Abstract: The OPCW Member states cover 98% of the global population and landmass. Regrettably, unanticipated chemical warfare agent assaults are reported during the last decades. In addition to the frequent threat situation, the sampling of bio-medical samples from these areas is critical and mainly depends on investigation opportunities of victims. Non-contact sensor technologies are desirable to enable a fast and secure estimation of a situation. Plants react on pollution because of their direct interaction with gases and it is assumed that chemical warfare agents influence plants, respectively. This impact can be analyzed for the detection and characterization of chemical warfare assaults. Nowadays technological progress in digital technologies provides new innovations in detectors, data analysis approaches and software availability which could improve the screening, monitoring and analysis of chemical warfare. Within this context hyperspectral imaging (HSI) is a promising method. Different applications from remote to close range sensing in medicine, food production, military, geography and agriculture do exist already. During the last years HSI showed high potential to determine and assess different plant parameters, e.g. abiotic and biotic stresses by recording the spectral reflectance of plants. Within the present manuscript, the basics principle of HSI as an innovative technique, aspects of recording and analyzing HSI data is presented using wild growing apple leaves which are treated with sulfuric acid, fire or heat. Resulting spectral signatures showed significant changes among the treatments. Especially the shortwave infrared was sensitive to changes due to the different treatments. Furthermore, the calculation of common spectral indices revealed differences due to the treatments which are not visible to the human eye. The results support HSI applications for the detection of chemical warfare agents and elucidate the impact of chemical warfare on plants.

Keywords: Chemical Weapons Convention 2017; optical sensors; public security; spectral reflectance; stress detection.

Introduction

To assess, monitor and control application events of chemical weapons, efficient methods are needed. These methods need to be able to proof the deployment also after some days of the application event. An unambiguous proof is essential for the public opinion and the legal evaluation of an incident. In terms of the Syrian civil
war, the UN and OPCW listed and demounted all chemical warfare agents until 2014 after a Sarin assault on the area of Ghouta 2013. BBC-World-Middle-East-News reported on 26th April 2017 suspected Sarin attack on Khan Sheikhoun in north-western Syrian. The incident occurred on 4th April with close to 100 victims. Many chemicals are volatile and only detectable indirectly by the caused symptoms at humans. The OPCW was able to collect bio-medical samples from victims on 19th April. The analysis took 15 days and revealed a chemical attack with Sarin or a Sarin-like substance. Existing therapeutic approaches after exposure to a nerve agent are limited and no synthesized oxime has reactivation potency against all kinds of chemical warfare agents (CWAs) [1]. To take countermeasures as soon as possible, fast and direct detection of chemical assaults and accidents are highly desirable. Jang et al. [2] presented an overview of chemical warfare agents and their possible detection and destruction mainly by biotechnological approaches. For acute treatments of people such approaches are very important, but additionally the investigation and monitoring of the whole environment of potential hazardous locations can improve countermeasures and personal protection. Such monitoring systems will consider important members of the whole environment such as plants. The determination and assessment of different plant parameters is performed by applying different sensors, especially optical sensors such as RGB, multi-hyperspectral imaging (HSI), thermography and chlorophyll fluorescence [3].

**Basic principles of hyperspectral imaging and the information content of plant reflectance**

The recently increased interest in optical sensing technologies for plants is triggered because light reflectance characteristics give us deep insights into the composition and structure of every object on our world. Matter consists of molecules which can reflect or absorb light, depending on the wavelength [4, 5]. Consequently, an object has a characteristic interaction pattern with the incoming radiance, and can be characterized by reflection, absorbance and transmission spectra of the incoming light. Humans and many organisms are able to recognize the visual part of light (400–700 nm), and by this they are able to evaluate important visual parameters of an object. Doing so, it is possible to distinguish between different colors and even more – based on cognitive abilities, instinct or experience – humans are able to assess a specific meaning of attraction or fear in nature and to derive material characteristics like roughness, solidity and composition. Plants show a high color diversity especially in leaves and flowers. Photo pigments such as anthocyanins, xanthophylls, carotinoids and chlorophyll characterize the leaf color and are important to convert energy from light and to protect plants against high-energy light such as ultraviolet A and B (280–380 nm) [6]. Plant senescence and stresses are visible as a reorganization or loss of these pigments [7]. To assess optical properties of plants and to analyze the effect of stress factors, optical sensors are used [3, 8]. Common RGB cameras can be applied to estimate chlorophyll content or to characterize foliar diseases [9–11]. But in many cases it is difficult to distinguish between the stress initiator, independently if abiotic stresses such as extreme weather scenarios or biotic stresses like plant pathogens appeared. To overcome this limit, hyperspectral cameras are applied in research for plant sciences and agriculture to detect and identify specific stress symptoms and the health status of plants [3].

