PHM Design Report

prepared for

WP 3.5 – Plant Health Monitoring

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1.3	2018-10-15	See List of Authors	Added new chapter on Bio-detection and Decontamination. Shifted previous chapter 2 into Annex A, B and C. Added multi-wavelength imaging system guide manual as Annex D. Added Decontamination Test Reports as Annex E. Minor updates to text. Final release version

Executive summary

EDEN ISS is a program funded by the EU having as main goal the development of a greenhouse to be installed in Antarctica at the Neumayer III station to test key technologies for a future space greenhouse. The Antarctic environment has been selected for an analogue test campaign because of its similarity with extraterrestrial outposts, with harsh ambient conditions, and similar limitations on, for example, power, number of human operators, and accessibility. Taking advantage of the limited accessibility and constraints on data transfer between Europe and the Antarctic, EDEN ISS has the objective to test the operation scenario, i.e. the planning of activities, the procedures, and the interaction between on-site and remote operators in a similar scenario as would apply for future planetary bases.

One of the key points of the greenhouse performance monitoring is the plant health monitoring, and most of all the early detection of plant disease and the subsequent activation of corrective actions. For that reason, a plant monitoring system is foreseen with the objective of collecting information suitable for analysis by a knowledge system (either local or remote) to assess and advise prophylactic measures to the local operator. As only one operator will handle the system, there is a need for objective assessment of plant welfare and performance, through automatic detection and expert assessment of remote information.

This document describes the final layout of the Plant Health Monitoring system. This system differs in several aspects from the one proposed at the CDR. In particular the mobile platform hosting the cameras for lateral views of the plants has been discarded due to budget issues. Nevertheless the capability of lateral image acquisition will be maintained by means of fixed cameras.

The system relies on several components:

1. Top view images by a fixed system of visual cameras one for each/two trays
2. One HD-video camera modified for multispectral imaging
3. An automatic image acquisition and distributor system

After some local pre-processing of information, the Plant Health Monitoring will provide data to remote experts from different disciplines, in order to implement their feedback into the overall control loop. Some information on the data acquisition and transfer is provided within this document.

Aside from plant health monitoring, the on-site operator will have options to control plant health by taking corrective actions to address issues with e.g. nutrient solution composition, environmental conditions or microbial contamination. This document details the equipment which will be available in situ to determine microbial contamination and, if necessary, to decontaminate the facility, to ensure safe and clean food production.

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Acronyms

Acronym	Explanation	Acronym	Explanation
AIT	Assembly Integration and Test	CDR	Critical Design Review
APH	Advanced Plant Habitat	FEG	Future Exploration Greenhouse
AS	Aero Sekur S.p.A.	HD	High Definition
ASI	Italian Space Agency	MTF	Mobile Test Facility
ATS	Analogue Test Site	NMIII	Neumayer III
BLSS	Bio-regenerative Life Support System	PHM	Plant Health Monitoring
C.R.O.P.	Combined Regenerative Organic-food Production	PoE	Power over Ethernet
CAB	Bio-regenerative Environmental Control	RAID	Redundant Array of Independent Disks
CAD	Computer Aided Design	SW	Software
CDH	Command and Data Handling	WLAN	Wireless Local Area Network

1 Plant Health monitoring

This document describes the components of the system for plant health monitoring that will be installed and operated in the Antarctic greenhouse, the Mobile Test Facility (MTF). As only one operator will handle the system, there is a need for objective assessment of plant welfare and performance, through automatic detection and remote expert assessment of relevant information. This document describes the system that will collect this information.

The main plant cultivation area within the MTF is named the Future Exploration Greenhouse (FEG). One of the key points of the FEG performance monitoring is the plant health monitoring, and most of all the early detection of plant disease and the subsequent activation of corrective actions. For that reason, a plant monitoring system is foreseen with the objective of collecting information suitable for analysis by a knowledge system (either local or remote) to assess and advise prophylactic measures to the local operator.

The system relies on several components:

1. Top view images by a fixed system of visual cameras one for each/two trays
2. Lateral view images by a fixed system of visual camera's one for each rack.
3. An automatic system for daily images acquisition, local storage and forwarding to the European sites.

Remark: One or more GOPRO cameras modified for multispectral imaging will also be used for PHM. This system is under responsibility of the University of Florida. A manual for operation of this system can be found in Annex D.

