended. In addition, because trained dogs do not respond to metal, soil or non-explosive objects, they eliminate much of the time required to investigate positive false alarms associated with manual mine clearance. However, a clearance task for dogs is set up by manual demining. The manual deminers clear safe access lanes about a meter wide around the task area. In addition, manual clearance around the task space is required to check for tripwires. The width of the task space, across the wind direction, must be not longer than the length of the leash on the dog. Mine detection dogs can work in almost all types of terrain. Trained dogs work best in clear open country with vegetation no higher than calf to knee height. They are easy to transport, highly reliable and can clear screening land up to five times faster than manual deminers. In addition, most trainers comment that the relationship between the handler and dog is essential to successful mine detection. Success has been reported from South Africa, Angola, Afghanistan, Bosnia-Herzegovina, Cambodia, Croatia and Mozambique in using dogs to detect individual mines and to locate edges of minefields (GICHD, 2003).

In spite of that, dogs can get tired and distracted, and can be effectively used as little as few hours a day. The presence of explosive vapor within the soil and vegetation is essential element for the dog to perform its detection duty. Dogs can be overwhelmed in areas with dense landmine contamination. Like their human handlers, they do not perform well under extreme weather conditions. Dogs can become confused if they can smell explosive coming from several sources at once. Dogs and other sniffers have high ongoing expenses, are subject to fatigue, and can be fooled by masked scents. Attempts to force dogs to search for long periods of time are normally unsuccessful as the search behaviour of a dog is essentially a voluntary effort, with a reward as its objective. The effectiveness of the dogs depends entirely on their level of training, the skill of their handlers, and on using them in the right place at the right time. More importantly, although dogs are quite effective at detection, their localization accuracy is usually poor. Furthermore, the documented limits of olfactory detection for the dog range from tens of part per billion (ppb) to 500 part per trillion (ppt), and the trainers/handlers should understand that dogs detect substances in term of vapor concentration, not weight, as dogs should be trained to alert to a wide range of concentrations, particularly at low concentrations (Johnston, 1999).

Dogs are normally used in the search mode, i.e. they are brought near the place where mine presence is suspected and they start searching for the source of a particular scent associated with landmines. In order to save dog's energies, another approach has been considered by bringing the source scent in

terms of collected air samples to the dog instead of using the dog directly in the search mode. Sampling is a common term used to describe procedures that are carried out by collecting air samples from genuine minefields with target substances from mines/UXO using vacuum suction and filters. Each air sample is represented by the molecules which remain after a volume of air has passed through the filter. Scent substances to be sampled and analyzed are in very low concentrations, and the quality assurance procedures of such process depend on detailed planning and preparations. A scent sampling team is normally made up of five to seven persons, including one person responsible for ensuring that procedures are followed properly. Scent sampling can be executed directly from a vehicle, but experience has shown that manual scent trapping is more reliable. A portable suction machine collects samples from a pump connected to a long tube (1.5–2 m) with a double-headed filter cartridge on the end, allowing the operator to collect two samples simultaneously (see Fig. 2C). With the long tube, the filters can be moved from side to side over the ground while the operator moves slowly forwards along a secure area (Fjellanger, 2003). At set intervals with focus on divided sectors, the team stop and the filters are changed by the assistant and replaced by new filters while cleaning the head of the tube. The assistant keeps a log of the different filters in association with the position of relevant sectors, and also responsible for monitoring movement patterns and walking speed of the team. When a stretch of road has been swept and the filter cartridges collected, the filter cartridges are then transported in their respective containers to central units where specially trained sniffer dogs sniff each filter cartridge. The sniffer dogs have been trained to detect trace substances emanating from mines in the filter cartridge. A group of six to twelve filter cartridges are attached to holders or stands and these are positioned in a line or circle for the dogs. The dogs are trained to move from one stand to the next and sniff each filter cartridge, and it will indicate a positive find by sitting/lying down if it has detected traces of TNT or other explosives emanating from a mine/UXO. After one dog has sniffed all the filter cartridges once, the order of the filter cartridges is changed by moving the order of the stands. The same dog sniffs the filter cartridges once again. After all filter cartridges have been sniffed twice by the same dog, more dogs are set to carry on the same task. The technique has proved successful for the demining of roads and for possible mined-area reduction.

3. African giant pouch rats

Trained African giant pouch rats have certain advantages over dogs (Verhagen et al., 2003). They have a better sense of smell, cheaper to keep and maintain, and they are suited to African climate with more resistant to tropical disease. In addition, once taught, the rats tend to perform repetitive tasks. Training time is much shorter for rats than dogs and cheaper as fewer resources are needed to raise a rat to adulthood. The training of a mine-sniffing dog costs 10 times more than training a rat (Nyambura, 2004). These rats are easily transferred between trainers/handlers in comparison with dogs. African giant pouch rats have a relatively long life span, and they can grow to be as big as a raccoon and can weight up to 4 kg. The rats are relatively small in size (40 cm/15 in.) and hence, it rarely detonates mines by walking over them. They have very poor eyesight and hence they depend on their senses of smell and hearing. A Belgian company (Apopo) has begun training these rats to locate buried bombs and mines due to their good sense of smell and tractable personality for mine detection (see Fig. 3A–C). The Geneva international center for humanitarian demining is examining aspects of the use of rodent detection as part of the dog study. African giant pouch rats are clicker trained from an early age to associate the smell of explosives with a food reward, usually banana or a peanut. In the field, the rat wears a harness connected to a rope suspended between two handlers. Handlers mark on a grid map the places that the rats signal for possible mine (usually by stopping and scratching). When an area has been thoroughly swept by the rats a team with metal detectors goes in to check and detonates all the marked mines in the area. A rat and its handler can search 150 m^2 (180 square yards) in about half an hour. In order to cut down on the time needed to search a particular area or to search larger areas, multiple rats can be used to search first the assigned area, and then the detonation team goes in to explode/remove the detected mine/UXO. In case of dogs, each landmine must be detonated or removed as it is detected, to avoid detonating the marked landmines.

The test area in Limpopo, Mozambique was a very dense minefield with 20 mines within an area of less than 30 m^2. Besides the mines, the area was highly contaminated with all kinds of war materials (bullets, detonator pins, mine fragments, etc.), which were also often indicated by the animals, especially the detonator pins. The construction of multilevel risk maps based on the indications of the animals seems to be a useful tool as 95% of the mines were found in the highly calculated risk area and the other mines in the second highest risk area. Using this method, more than 80% of the total area evaluated by the rats could be declared free of mines (Verhagen et al., 2006). Some limitations are associated with the use of the African rats, such as rats are not able to tell the handler whether smokeless powder or Royal Demolition Explosive (RDX) was detected. In addition, unlike dogs, selection breed of rats should be considered carefully. Further studies and evaluations are needed to yield more information on the performance and effectiveness while avoiding possible side effects.