Hyperspectral imaging (HSI) includes high resolution optical techniques similar to conventional RGB cameras but with an increased spectral resolution. In addition to red, green and blue wavebands, HSI can assess narrow wavebands in the visual light from 400 to 700 nm (VIS), in the near-infrared from 700 to 1000 nm (NIR) and in the shortwave infrared from 1000 to 2500 nm (SWIR). The VIS reflectance of plants is mainly characterized by absorption of the leaf pigments like chlorophyll, carotenoids and xanthophylls [12] (Fig. 1). The NIR and SWIR stimulate molecular motion that induces a strong absorption or reflection by compounds that show characteristic spectral pattern [4]. Due to internal scattering effects by the cell structure and air filled space, and the interaction of light with starch, oil, proteins and further compartments inside the cells, cell walls and membranes, NIR reflectance of plants is mainly influenced by the leaf and cell structure [5, 13]. Wavelength bands in the SWIR can be used to determine water content of plants and further plant chemicals [12]. Because of this enormous amount of information in hyperspectral images that are invisible
for the naked eye and the continued improvement in technique, methods, material and data analysis [3, 14–16] we state, that this innovative technology could be used for the indirect determination and assessment of chemical assaults and accidents.

**Chemical warfare agents and their known impact on plants**

Some distinct changes of plant characteristics after CWA exposure suggest the use of vegetation for the verification of presence of CWAs, even after days or weeks. Like soil, plants are present at most of the sites and will be still present when samples are taken and investigated. Plants can take up volatile substances directly via their leaf surface or from the soil via their root system. Research in the context of OPCW and beyond, suggests that CWAs have an impact on plants. A recent study with white mustard plants show that VX nerve agent and its degradation products can be detected in plant samples even longer than in the soil samples [17]. The active uptake suggests also the cultivation of suitable plants for the decontamination of soil. Based on the mode of action, multiple CWAs are phytotoxic or harm plant organs in a specific way. Early CWAs like chlorine gas and phosgene gas induce chemical burning by reacting to strong acids that can harm mammals and plants in similar way [18]. Typical symptoms after exposure of plants to chlorine gas are chlorosis, necrotic mottling and necrosis of the leaf tissue. Studies by Schreuder and Brewer [19] showed also a slightly long term negative effect on coniferous species. They studied a forest in a valley of the Rocky Mountains where a derailed 72-car train released ~55 metric tons of chlorine gas in 1996. In addition to the direct foliar injures, they identified species-specific loss in photosynthetic biomass and efficiency and hypothesized negative influence of tree growth, drought susceptibility and chloroplast damage due to chlorine gas exposure. Further CWAs are targeted directly at the vegetation, e.g. Agent Orange induces senescence by interfering with plants’ growth regulation system [20]. The resulting defoliation is obvious but the inducing agent needs to be identified by further analysis.

Nerve agents that interfere with the central nervous system of mammals have also shown effects on the reproductive process of plants [21]. In germination experiments with wheat seeds and methylphosphonofluoridates, a significant negative effect has been verified and also the growth of seedlings was reduced by this substance and its degradation products [21]. Solomos and Laties [22] investigated the effects of cyanide and ethylene on the respiration of fresh peas, 5 day old pea seedlings and cherimoya fruits. They revealed...
that the respiration is strongly inhibited by cyanide in fresh green peas and germinating pea seedlings. In addition to a Pasteur effect, oxygen uptake was decreased 40% in germinating pea seedling and the CO₂ evolution was increased up to 20%. The following calculated respiratory quotient indicated a high fermentation and consequently the production of ethanol and lactate with an increased glycolysis. Such anaerobe metabolism for a longer period is toxic for higher plants [23]. This type of interaction suggests a vague initial indication that also target specific CWAs may be identified by a characteristic effect on the development of living plants. Even agents with no visible effect on the phenotype of plants (e.g. VX) are analytically detectable in plants for longer periods because of hydrolyzed products inside the plants [17]. For a further overview of gas damage to plants, we refer to [24].