1.1 FEG Configuration and Structure

The whole EDEN ISS greenhouse (named the Mobile Test Facility or MTF) consists of two 20 ft. shipping containers (Figure 1-1) which will be placed on an elevated platform a couple of hundred meters from the Neumayer III Station in the Antarctic. One of these containers will house the Future Exploration Greenhouse (FEG), which is the main plant cultivation area of the MTF.

Figure 1-1: Mobile Test Facility.

The FEG houses 8 multi-level growth racks which will be used to cultivate the crops selected for the EDEN ISS project. In Figure 1-2 a top view of the FEG is presented. As seen in the image, each rack will have two growth trays (green rectangles in the image) per level, up to a maximum of eight growth trays per chamber. A total of 42 growth trays can be used within the FEG at a time, according to the layout shown in Figure 1-3.

Figure 1-2: Future Exploration Greenhouse – Top view.

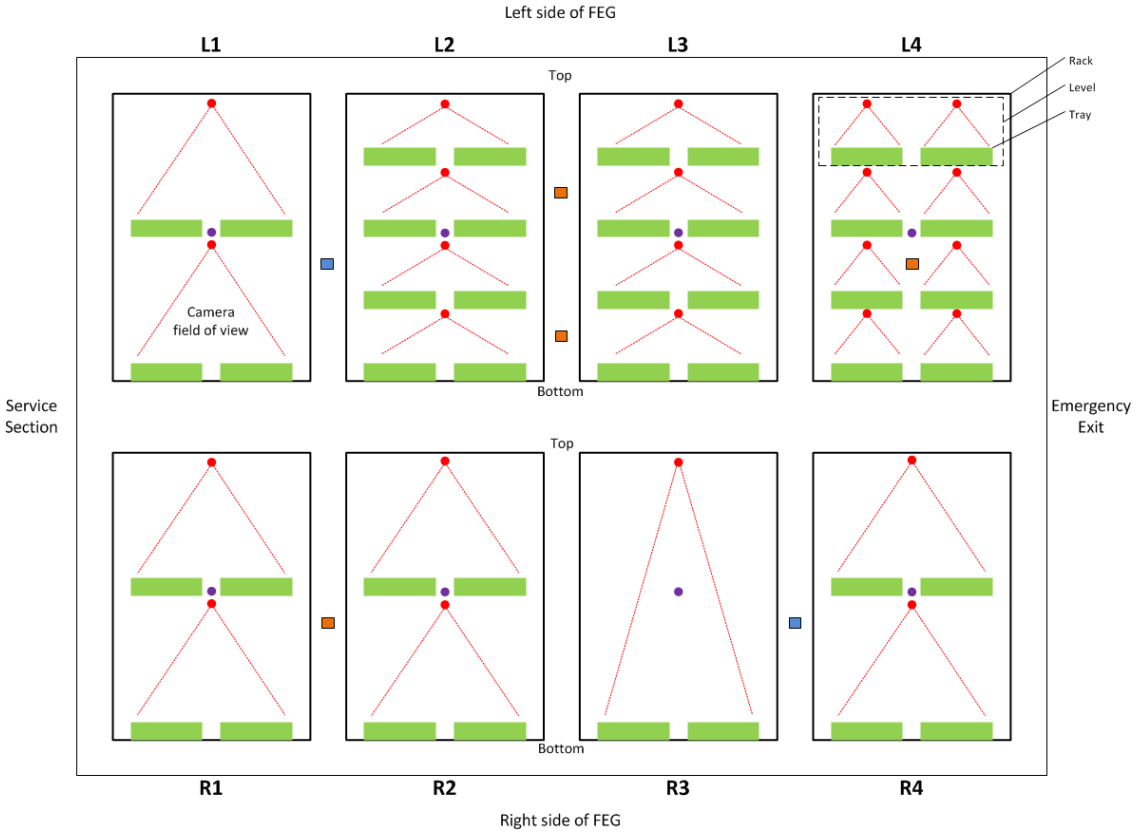


Figure 1-3: Future Exploration Greenhouse: plant tray configuration – A mix of top view (the chamber) and lateral view (the racks). This figure defines: chamber; rack; unit; level and tray.