4. Pigs

Pigs are thought to sniff better than dogs and their use to find truffles is well known. No open literature has been seen describing any tests or trials for the use of pigs to locate landmines. In comparing dogs to pigs, dogs are excitable, but not as focused as pigs are. Pigs are always focused on eating and sleeping. They are very calm and relaxed animals. It is important to notice that training with punishment is not going to work with pigs. Loud voices and encouraging words are not going to be effective with pigs as been used for dogs. The pig wants to find the mine because it will be rewarded by food as this represents its main goal. Experimental tests show that only the female pigs are suited for the job as pig males are very aggressive and hard

Fig. 3. African giant pouch rat trained for mine detection (VDT-Apopo, 2007). (A) The African giant pouch rats. (B) Trainer treats his rat with a banana during the training for mine-detection. (C) A rat maneuvers a test course during an indication of a buried mine.

to train. The training of pigs takes place at multiple stages. In the first stage of training, every time the pig detects the scent of visually exposed explosive as shown in Fig. 4, she is rewarded with food. In the second stage of training, the pig must find the explosive without seeing the explosive. In the third stage, the pig trained to search a defused buried mine at 10 cm depth, and then is rewarded when she finds the mine, indicated by normal rooting behavior. In the final stage of training, the pig been taught to sit when she finds a mine. This is difficult because it is not a natural response for pigs to sit (Townsend, 2003).

Pigs, like dogs, could be used for quality assurance. Another option for their use is to detect mines with low metal content. The research is not complete yet and no field test has been conducted and reported. Climate effect on the animals alone with the local disease should be considered properly before using it.

5. Honeybees

Researchers are trying to determine whether trained foraging bees can reliably and inexpensively search wide areas for the presence of the chemical signatures associated with landmines, such as TNT, at very low concentration, and possibly other explosive materials in UXO and mine-like objects. In addition, the researchers are looking to search for other chemicals of interest, including drugs and even decomposing bodies. Bees are free-flying organisms and have acute sense of smell. When properly conditioned, it has been found through a series of repeated trials conducted in 2001 and 2002 that bees behave like a fine-tuned detector at vapor levels higher than 10 pptr from 2.4-dinitrotoulene (2.4-DNT) mixed in sand with low probability (less than 2%) of either false positive or negative (Bromenshenk et al., 2003b; Burlage, 2003). Bees are analogous to dogs for mine detection, except that thousands of bees can be trained within a very short time to fly over and search a field for explosives without having a direct contact with any mines. Honeybees inhale large quantities of air and bring back water for evaporative cooling of the hive. As such, bees sample all media (air, soil, water and vegetation) and all chemical forms (gaseous, liquid and particulate). A honeybee's body has branched hairs that develop a static electricity charge, making it extremely effective collector of chemical and biological particles, including pollutants, biological warfare agents and explosives (Bromenshenk et al., 1985).

Researchers at the University of Montana (UM) while cooperating with Sandia laboratory and a consortium of Defense Advanced Research Projects Agency (DARPA) are using honeybees to screen large areas for mines and UXOs including the localization of volatile and semi-volatile organic chemicals, radioactive materials, explosive materials and landmines (Anderson et al., 1999). At the early stage of this research, Pacific

Fig. 4. A pig to sniff out explosive on a trail test (Townseed, 2003).

Northwest engineers have modified commercially available passive radio-frequency tracking tags (half size of a grain of rice as shown in Fig. 5), which store information that can be used to track items such as clothing, to serve as high-tech "backpacks" for bees. The radio tracking tag is attached to the backs of the bees to boost bees' natural abilities by providing timely and accurate information. The aim is to monitor the daily journey of the bees, to and from an electronically monitored manmade hive. A special landing pad was designed for the bees just outside the hive that cause them to pass through two coils as they enter and exit the hive while saving a timestamp for each event. These coils allow the system to detect direction and to uniquely identify each bee entering and leaving the hive using a code in the passive tag, and save a timestamp for each event accordingly (Anderson et al., 1999).

As a bee leaves the hive, it will trigger the code reader, which scans a 10-digit code on each tag and sends the bee's identification code, direction of flight and the time to a modem. The process is repeated when the bees return back to the hive. Members from a single colony make tens of thousands of foraging trips per day over areas as far as 1–2 km. During these foraging trips, the insects are in a direct contact with different environmental media, and during foraging they encounter contaminants in gaseous, liquid and particulate form. These contaminants are carried back to the hive where a system with analysis tools scans for chemicals found in explosives. Researchers plan to use this information to gather data about the behavior of the insects under different conditions and determine how far the hive is located from a potential chemical source. The passive collection of information is helping to provide an initial survey of landscapes and to determine the presence of any chemical and biological threats. It generally identifies regions where materials of concern can be found and, with appropriate relocation of hives and re-sampling, it can help to narrow down the search to areas of a few hundred meters (Bromenshenk et al., 2003a). No important success has been reported at this stage.

The DARPA program extends this work to include the possible training of honeybees to actively search for contaminants, such as the explosive residue released by buried landmines. UM have developed methods that help to train bees to detect explosives and chemical agent surrogates. Sandia provided the explosives expertise, test facilities, electronics support, and state-of-the-art analytical instrumentation (Rodacy et al., 2002).

The training approach of bees focuses on associative learning in which the scientists took sugar-soaked sponges mixed with traces of TNT to see if bees would swarm to them. The training of the bees is done by injecting trace amounts of target chemical into their feeders. They gradually reduced the sugar and increased the TNT so the bees would begin to associate the smell of TNT with a possible food source. When trained, foraging bees locate vapor plume of the same odor of the trained food, and they tend to fly along the plume until they reach its source. At the source the plume, they hovers over a potential mine site for a few seconds at the most (Bromenshenk et al., 2003b; Hewett, 2005). The next part of the puzzle was to focus on how to track the bees, and then be able to recognize the source of plume within the mentioned hovering time.