Detection of CWAs by observing biological material

Sanders et al. [25] developed and established a closed system aeration apparatus using Nostoc commune and Chlorella vulgaris; two autotrophic organisms, as biosensors and a commercial fluorometer to detect CWAs in suspected danger zones. Authors concluded that this system can be integrated into hand-held fluorescence sensors for field applications, respectively. But like chlorophyll fluorescence measurements being highly sensitive, the changes are unspecific which hinder the identification of the causal agent. However, such observations can provide an indication of potential hazard locations. Using HSI, the impact of CWAs can be analyzed to characterize the specific effect of individual active substances directly on the plant and in a next step to determine significant wavelength bands for the specific detections. The assessment of these wavelength bands can be used for further development of tailored optical sensors where the amount of data recorded is reduced to what is relevant for specific field applications. However, the overall impact of chemical warfare agents on plants is little investigated until now. Known and putative impact of CWAs on plants is summarized in Table 1, which also indicates a lack of important knowledge. To the best of our knowledge, a systematic investigation of the effects of CWAs on plants and their detection by optical or spectroscopic or mass spectroscopic sensors has not been reported till this day.

Plants could serve as a new indicator for CWA application events, because plants react on many active compounds which may influence the characteristic plant reflectance signature. This would accelerate the sampling and analysis on locations which are difficult to assess and will improve medical treatments, protective measures and will result in a gain of time. This manuscript shows the potential of HSI to determine and assess different plant stresses which cannot be differentiated with the human eye. As scenarios, an incident with massive heat emission was investigated and it should be proven if, in addition, CWAs have been released. Therefore, leaves of a wild growing apple tree were treated with sulfuric acid, fire or heat and were hyperspectrally measured in the VIS, NIR and SWIR range.

Table 1: Overview of mortal chemical warfare agents and their effect on plants.

<table>
<thead>
<tr>
<th>Name</th>
<th>Reactive substance</th>
<th>Effect on plants</th>
<th>Effect on mammal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine</td>
<td>Cl₂</td>
<td>Chlorosis, tipburn, intercostal necrotic spots, necrosis</td>
<td>Chemical burn</td>
<td>[18, 19, 26]</td>
</tr>
<tr>
<td>Phosgene</td>
<td>COCl₂</td>
<td>Unknown, possible chemical burn due to hydrochloric acid</td>
<td>Chemical burn of alveolus</td>
<td>[27]</td>
</tr>
<tr>
<td>Sulfur Mustard</td>
<td>C₆H₄Cl₅S</td>
<td>Necrosis, reduced germination</td>
<td>Diverse</td>
<td>[28]</td>
</tr>
<tr>
<td>Cyanide</td>
<td>KCN, NaCN</td>
<td>Inhibition of cytochrome oxidase, induction of anaerobe metabolism, increased glycolysis</td>
<td>Inhibition of cell respiration</td>
<td>[22, 29]</td>
</tr>
<tr>
<td>Agent Orange</td>
<td>C₂H₆Cl₂O₃, C₆H₆Cl₂O₃, C₆H₄Cl₂O₂</td>
<td>Excessive growth due to extreme induce of auxin (accelerated senescence)</td>
<td>Dysplasia</td>
<td>[20]</td>
</tr>
<tr>
<td>Sarin</td>
<td>C₆H₆FO₃P</td>
<td>Unknown, putative germination inhibitor</td>
<td>Asphyxiation</td>
<td>[21]</td>
</tr>
<tr>
<td>Tabun (G agent)</td>
<td>C₆H₇NO₃P</td>
<td>Unknown, putative germination inhibitor</td>
<td>Nerval palsy</td>
<td>[21]</td>
</tr>
<tr>
<td>VX</td>
<td>C₆H₇NO₅PS</td>
<td>Unknown, putative germination inhibitor</td>
<td>Asphyxiation</td>
<td>[21]</td>
</tr>
</tbody>
</table>
Plant material and treatment