1.2 Plant Health Monitoring System Layout

Following the analysis done before the CDR phase and in the post-CDR iterations, the *HIKVISION DS-2CD2542F-I (4 Mpx, 2.8 7mm)* has been selected for both top view and lateral images acquisition.

The HIKVISION DS-2CD2542F-I is a camera with a fixed focus (Figure 1-4) whose functionalities are described in Annex A.

Figure 1-4: HIKVISION DS-2CD2542F-I

A total of 40 HIKVISION DS-2CD2542F-I cameras have been acquired for the project, they will be used as follow:

- 17 are mounted on the ceiling of each rack level (with the exclusion of the nursery), pointing downward towards two trays for top view imaging
- 8 are mounted on the ceiling of each nursery level (two per level, one for each tray), pointing downward towards the trays, for top view imaging
- 8 are mounted along the corridor, on the rack structure pointing at the opposite rack for lateral view imaging
- 4 are used as internal ambient monitoring

- 3 cameras will be used as spares to replace active cameras in case of failures.

Figure 1-5: **Camera placement in the FEG**

In addition, two other HIKVISION cameras (Figure 1-6) have been acquired for exterior video-monitoring purpose. In principle the description of this system is out of the scope of this document, nevertheless, since this camera will be part of the same PHM network and managed with the same systems and tools, it is worth to include their description in this document.

As already foreseen in the CDR study and part of the trade-off analysis, for exterior monitoring once again a HIKVISION camera has been selected. Specifically the DS-2CD4026FWDA (Telecamera boxed IP 2 Mpx. P-Iris. 0,01lux) model has been selected, which will be equipped with the multifocal lens of type HV3816D-8MPIR (Varifocal Lens 3,8 / 16mm F 1.5). This camera has the capability to work in harsh environment and extremely low temperatures (-30 °C) but that is not enough to survive and operate in the Antarctic site where temperatures could drop to -50 °C. For that reason, this camera will be housed in a box equipped with heaters (the VIDEOTEC HPV42K2A160) to increase its operating range to lower external temperatures.

Camera DS-2CD4026FWDA	Lens HV3816D-8MPIR	Housing HPV42K2A160

Figure 1-6: External Camera

Figure 1-7: Camera's Network

The aforementioned cameras are connected to the MTF network via Ethernet switches located in the FEG and the Service Section. They are controlled by a **Camera Control PC** which is equipped with dedicated camera management software (SW).

Figure 1-8: Cisco-SLM2024-Gigabit-Smart-Switch with 24 ports

The Service Section, located within the other 20 foot container, will house a 24 port switch from Cisco (model: SLM2024T-EU 200 Series SG200-26), which is installed in the rack system of the Service Section. The 24 port switch will be connected to the MTF camera system, as well as to two computers and a laptop in the Service Section for monitoring and control purposes. Furthermore, the WLAN access point, patch antenna, VOIP telephone and the LED lamps in the FEG (over two 48 port switches) will also be connected to this switch.

The aforementioned two 48 port switches (model: CISCO SG500-52P with 48 POE-ports) are mounted near the ceiling on the right and left side of the FEG. The dimensions of the 48 port switches are 440 x 350 x 44 mm (B x H x T) and they are fully backwards compatible with IEEE 802.11af PoE, which is needed for the camera system of the FEG. These switches will serve to connect the EDEN ISS network to the FEG cameras and the LED lamps. In total there are 35 cameras and 42 LED lamps connected to the 48 port switches.

Both switches are equipped with PoE ports, and for this reason all the cameras are directly powered via the LAN cable. Only two power lines are needed (12VDC/24VAC), for the heaters in the external camera boxes.

Figure 1-9: CISCO SG500-52P with 48 POE-ports.

Two FUJITSU Server PRIMERGY RX1330 M2 computers are available at the Antarctic site for the camera system configuration and control, one located in the MTF, the other in the NM-III station. They have the same characteristics and features and will be configured in a similar way, such that one is the backup of the other, providing the capability to replace a failed PC with minor or no modification.

Both have installed the same software for the camera system configuration and control, i.e. the operator can do the same operations independently of the location. But only the computer in the NM-III station will be used for image storage, even if both are equipped with the same RAID system.