Practical use of bees in mine detection requires a method of monitoring bees' location and dwell time throughout a test region, with significant operator stand-off distance. Researchers from MU come in with their horizontal polarized scanning light detection and ranging (LIDAR) system to detect flying honey

Fig. 5. A passive RF tag integrated as backpacks with each bee (Anderson et al., 1999).

bees trained to locate buried landmines through odor detection. The polarized LIDAR is a remote sensing technique that uses laser light in much the same way that sonar uses sound or radar uses radio waves. Laser light is transmitted over the area where bees are trained to fly. Some of the laser light, which strikes the bees, is scattered back to through a receiver with a linear polarization parallel to that of the emitted light and collocated with the laser source. The time between the outgoing laser pulse and the return signal is used to measure the distance from the bees to the LIDAR. By using a narrow laser beam and scanning this beam over time, one can produce an accurate map of the location of the bees. Since LIDAR can provide both the range and the coordinates of the bees over targets, the location of buried munitions can be mapped for subsequent removal (Bender et al., 2003; Shaw et al., 2005). A LIDAR mapping of bee density shows good correlation with maps of chemical plume strength, and with the bee density determined by visual and video counts. The co-polarized LIDAR backscatter signal was found to be more effective than the cross-polarized signal for detecting honey bees in flight (Shaw et al., 2005). In 2002, a team from Sandia National Laboratory demonstrated that a LIDAR transmitting 30 pulses per second of 355-nm light (1–40 mJ per pulse) was able to detect bees from a distance of 1.33 km by pointing the LIDAR beam over the top of a feeder (Bender et al., 2003).

To test the feasibility of the developed approach, the research team carried out experiments on a live minefield. By using tens of thousands of bees, the researchers have concluded that the scanning LIDAR is consistently able to detect a higher bee density near most of the significant chemical plumes. The bees hover over a potential mine site for a few seconds at the most. This short dwell time places a requirement on the sensors to use fast scanning algorithms or fast beams. The primary limitation was the difficulty to identify bee's specific signatures from grass and other interfering objects (Hewett, 2005). In 2003, field trials were conducted in a relatively flat field at Fort Leonard Wood in Missouri. Ten full-size bee colonies were conditioned to search for explosive vapor and the hives were placed in the test area. The trial results were positive in locating the relevant mined area but there was no indication about the density of the landmines or the level of contamination within the testing area. The significant operational limit in such experiments was the need to have a clear line of sight from the LIDAR to every point in the minefield. In addition, the beam had to be swept over the ground at a level that is high enough to avoid the high spot, thereby causing the height of the bottom edge of the beam to vary over a range of approximately 1–60 cm. This is significant because bees tend to fly quite near the surface. In addition, the LIDAR cannot distinguish between scattered signals from bees and vegetation with its current design capabilities. This problem is critical and it is solvable at the same time. Researchers are now exploring ways to make their procedures simple enough to be used by local beekeepers anywhere in the world. Similar research is under way to determine if bees might be useful in detecting other chemical and biological agents.

In relation to the performance of the bees themselves, bees perform well in open fields under hot and dry conditions. The other advantage of honeybees over canine detection is the fact that humans are needed only to condition and maintain the colonies, and there will be no need to assist the bees in detecting landmines or other UXOs. Bees can be trained in one or two days to pick up the scent and seek out buried explosives.

Several issues have been raised, such as bees might have problems when faced with multiple chemical sources in an area. Would the bees go to the highest vapor sources and ignore others? In addition, it was difficult to track bees and determine precisely where the targets are located. LIDAR system could see bees, but did not show that it could track bees. Bees, dust, other insects and hard objects produce a back-scatter signal that is larger than the typical atmosphere. High trees and other barriers, such as walls within the field will block the sensor from detecting the bees.

LIDAR was able to detect individual bees at long ranges of hundreds of meters. Fixed and scan modes were tested and proved capable of providing bee location and range data within a few centimeters' resolution. In the summer of 2003 a blind field trial was conducted at an active minefield with a horizontally scanning LIDAR to measure bee density as a function of time and space over the minefield and an adjacent mine free control region. This experiment demonstrated that the LIDAR measured the highest bee density near the maxima found by visual observations and nearby cameras, which also correlated well with regions of high vapor plume density measured by chemical sampling.

Finally, bees do not fly at night, during heavy rain, cold weather or wind, or when temperatures drop to near or below freezing. As such, the use of bees is seasonal in temperate climates. Other environmental conditions as open areas versus dense forests, different climatic conditions, interference or complicating factors such as, multiple targets, mine fragments, exploded ordinance, chemical smokes have not been examined. The key difficulty with bee based demining is that tracking bees is difficult beyond a few meters and this limitation applies to the human eye as well as sophisticated audio/video equipment. Efficient tracking approach is required to track the bees at ranges on the order of tens of meters. It is necessary to have a sensor that can detect and cover the bees within their flying range 2–5 km.

6. Biotechnology and microbial techniques for mine detection

6.1. Bacterial biosensors

In general biosensors are analytical sensing device that couple a biological recognition element, such as tissue, microorganisms, organelles, cell receptors, enzymes, and antibodies, to a physiochemical transducer. Fig. 6 shows a diagram of typical biosensor mechanism. The transducer is typically optical, calorimetric, acoustic, electrochemical, magnetic, etc., Specific interaction between the target analyte, such as biological recognition reaction or biocatalytic process and the biological element layer, produces a physicochemical change that is detected by the transducer component to yield a measurable signal proportional to the concentration of a specific analyte or group of analytes

(Hitt, 2004; Schmidt et al., 1993). Biosensors come in a large variety of sizes and shapes, and the can monitor changes in environment conditions, detect and measure concentrations of specific bacteria or hazardous chemicals, measure acidity levels (pH), etc. (Jacobson, 1996). There are many different ways to combine chemistry, physics, information and biology with suitable transducers that can lead to develop variety of biosensors supporting an unlimited number of applications.

Biologists and engineers have used the fact that common, naturally occurring microorganisms have developed systems for detoxifying or expelling toxic substances, such as bacteria that can consume chemical compounds in soils to accomplish hazardous chemical cleanup objectives. The field of bioremediation evolved from this understanding. These microorganism systems can be used as the contaminant-sensing component of a biosensor by detecting the substance it is designed to detoxify, eliminate or detect. The sensing-component determines the specificity of the biosensor (Daunert et al., 2000), and it is coupled with reporter genes to create biosensors with capabilities to identify toxic substances at very low levels (Tauriainen et al., 1999). A reporter gene (often simply reporter) is a gene that researchers attach to another gene of interest in cell culture, animals or plants. Certain genes are chosen as reporters because the characteristics they confer on organisms expressing them are easily identified and measured, or because they are selectable markers. Commonly used reporter genes that induce visually identifiable characteristics usually involve fluorescent proteins; examples include the gene that encodes jellyfish green fluorescent protein (GFP), which causes cells that express it to glow green under UV light, and the enzyme luciferase, which catalyzes a reaction with a luciferin to produce light. Another common reporter in bacteria is the *lacZ* gene, which encodes the protein β-galactosidase This enzyme causes bacteria expressing the gene to appear blue when grown on a medium that contains the substrate analog X-gal.