Leaves were detached from a wild growing apple tree at the street side in Bonn, Germany (50°43′33.2″N, 7°05′22.8″E). To prove different stress scenarios which can be induced by an explosion with CWA release, leaves were exposed to the following treatments (Table 2):

One half of a green and a senescent leaf were treated with 95 % sulfuric acid for 2 or 10 s, respectively. The other half was touched with a red-hot coin and the senescent leaf was charred with the glowing coin, respectively. A fourth green leaf was moved two times through the flame of a full running Bunsen burner (Gasprofi 1, Wartewig Labor, Göttingen, Germany). The last green leaf was exposed to high temperature in a drying chamber (Memmert GmbH, Schwabach, Germany) at 55 °C for 7 min. Treatments were simultaneously conducted.

Hyperspectral imaging, data preprocessing and analysis

Spectral reflectance from 400 to 2500 nm was acquired with two hyperspectral imaging line scanners. Hyperspectral camera ImSpector PFD V10E (Spectral Imaging Ltd., Oulu, Finland) with a spectral range of 400–1000 nm and a spectral resolution of 2.73 nm was used to determine the VIS-NIR range. A SWIR-Camera (ImSpector N25E, Spectral Imaging Ltd., Oulu, Finland) with a spectral resolution up to 5.8 nm was used to record the spectral range from 1000 to 2500 nm. The hyperspectral cameras were mounted on a line stage. For image recording the leaf samples were placed nadir to the camera system and were illuminated by six 250 W halogen tungsten lamps (Analytical Spectral Devices Inc., Boulder, CO, USA) with a vertical orientation of 45°. The samples were placed at a distance of 35 cm to the foreoptics of the cameras. Hyperspectral measurements were performed without environmental illumination. Spectral binning and spatial binning of the cameras were set to 1. Frame rate was set to 10 frames per second and exposure time was adjusted to the object. For detailed description of the measuring setup refer to [30].

Hyperspectral data cubes were acquired immediately after the different treatments. To receive the relative reflectance (R), a white reference bar (SphereOptics GmbH, Uhldingen-Mühlhofen, Germany) was recorded (W), followed by a dark current image (B). Subsequently, the leaf samples (I₀) and a corresponding dark current image (B₂) was recorded. Calculation of relative reflectance was according to the formula \[ R = \frac{(I₀ - B₂)}{(W - B₀)} \], using the software ENVI 5.1 + IDL 8.3 (ITT Visual Information Solutions, Boulder, CO, USA). Spectral reflectance from 400 to 2500 nm was acquired with two hyperspectral imaging line scanners. Hyperspectral camera ImSpector PFD V10E (Spectral Imaging Ltd., Oulu, Finland) with a spectral range of 400–1000 nm and a spectral resolution of 2.73 nm was used to determine the VIS-NIR range. A SWIR-Camera (ImSpector N25E, Spectral Imaging Ltd., Oulu, Finland) with a spectral resolution up to 5.8 nm was used to record the spectral range from 1000 to 2500 nm. The hyperspectral cameras were mounted on a line stage. For image recording the leaf samples were placed nadir to the camera system and were illuminated by six 250 W halogen tungsten lamps (Analytical Spectral Devices Inc., Boulder, CO, USA) with a vertical orientation of 45°. The samples were placed at a distance of 35 cm to the fore-optics of the cameras. Hyperspectral measurements were performed without environmental illumination. Spectral binning and spatial binning of the cameras were set to 1. Frame rate was set to 10 frames per second and exposure time was adjusted to the object. For detailed description of the measuring setup refer to [30].

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signals are smoothed by employing the Savitzky-Golay filter [31]. Parameters for the smoothing process were 12 supporting points to the left and right and a third degree polynomial.