Figure 1-10: Fujitsu Server FUJITSU Server PRIMERGY RX1330 M2

Both the computers are equipped with the following software:

- Windows 10
- MS Office
- FTP Server (Filezilla Server)
- FTP Client (Filezilla Client)
- HIKVISION SADP SW
- HIKVISION iVMS SW
- Phyton Source Code
- Geany Editor
- TPZ Camera Management SW

1.3 Camera Networking

The camera system, including the switches and the computers for camera control, is part of the EDEN ISS network that includes, of course, all the other systems and components for the EDEN ISS operations.

This chapter deals with the definition of all the network parameters that have to be assigned to the camera system such that the communications between the different parts of the Plant Health Monitoring System are correctly defined, and are compatible with the existing NM-III Network.

The AWI IT-expert responsible for the NM-III network in Antarctica informed us that the subnet IP 192.168.39.0/24 has been reserved for the EDEN ISS operations and that it only is possible to use the static IP addresses, i.e. it is not possible to use DHCP.

Therefore the following network parameters have to be used for the camera system:

- IP-Address: 192.168.39.xxx
- Netmask: 255.255.255.0
- Gateway: 192.168.39.254
- DNS: 192.168.38.46
- Timeserver (NTP): 192.168.33.40

Item	Position	IP Address	Server Port	Subnet Mask	Gateway	HTTP Port
48 PoE Switch	MTF/FEG					
48PoE Switch	MTF/FEG					
24 PoE Switch	MTF/SS					
Camera PC	MTF/SS	192.168.39.102	8000	255.255.255.0	192.168.39.254	80
Camera PC	NMIII					
Top View Cam	L1-2C	192.168.39.111	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L1-4C	192.168.39.112	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L2-1C	192.168.39.113	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L2-2C	192.168.39.114	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L2-3C	192.168.39.115	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L2-4C	192.168.39.116	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L3-1C	192.168.39.117	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L3-2C	192.168.39.118	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L3-3C	192.168.39.119	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L3-4C	192.168.39.120	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L4-1L	192.168.39.121	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L4-2L	192.168.39.122	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L4-3L	192.168.39.123	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L4-1R	192.168.39.125	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L4-2R	192.168.39.126	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L4-3R	192.168.39.127	8000	255.255.255.0	192.168.39.254	80
Top View Cam	L4-4C	192.168.39.128	8000	255.255.255.0	192.168.39.254	80
Top View Cam	R1-2C	192.168.39.129	8000	255.255.255.0	192.168.39.254	80
Top View Cam	R1-4C	192.168.39.130	8000	255.255.255.0	192.168.39.254	80
Top View Cam	R2-2C	192.168.39.131	8000	255.255.255.0	192.168.39.254	80
Top View Cam	R2-4C	192.168.39.132	8000	255.255.255.0	192.168.39.254	80
Top View Cam	R3-4C	192.168.39.133	8000	255.255.255.0	192.168.39.254	80
Top View Cam	R4-2C	192.168.39.134	8000	255.255.255.0	192.168.39.254	80
Top View Cam	R4-4C	192.168.39.135	8000	255.255.255.0	192.168.39.254	80
Side View Cam	L1-S	192.168.39.141	8000	255.255.255.0	192.168.39.254	80

Side View Cam	L2-S	192.168.39.142	8000	255.255.255.0	192.168.39.254	80
Side View Cam	L3-S	192.168.39.143	8000	255.255.255.0	192.168.39.254	80
Side View Cam	L4-S	192.168.39.144	8000	255.255.255.0	192.168.39.254	80
Side View Cam	R1-S	192.168.39.145	8000	255.255.255.0	192.168.39.254	80
Side View Cam	R2-S	192.168.39.146	8000	255.255.255.0	192.168.39.254	80
Side View Cam	R3-S	192.168.39.147	8000	255.255.255.0	192.168.39.254	80
Side View Cam	R4-S	192.168.39.148	8000	255.255.255.0	192.168.39.254	80
External Cam.	EAST	192.168.39.171	8000	255.255.255.0	192.168.39.254	80
External Cam.	WEST	192.168.39.172	8000	255.255.255.0	192.168.39.254	80
Observ. Cam.	MTF/CP	192.168.39.181	8000	255.255.255.0	192.168.39.254	80
Observ. Cam.	MTF/SS	192.168.39.182	8000	255.255.255.0	192.168.39.254	80
Observ. Cam.	MTF/SS	192.168.39.183	8000	255.255.255.0	192.168.39.254	80
Observ. Cam.	MTF/FEG	192.168.39.184	8000	255.255.255.0	192.168.39.254	80