Bacterial biosensors are bacteria that specifically engineered to react to the presence of chemical compounds with the production of an easily quantifiable marker protein. A whole-cell bacterial biosensor can be created by placing a reporter gene under control of an inducible promoter to signal a particular environmental condition. Reporter genes are coupled to a sensing-component, which recognizes a substance or chemical and thus confers selectivity to the system. When a bacterial biosensor is exposed to a contaminated environment, the bioavailable substance or chemical is able to penetrate the membrane of the bacterial biosensor and stimulates the reporter gene. The response made by the biosensor is specific to its membrane. The reporter gene then produces detectable and measurable response which is proportional to the concentration of the chemical or physical change (Daunert et al., 2000; Strosnider, 2003; Biran et al., 2003; Turpeinen et al., 2003). The produced visible light can be measured with a variety of instruments including a luminometer and optical fibers.

Bacterial biosensors are used for a variety of applications in environmental analysis. During the last two decades of environmental engineering progress, biologists and engineers have used bacterial biosensors to measure the bioavailable concentration for the contaminant they are designed to detect within a variety of environmental media including soil, sediment, and water by coupling bacteria to transducers that convert a cellular response into detectable signals (Biran et al., 2003). New bacterial biosensors are being developed to respond to heavy metals and metalloids including arsenic, cadmium, mercury, and lead. It is also possible to have bacterial biosensors measuring volatile compounds in the gas phase. In addition, bacterial biosensors have been created to detect naphthalene, benzene derivatives including toluene and xylene, certain toxic metals, etc.

The reporter gene that intended to be used in a biosensor should have the following characteristics (Daunert et al., 2000),

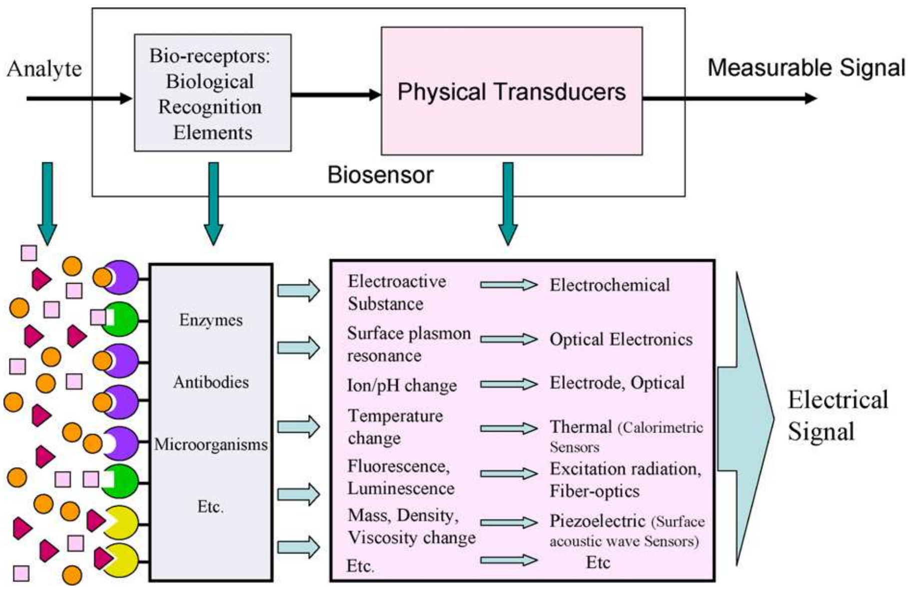

Fig. 6. General concept of typical biosensor mechanisms.

(a) The gene must have an expression or activity that can be measured using a simple assay and reflects the amount of chemical or physical change.
(b) The biosensor must be free of any gene expression or activity similar to the desired gene expression or activity that is being measured.
(c) Ensuring that the biosensor is free of any similar gene expression or activity to prevent misinterpretation of the response and guarantees accurate measurement relevant to the desired chemical or physical change.

6.2. Advantages and challenges

The main advantage of developing and using bacterial biosensors is the ability of such biosensors to detect and provide a real-time measurement of the bioavailability of fraction of substances and chemicals, as opposed to the total concentration. They are particularly suitable for field work, since the procedure for using bacterial biosensors does not involve expensive and fragile equipment for storage, daily cultivation, handling or specialized training that most analytical methods require. In addition, bacterial biosensors are also fast, less expensive and less labor intensive than other traditional methods. Bacterial biosensors can be freeze-dried so they are easier to handle and store. The results obtained from bacterial biosensors are compatible with and comparable to chemical analysis while it is more sensitive than chemical methods.

Several challenges, such as longevity, reproducibility, and linearity must be overcome in order to fully implement the use of biosensors in the field. In addition, the construction and use of bacterial biosensors has been restricted by our limited understanding of the genetic systems that control bacterial responses to polluting chemicals. Bacterial biosensors perform best under conditions similar to their natural environment (Petänen and Romantschuk, 2003). Unfortunately, little is understood about the relationship between the microorganisms and their natural environment. Hence, it is difficult to determine the effects that certain procedural conditions have on the performance of biosensors. The temperature, pH, incubation time, medium, and reagents all can have effects on their performance that can only be understood with further research and testing.

With more testing of bacterial biosensors in the field and a standardization of biosensors along with their procedures, bioavailability can be incorporated into wider range of applications. In addition, improvements on the applicability of biosensors may include the development of a multi-analytical biosensor. By genetically labeling the bacteria used in the biosensor, a multi-analytical biosensor can be developed to test more than one contaminant at one time.

6.3. Microbial mine detection system (MMDS)

The microbial mine detection system is an example that makes use of common bacteria responds to explosives and provides signal to identify and locate explosive compounds in large areas of potential contamination (Burlage et al., 1999). ORNL took the advantage offered by such microscopic creatures to genetically engineer common microorganisms for the possible application to waste management technologies (Burlage et al., 1996; Burlage et al., 1999). They found that such bacteria, when applied to soil, will glow if the soil is contaminated with solvents like toluene or xylene. As the TNT is chemically related to these solvents, and since most landmines leak slightly and leave on the soil traces of explosive chemicals such as TNT shortly after they are laid in the ground, it was fairly simple to expand on this concept. Microbiologists were able to culture a strain of bacteria with a modified gene that will cause the microbe to fluoresce under laser light when in contact with TNT.

The plan involves spraying microbes over each of three test sites, then allowing the bacteria to dine on any chemicals present for 3–6 h before the deployment area is checked. If there is any TNT available for consumption, the area where it is concentrated should emit fluorescent light when a laser is scanned across the ground surface.

A photoprotein, green fluorescent protein (GFP), and its encoding gene from jellyfish (*Aequorea victoria*) have been used in the biosensors. The production of GFP in the jellyfish results in the emission of a green fluorescence in which its luminescence can be measured. Due to the characteristics that featured GFP system, it can be used as a reporter gene for the biosensors (Tauriainen et al., 1999; Daunert et al., 2000). ORNL has developed genetically engineered *Pseudomonas putida* a common soil microorganism that had a TNT inducible promoter fused to GFP with the aim of recognizing an explosive signature and responding to it by producing a fluorescent protein, which appears as an extremely bright green light when they are illuminated by ultraviolet (UV) light. The engineered bacteria will scavenge the explosive compound as a food source, activating the genes that produce proteins needed to digest the TNT. The fluorescent signals are mapped, and the area is examined for the source.