Spectral signatures of pixels from healthy and treated regions were extracted manually. Depending on the treatment size, a polygon region of interest of about 500–80,000 pixel was extracted. To calculate the vegetation index, Normalized Differences Vegetation Index (NDVI), spectral reflectance at 800 nm (NIR) and 680 nm (Red) were used. The NDVI is a normalized difference calculation of reflectance from the NIR and from the red range [NDVI = (800 nm–680 nm)/(800 nm + 680 nm)] and shows the vitality of a plant due to green biomass [32, 33]. A second spectral index was calculated in the SWIR using reflectance at 2000, 2100 and 2200 nm. The index is described as the cellulose absorption index [CAI = 0.5 × (2000 nm + 2200 nm)–2100 nm] which indicates the depth of lignocellulose absorption [34]. Lignocellulose is the main compartment of cell walls of woody plants and the index shows the possibility to detect dried and degraded plants in remote sensing experiments independently of the soil background [34].

Results and discussion

Hyperspectral images and spectral indices

Healthy and treated leaves can be distinguished in the RGB image (Fig. 2). Black areas on the left hand side of the second green and the senescent leaf is caused by 95% sulfuric acid for 10 s (LA) [35]. The adjacent brown discoloration came from 95% sulfuric acid for 2 s (SA). Further brown to black discoloration on the right hand side of this leaves was caused by a glowing coin (R). The fourth leaf shows combustion characteristics with burst leaf tissue due to a Bunsen burner (B). A dark green color is showed by the last leaf which was dried by high temperature (D). In the given color-infrared (CIR) visualization of the VIS-NIR hyperspectral image, significant differences are mainly visible for the senescent leaf. This is because the senescent leaf shows a heterogeneity in chlorophyll content that is indicated in remaining vegetative tissue at the leaf base. The CIR visualization represents vegetation with a large amount of chlorophyll in intense bright red. Lighter tones of red and pink represents vegetation with less chlorophyll. These differences can now be analyzed pixel per pixel to assess the plant health status [30]. Obvious differences are visible in the pseudo RGB visualization of the SWIR image. Treated leaf areas are visible and also differences between the treatments appear. Beside the black area due to the longer sulfuric acid treatment, the short acid treated area can be distinguished from the burned area. Charred (HB, heat burned) leaf area can be distinguish from the corroded area though these parts are only black on RGB visualization. This effect is derived from the different treatment impact on the leaf water content which mainly characterized the SWIR reflectance of plants [36]. Calculation of two different spectral indices increased the contrast of differences between the leaves and the treatments. The NDVI visualized the corroded and burned leaf areas but only on green leaves. This limits the NDVI for analysis of chemical treatments only on green and healthy plants, because this index is characterized by the green biomass and photosynthetically active radiation [32, 33, 37]. In contrast, the CAI indicates also differences of the same treatment on green and senescent leaves. In addition, stronger dried areas on the dried treated leaf are visible, because the index estimates non-photosynthetic biomass [38]. Interestingly, the burned and roasted areas have a higher CAI to corroded leaf area which indicates decreased photosynthetically activity due to heat stress. This results are in accordance with investigations by [39]. They showed a declined ribulose 1,5-bisphosphate concentration due to heat stress and hypothesized an increased thylakoid membrane leakiness which inhibit the photosynthesis.
Fig. 2: Apple leaves treated with 95% sulfuric acid for 10 (LA) or 2 (SA) s, roasted (R) or charred (HB) by a glowing coin, moved through a Bunsen burner (B) or dried at 55 °C for 7 min (D). RGB image were taken immediately after treatment with a digital camera (EOS 6D, Canon, Tokyo, Japan). Hyperspectral images were taken in the visual-near-infrared (VIS-NIR) range with the ImSpector PFD V10E (Spectral Imaging Ltd., Oulu, Finland) in the shortwave-infrared (SWIR) with the ImSpector N25E (Spectral Imaging Ltd., Oulu, Finland). Normalized Difference Vegetation Index (NDVI) was computed to assess leaf vitality and the Cellulose Absorption Index (CAI) to analyze non-photosynthetically biomass (CIR, color-infrared).
Spectral signatures

For a detailed analysis of the treatment impact on the spectral characteristics of plants, spectral reflectance of the symptom spots were manually extracted (Fig. 3). Hyperspectral signature of the cropped, healthy apple leaf showed a characteristic spectral pattern of healthy tissue (Fig. 3). The treated samples revealed