The plan involves spraying a suspension of the genetically engineered bacteria over a field (see Fig. 7A), and allow it to contact the explosive that resides in the bulk phase of the soil. Once the engineered bacteria pseudomonas contact the explosive, the genes that are involved in TNT metabolism are activated. The concentrations of explosive are much higher here than in the vapor phase, and research has shown that ppm concentrations are available to the microbes, which is ideal. As fluorescent protein is produced through the reporter gene, the bacteria become detectable using any of several fluorescence detection techniques (see Fig. 7B). The bacteria require about 4–6 hours before they maximize their output of fluorescent protein. About 10^6 bacteria per square centimeter are preferred. The signal is then stay stable for the next 24 h, although the bacteria die off exponentially over the next several days (MacDonald et al., 2003).

The method has been tested mainly in lab environment. A simulated minefield demonstration was conducted around prepared landmines in October 1998 in South Carolina. During these test, genetically engineered *Pseudomonas putida* detected five out of five simulated mine targets in a 300-m^2 field, however, they also produced two false positive signals, indicating the presence of a landmine where none existed. Testing with real-world conditions of a real minefield has not been conducted.

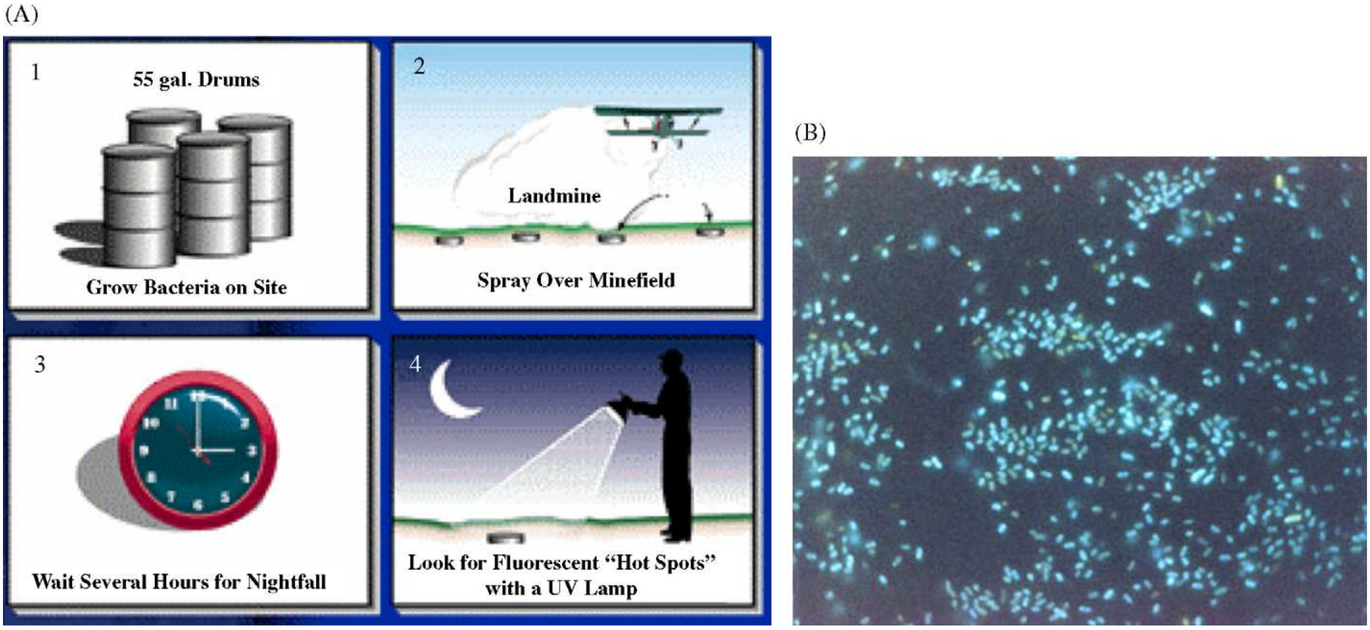

Fig. 7. Bacterial biosensors for mine detection. (A) The cycle of growing and testing the use of bacteria for landmine detection (O'Donnell et al., 1998). (B) The fluorescence of the bacteria makes them easily visible (McDonald, 1999).

This approach could allow for searching hundreds of acres in a few hours, which is much faster than other techniques, and could be used for area reduction and on a variety of terrain types. In addition, vegetation tends to take up the chemicals (on its leaves and through roots), so the bacteria glowing on the vegetation due to the presence of the relevant chemicals could even localize the explosives more by magnifying the signal (ORNL, 1998). It would be possible to detect landmines remotely from rolling towers or helicopters by looking for glowing microbes on soil illuminated with UV light.

This technique would not work in places that include areas with standing water, such as rice paddies, consistently low or high temperatures areas, and snow covered soil, which would disperse the bacteria. Rain would change the location of the signal. In addition, there are problems with dispersal of the bacteria over dry ground where the bacteria will be quickly absorbed by the soil and the signal lost to detection. There are some false positive indicators, especially near plants and water drainage. Unfortunately, there is currently no known strain of bacteria capable of detecting RDX. Other barriers to expansion of the bacteria into the environment include the natural heat of the desert and the lack of moisture. Moreover, while the genetically engineered bacteria have little chance of surviving much beyond the length of the test, there is a need to study the safety and the effectiveness of using bacteria in real mine infected area while addressing social and health concerns. Important issues that need to be answered are, the stability of the genetic construction, partitioning of explosive residue in various soil types, the role of vegetation in uptake and magnification of the signal, and reliable dispersal methods that allow the system to be used in many soil types (MacDonald et al., 2003). Although this technique has been demonstrated in the field, it remains a largely unproven approach, but it holds promise and needs further development and evaluation.

7. Genetically engineered plants for mine detection

Plants, like all organisms, naturally sense the chemicals in their environment. In addition, genetically engineered plants have been developed with focus on crops with engineered production qualities, such as insect resistance and herbicide tolerance. The main concerns associated with genetically engineered plants are those related with food safety issues and ecological consequences. Monitoring environmental hazards is another area where genetically engineered plants can be used as biosensors to perform a crucial role, such as monitoring radioisotope levels around nuclear power plants, and unwanted and dangerous substances in the environment. In addition, researchers have genetically engineered plants that can transform toxic mercury to a much weaker, less toxic and volatile form of mercury known as elemental mercury. Genetic engineering has been applied to isolate and transfer the mercury-eating traits from bacteria's DNA using a high-pressure machine called a gene gun to "shoot" the genes into plant tissue. Then, the seeds of a mature genetically engineered plant are taken and planted in mercury contaminated area (Rugh et al., 1998).

Several research groups are currently performing research using genetically engineered plants as biosensors. Plants have thousands of genes. But, depending on the environment, not all genes are expressed at the same time. By identifying responsive genes, it would be possible to identify the promoters. A gene has three parts: the promoter, the coding portion and the terminator. The promoter is the part of the gene that initiates the ultimate production of protein. The activation of the promoter initiates the transcription of the DNA coding sequence into RNA, a biochemically different copy of DNA. If a gene responds to explosives, then its promoter can be fused with the coding portion of a different gene (reporter gene, such as a GFP gene typically obtained from the Pacific jellyfish (*Aequorea vic-*

toria)). This fusion results in a combined gene whose promoter would activate in the presence of relevant explosives with landmines, hence producing the green fluorescent protein would then visibly signify the location of a landmine when excited by UV light. It is possible to genetically manipulate plants to change their behavior in the presence of TNT or other explosive material, for example changing color, growing up fast and high or display any other detectable sign. The TNT would be absorbed by plant roots and then transported to leaves (French et al., 1999) where the fluorescence could be readily observed. The root structures would also more effectively mine the soil for trace explosive, resulting in increased mapping accuracy. These signs need to be visible to the human eye and/or recognized from aircraft. Other signs, such as changes in UV reflection are also usable and measurable by simple tools. Genes that are found to be responsive to TNT or RDX can guide researchers to the promoter element, a key to genetically engineering the plant that would fluoresce in the presence of landmines. An important component of any detection system is the photonic device used for picking-up the fluorescent biosensor signals.

A group of researchers at the University of Tennessee have considered using biology to address the problem by genetically engineering plants to detect the explosives most commonly used in landmines, such as TNT and RDX. The tobacco plant was chosen as candidate because it is easy to manipulate, while in real minefield, other crop-type plants that are indigenous to the minefield's environment could be used. The research group has manipulated the promoter and the marker gene. This research, however, could lead to the production of sterile plants, ones that would not produce any seeds. Helicopters, air balloons and even satellites could utilize laser-based instrumentation to detect the changes in the plants, reducing the need for people on the ground. Currently, the green of the fluorescing plant could only be seen at night. If a correct marker can be found to make the plant simply change colors when it comes in contact with explosives, it would be possible to give the seeds away to people to plant in strategic areas, and accordingly differently colored plants could mark landmines. The same genetic engineering process could be used to fuse a promoter with a gene coding sequence that would lead to the development of plants which could remediate contaminated soil, converting pollutants into lesser toxins, a process called phyto-remediation. For example, in a case where the promoter has been isolated and modified so that it is responsive to copper (Mett et al., 1993) plants containing this construct might be deployed around the periphery of a copper mine to monitor copper movement into the surrounding countryside. When copper was present in sufficient concentrations to trip the promoter, plants would fluoresce green at that location. Ultimately biosensors of nature could be used in conjunction with bioremediators. The perfect scenario is to have an individual plant that performs both functions.

Another approach has been introduced by Aresa Biodetection Company. They genetically engineered thale-cress plant, commonly called *Arabidopsis thaliana* (see Fig. 8A). This plant is a small flowering plant, it occurs naturally around the world, and has a fast growth rate). The plant, which is sensitive to nitrogen dioxide (a product released by landmines as they buried in soil), has been genetically coded to change color when its roots come in contact with nitrogen dioxide evaporating from explosives buried in soil. In order to plant seeds in a large area (such as a minefield), Aresa uses a seeding hose, known as a "hydroseeder", which can seed the size of a football field in a day. In addition, the plant can be transplanted via planes or by a number of other methods. Within three to six weeks from being sowed over mine infested areas, the small plant will turn a warning red when close to a landmine or an associated explosive (see Fig. 8B). So far the thale-cress plant has turned red in all experiments, but it does not grow to be very large, making it difficult to see the results.

However, because nitrous oxide can also be formed by denitrifying bacteria, there is some risk of generating false indicators through the use of this technique. In addition, no reported study has been conducted with actual landmines, though successful studies have been done in greenhouses (ACF, 2006). Some scientists raised their concern that such bioengineered plants could escape into the wild and confer undesirable traits on wild plants. Proper consideration should be given to water requirements and pollution issues. In addition to Aresa's scientists, other independent research groups were trying to use different genetically modified plants to detect landmines.

Finally, the developed technology may be used to genetically engineer other plants that are indigenous to each of the areas afflicted by landmines.

8. Antibodies and mine detection

Antibodies are Y-shaped proteins used by the immune system to identify and neutralize foreign objects like bacteria and viruses. Each antibody consists of sites called paratopes that recognize a specific target which is called antigen. Antiboidies have the ability to bind very specifically to a certain biological compound in the body. An interaction occurs when an antigen combines with a corresponding antibody to produce an immune complex. An antigen is a molecule that stimulates an immune response. A molecule of antibody has two identical binding sites for one antigen or more, depending on its class. The interaction occurs by non-covalent forces between the antigen-combining site on the antibody and a portion of the antigen called the antigenic determinant or epitope. The binding of antibody to antigen is a reversible process, involving non-covalent bonds.

For many years antibodies have been used to determine and diagnose different diseases, and today thousands of antibodies are available for this purpose. The binding specificity represents a measure of the usefulness of the antibody. An antibody with improved specificity, could provide the ability to correctly determine or treat diseases. Vapor and trace analysis has been used for forensic analysis procedures for decades. But, it is technically demanding to bring these technologies from the laboratory to the field, and to make them cheap enough for cost-sensitive applications. Explosive trace detection systems have been developed for applications to security, inspection and monitoring of harmful substances. Different research groups are developing new detectors based on a fundamental understanding of biosensor systems with an emphasis on new biorecognition prin-

Fig. 8. Plant as indicator for explosive detection. (A) Thale Cress (*Arabidopsis thaliana*) (Wikipedia, 2007). (B) Red plants growing in soil containing solid explosive (TNT), and green plants growing in soil without explosive (Aresa, 2007).

ciples that involve antibody–antigen interaction. Formation of antibody–antigen complexes has to be detected under conditions where non-specific interactions are minimized. Each antigen determination requires the production of a particular antibody, its isolation and, usually, its purification. In an effort to use such technique for drug and explosives detection, a detector is set up so that as an antigen, a specific molecule of an explosive reacts with a specific antibody. Detecting this antigen–antibody reaction forms the basis of this detection method (Hitachi, 2004).