Fig. 3: Spectral signatures of non-treated (healthy) apple leaf and apple leaves treated with 95 % sulfuric acid for 10 (LA) or 2 (SA) s, roasted (R) or charred (HB) by a glowing coin, moved through a Bunsen burner (B) or dried at 55 °C for 7 min (D) with their corresponding phenotype. Spectral signatures changed specific to the treatment and can be characterized. Similar treatments on a senescent leaf showed differences in the spectral reflectance of the VIS-NIR range (b). Spectral signatures of the healthy leaf correspond to one green apple leaf (a, b, c). (n = 1).
specifically changed spectral signatures. Green leaf, treated with sulfuric acid showed different changes in the spectral signature depending on the treatment time (Fig. 3a). The leaf area, treated only for 2 s showed an increased spectral reflectance 550–670 nm and over the whole SWIR range. The 10 s treated leaf area showed reduced reflectance intensity in the visual, near-infrared and SWIR range (Fig. 3a). These differences are caused firstly by structural changes due to the corrosion levels [13]. The lower reflectance intensity and changed reflectance pattern after the longer sulfuric acid exposure is because the leaf tissue was almost carbonized. In contrast, the roasted leaf area showed a decreased reflectance 750–1000 nm, but an increased reflectance 1000–2500 nm. Here the leaf structure is stable and showed the characteristic reflectance pattern. However, the water content is minimized which is revealed in an increased reflectance of the SWIR.

Interestingly, treatments on a senescent leaf revealed a different spectral pattern with the exception of 10 s sulfuric acid treatment (Fig. 3b). This leaf tissue was strongly carbonized which is linked to the loss of characteristic plant reflectance pattern. The spectral reflectance was increased from 450 nm to 2100 nm for the shorter treated leaf area. This pattern revealed non-photosynthetically active plant tissue, because of reduced or missing chlorophyll [40]. A similar spectral pattern was shown by the roasted leaf area with a higher increase in the SWIR. Nevertheless, comparing images of corroded leaf tissue using sulfuric acid with leaf area that was charred by a glowing coin, no differences are visible for the naked eye (Fig. 2). Interestingly, this charred leaf area (HB) showed an exceptional spectral pattern compared to the other tested treatments (Fig. 3b). Spectral reflectance intensity increased 1000–1700 nm in a ramp pattern. This phenomenon indicates that the structural changes due to these treatments are different but not obvious for human eyes. This spectral range has therefore a high potential for the assessment of the impact of CWAs on plants by HSI.

The green leaf which was moved through a Bunsen burner had a decreased reflectance intensity 750–1000 nm (Fig. 3c). In the SWIR range reflectance was increased and water absorption bands were not clearly visible anymore. In contrast, the dried healthy leaf showed a decreased reflectance in the VIS range and from 700 to 1000 nm. This showed the impact of heat on the photosynthesis apparatus [39]. The reflectance intensity was also increased in the SWIR, but water absorption bands were visible as well as characteristic plant SWIR reflectance pattern. Overall, probably wavelength bands for high accuracy in determination and differentiation of the tested treatments and for further investigation are 600, 800, 1450 and 2200 nm.

Conclusion and outlook

Hyperspectral imaging is a unique method and technique to determine and assess the plant status, because structural characteristics and the leaf chemistry are reflected in the optical properties of plants. Beside biotic stresses, acidic solutions, fire and heat can change or destroy the plant structure and chemistry. This results in characteristic spectral reflectance pattern which can be used to assess the influencing factor and impact level. The results of the present study underline the high potential of HSI for the detection and characterization of chemical warfare agents. Implementation of HSI in research projects like the Advances Plant Technologies project by the Defense Advanced Research Projects Agency could bring a sophisticated method for a ground-based sensor solution forward. We believe that HSI will provide the spectral information of interest to develop adapted sensors for faster and secure acquisition of chemical warfare. Therefore, the impact of common CWAs on plants must be investigated and further validated on different plant species. Based on a spectral library, data driven analysis will establish an accurate and fast acquisition of relevant spectral information for specific chemical substances. To utilize the full potential of these highly sophisticated, innovative technology and high dimensional, complex data for plant sciences, a multi-disciplinary approach – including plant physiology, chemistry, engineering and informatics – is required. In addition to the tailored spectral sensors, a final and collective goal will be the implementation in remote sensing for an fast warn and secure system for employees and the public in case of chemical warfare.
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