Antibodies are very selective to other molecules and can therefore be used to develop sensors that react in the presence of specific molecules. Bofors (Sweden) launched a program in 1995 targeted specifically at detecting antipersonnel mines through odor sensors based on antibodies (Brink, 1996). They were using antibodies coupled with other sensor technologies, such as ground penetrating radar (GPR). Basically, the sensor is a piezoelectric crystal coated with antibodies that react to TNT vapor. The piezoelectric crystal has a specific oscillating frequency that can be measured using an electronic circuit. When in the vicinity of a mine, some antibodies detach from the crystal to join with TNT molecules and the oscillating frequency of the crystal changes measurably. One of the antibodies on the market that can be used for this type of sensor is directed against DNP (dinitrolphenol).

In addition, an antibody-based immunoassay has been developed using a surface plasmon resonance (SPR) sensor platform for the detection of TNT in soil extract solutions. The main objective was to develop technology for real-time detection of landmines. The immunoassay combines very simple bio-film attachment procedures and a low-cost SPR sensor design to detect TNT in soil extracts. The active bio-surface is a coating of bovine serum albumin that has been decorated with trinitrobenzene groups. This development has illustrated that a SPR sensor could be used to screen soil samples for TNT and related materials using a simple bioassay and aqueous soil extracts. The study shows that the sensitivity of the assay needs further enhancement by seeking other methods. Furthermore, for such a methodology to be useful in the field, a soil collector/extractor is required. The collector/extractor would have to perform its duty rapidly and efficiently (Strong et al., 1999).

Finally, new approaches for faster antibody development are required with improved methods of selection, optimization and evaluation for novel applications.

9. Molecular imprinting biomimetic

Biosensors, in particular, have attracted considerable attention because of their extraordinary sensitivities and specificities. However, they often lack storage and operational stability because they are based on a fragile biological recognition element: an enzyme or antibody (Kriz et al., 1997). Computational method has been developed to engineer proteins that can specifically detect a wide array of chemicals from TNT to brain chemicals involved in neurological disorders. This may constitutes an important step toward a new technology of synthetic biology, in which scientists will be in position to construct tailor-made organisms for a variety of tasks. A rapidly developing field that takes advantage of nanoscale templating is known as molecular imprinting. This field could provide an alternative to biosensors because it leads to highly stable synthetic polymers. The emergence of molecular imprinting has its original source in the area of immunology. It is well known from biology and chemistry that molecules tend to stick to receptors or surfaces that fit the molecular contours like a key fits a lock. Molecular imprinting is an approach to making molecularly selective cavities in a polymer matrix. These cavities function much as

enzyme receptor sites: the chemical functionality and shape of a cavity in the polymer matrix cause the cavity to bind to specific molecules. The majority of molecular imprinting requires three basic ingredients to work: the template molecule, functional monomers, and a cross-linker (Markowitz et al., 2000). A molecule imprinting polymer matrix is made by polymerizing monomers in the presence of the compound of interest (template molecule). The template molecule is dissolved in a solvent together with polymerisable monomers, which interact with the template, either via non-covalent interactions (electrostatic interaction, hydrogen bonding, and hydrophobic interactions) or via reversible covalent bonds. The polymer forms around the template. After the polymer solidifies, the template molecules are extracted from the polymer matrix by decomplexing them from their binding sites and then dissolving them, leaving cavities (recognition sites) that are matched in size, shape, and chemical functionality to the original template molecules. The resulting cavities thus become molecular-recognition sites that can selectively bind to molecules of interest, i.e., molecules identical to the original template fit into the recognition sites and are bound strongly, while molecules which differ in structure are excluded as they are unable to bind (Kriz and Ansell, 2001). Hence, it can be applied in a sensor or in chromatography. Fig. 9 shows the principles of molecule imprinting process. A wide variety of print molecules have been used in various imprinting protocols. Compounds such as drugs, amino acids, carbohydrates, proteins, nucleotide bases, hormones, pesticides and co-enzymes have been successfully used for the preparation of selective recognition matrices. Several polymer systems have been developed for use in molecular imprinting technology. The most readily used are either polyacrylate-based or polyacrylamide-based systems. Another approach is the polystyrene-based systems, used to a lesser extent. Typical functional monomers used are carboxylic acids while limited number of crosslinkers has been tried with different degrees of success because the solubility of the crosslinker itself in the pre-polymeric solution and the solubility of the monomerised template species reduce the number of possible alternatives. The "molecular key" may, in principle, be any type of molecule—ranging from small molecules such as drug substances, amino acids or steroid hormones to larger molecules such as nucleic acids or proteins. Large molecular assemblies such as cells and viruses may also be perceived. In general, though, the difficulty of making the imprinted materials increases with the size of the selected key molecule.

A wide range of disciplines are showing the interest in molecular imprinting, such as biology, pharmaceutical, waste management, food processing, and virtually any other process where inexpensive selective sorption is valuable (Lenhart, 2001). NASA has used this technique to develop Biomimetic/optical sensors as means of real-time detection of bacteria in liquid samples through real-time detection of compounds, such as acylhomoserine lactones and peptides secreted by the bacteria. Bacterial species of interest would be identified through detection of signaling compounds unique to those species.

A sensor would include a specially formulated biomimetic film, made of a molecularly imprinted polymer that would respond optically to the signaling compound of interest (NASA, 2006).

10. Biomimetic sensor systems

Biomimetics refers to an interdisciplinary effort aimed at understanding biological principles and then applying those principles to improve existing technology or to create entirely new technologies. The goal is to produce hybrid materials and/or sensors, devices, etc. that possess superior properties compared to either entirely synthetic or biological alternatives.

Advances in biotechnology and nanotechnology have opened the possibility to develop bio-electronic sensors based on the properties of single biomolecules. These elementary nanobiosensors will represent the ultimate limit in miniaturization, specificity and sensitivity. An array of these elementary nanobiosensors could constitute the closest bio-electronic mimic of the animal sensing systems. Many biomimetic sensor systems are under development as potential, highly selective and sensitive trace/vapor explosive detection systems. Of all sensor technologies, taste and smell in terms of the electronic tongue and nose have been widely considered as the most difficult to

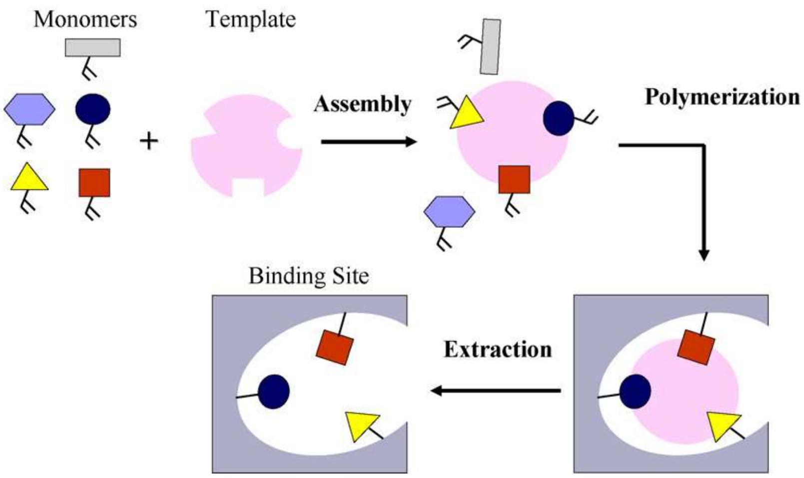

Fig. 9. The principles of the molecule imprinting process.

realize and the development of these sensors significantly contributes to the understanding of the reception mechanisms in gustatory and olfactory systems in human and other species. Hence, biomimetic sensor technology is of high interest to researchers in food science, biochemistry, biophysics, medical science, biotechnology and electronic and mechanical engineering. The current vapor sensors for mine detection are facing several challenges that need to be addressed properly,

(a) Determine the exact position of a mine, because explosive's odor penetrates the ground and vegetation over an area up to 10 m from the real position of the mine.
(b) Biomimetic and other artificial vapor sensors cannot be used for several days after a mine exploded, because it pollutes the air and affects the use of biomimetic sensors.
(c) A major problem for any chemical sniffer system is the need to understand the explosive signature of the target compound.
(d) It is required to have knowledge about the concentration level of the targeted chemical compound.

10.1. Biomimetic nose

Trained dogs or rats possess a high TNT-sensitive nose that can outperform even the most-advanced landmine detection technology and an artificial snout to mimic them would have significant advantages. A major problem in bio-mimic sensory is that we do not know what exactly the dog, rats, bees, etc. are really responding to the odor. Therefore, understanding the mechanism of how dogs and other animals actually detect mines will allow developers to duplicate/approximate animal smell and taste organs. This will lead to an enhanced ability to find a particular odor against a complex background environment, and will be a major step towards moving away from the electromagnetic spectrum as the only promising device for locating minefields and individual mines. Advances in the understanding of olfaction are leading to artificial electronic noses based on an array of sensors that bind airborne molecules with only modest specificity. Detection is based on unique patterns of responses generated by the sensor array in the presence of an odor. Systematically, the olfactory process of the dog and consequent detection of materials has been investigated. Dog breeders and trainers are working closely with biologists, biochemists and physicists. So far, it has not been possible to develop measurement instruments sensitive enough to indicate the small concentrations of chemicals that dogs can clearly identify. These research programs are very important to establish new direction that could in future provide some very useful results. Biosensor systems are under development as potential portable highly selective and sensitive trace/vapour explosive detection systems and if proven to be sensitive enough, these could be used for verification and area reduction purposes. A variety of disciplines must come together effectively in order to obtain successful results.

Many research activities are adopting creative interdisciplinary technological approaches to help understand, inspire and mimic as possible human, animals and other species olfaction capabilities with aim to develop an efficient and tiny bio-electronic sensors. In Europe, research efforts led to develop electronic noses based on natural olfactory receptors that could be used to detect the early warning signs of different illnesses. In addition, it could be used in agriculture, industry, environmental protection or security. The developed sensor consists of an array of elementary nanobiosensors, each of which consists of two electrodes a few nanometres apart (nanotransducers) with an olfactory receptor (protein) monolayer anchored in between. The array structure will increase the sensitivity and specificity of the whole olfactory sensor. The nanoelectrodes will be in charge of detecting electrically any conformational or chemical change in the olfactory receptors when these receptors bind a given odorant molecule (SPOT-NOSED, 2006). The developed system is said to be capable of detecting odorants at concentrations that would be imperceptible to humans.

11. Summary and conclusions

New approaches to landmine and UXOs detection are being studied to improve the detection rates and to reduce the false alarm rate of the existing detection techniques. An interesting departure from the use of electromagnetic radiation involves approaches focusing on developing and using detection tools that can identify explosives residue in mined areas as a robust primary indicator with no regards to the mine container. These techniques take advantage of the fact that all munitions leak with time some amounts of their chemical constituents as vapor, soluble and non-volatile components that can be found in and on the surrounding ground and nearby plant life. Even very slow, low concentration, vapor emissions will be sufficient to allow interception and identification. Being conditioned on explosives, they will also pick up the scent from UXO. Accordingly, the vapor and soluble components of explosives signature, such as TNT, DNT, RDX, etc. as well as vapor from their casing materials of various types of plastics, metal, wood, or paint, can be checked in the air above or near buried mines or UXOs. Typically, humidity and temperature are key factors affecting vapor availability. The increase of temperature significantly increases the vapor availability above a given substance. In addition, signature scent above the soil is complex and can vary according to the amount of buried time.

Understanding the behaviors and capabilities of animals, insects and other living creatures, along with close collaboration between biologist and engineers, present unique opportunities for enhancing, genetically manipulating, and creating new capabilities through mimicry and inspiration, integration of living and non-living components, and direct use of complex biological systems; with focus to support wide range of applications throughout the process of humanitarian demining.

The presented biological and biologically inspired techniques and technologies are promising approaches that can contribute to help detect individual landmines and support area reduction. Some of the presented techniques are used in real minefields, and other techniques are still years away to be effectively applicable in a real minefields. Landmines that might be manufactured to be completely sealed (which are not currently the case) cannot be detected by any of the methods listed in this paper. The

role of trace vapor detectors in most countermine missions is likely to remain limited due to the limited standoff that can be achieved.

Important care should be applied to safety, health and environmental concerns when developing genetically engineered bio-systems. Many technologies are promising, but none is in the sensitivity, size, weight, manufacture-ability and price range required for humanitarian demining yet. It is a huge challenge to seek and optimize new technology and to make a meaningful difference in the elimination of landmines threat.

12. Future perspectives

Although many studies have been conducted with promising results, and while there are additional studies are underway, there are still many more remain to be done in this area to,

(a) Have better understanding about the natural capabilities of bio-systems. This will lead, to enhance the development at the other three categories.
(b) Coordinate and develop efficient interdisciplinary research teams.
(c) Create new ideas and innovative technologies to boost the performance of already available techniques and new approaches as well.
(d) Fulfil the high level of interest in the detection of low-level concentrations of explosives such as TNT and RDX.
(e) Have better understanding the key features that identify the specificity of explosive and chemical signatures, and study the effect of humidity, temperature and other climatic parameters on them.
(f) Improving the sensitivity and fine resolution of sensors because it has a direct effect to determine what can be detected, at what location, and how quickly.

In addition, further studies, test, and analysis relevant to the presented technologies and techniques are required to yield more information and better understanding that lead to enhance performance and reduce possible side effects if any on human and environment.

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