Remarks:

For top view camera position the position code (XY-ZK) is identified in this way:

- X (L = Left, R =Right) identifies the side of the corridor, with respect to the FEG entrance, where the rack is located
- Y (1 to 4) identifies the rack number (1 is close to the FEG entrance, 4 is on the opposite side of the FEG)
- Z (1 to 4) identifies the rack level (starting from the bottom, 1 is the first level, 4 is for the upper level)
- K (L = Left, R = Right, C = Center) defines the position of the camera with respect to the level.

For side view camera the position code (XY-Z) is identified in this way:

- X (L = Left, R =Right) identifies the side of the corridor with respect the FEG entrance where the rack is located
- Y (1 to 4) identifies the rack number (1 is close to the FEG entrance, 4 is on the opposite side of the FEG)
- Z (S) identifies the camera as a side view camera.

Information on the software used to control the cameras can be found in Annex C, while further specifications on the camera hardware is presented in Annex A. Additionally, Annex B and C contain information on software developed by Telespazio for the automatic image acquisition and image transfer processes, which is used as part of the plant health monitoring system.

2 Bio-Detection and Decontamination

2.1 Introduction

Microbiological contamination is an issue for crew and hardware in space. Fungi and bacteria will become a great safety related-danger on the ISS or on long-term missions. This will affect the crew, materials, environment and plants (if on-board during flight). Countermeasures have to be developed and deployed to prevent damage like microbial attack of materials or crop failure. Figure 2-1 shows leaves which are affected with fungi.

Figure 2-1: Fungi on leaves.

All materials can be affected by microorganisms. Besides metal - also polymer, glass and ceramic can be attacked and modified. This process is also called microbial induced corrosion (MIC). It is estimated that 20 % of all costs caused by corrosion are microbial induced. (E. Heitz, H.-C. Flemming, W. Sand, 1996).

Sulfate Reducing Bacteria (SRB) are basically the main source of the problems, although other bacteria, like iron oxidizing bacteria and fungi, could cause problems too.

A generated hole in aluminum by a fungus on the MIR

Figure 2-2: Microbial Induced Corrosion (MIC).

Examples of materials affected by microbial contamination are aluminum panels on the Russian MIR space station (Figure 2-2) and 15 year old cables within the ISS (see Figure 2-3). The affected cables (could) have an increased risk of smoldering or fire.

Figure 2-3: Microbial attacked material.

During the concurrent engineering Study the Bio Detection and Decontamination was discussed with the involved disciplines (Food Quality; Plant Health Monitoring; Microbial Investigations) regarding the different requirements / limitations given by the plants.

2.2 Bio detection

For the detection of the biological load on surfaces but also on the plants the commercial E-Nose PEN3 of the company AIRSENSE (see Figure 2-4) will be used within the greenhouse. But it first had to be checked if the available sample taking is sufficient for the required measurements.

2.2.1 E-Nose

This portable device is based on MOS-Technology (metal oxide semiconductors). An electronic nose possesses an array of chemical sensors, which are sensitive to gas molecules. This sensor array is in connection with a data acquisition unit.

Surfaces or samples can be analyzed by this device and existing fungi and bacteria can be located. This device is optimized to analyze the VOCs (Volatile Organic Compounds) which are produced by the bacteria or fungi.

Figure 2-4: E-Nose.

The device has internal pumps for taking samples or purging the system. During the purging phase, air is sucked through an active charcoal filter and purges the sensors of an earlier sample. Sample gas is sucked in by a sample taking unit and is analyzed by the sensor array of 10 metal oxide semiconductors. The status of the device is tracked on the display on the front side. All generated data is stored onto internal data storage and can be transferred to an external PC (Figure 2-5). For the data transfer and the data evaluation the commercial software WinMuster will be used.

Figure 2-5: Schematic Diagram.

Figure 2-6 shows an exemplification of a possible use case. The electronic nose is located inside a habitat and is taking samples on different surfaces or on plants.

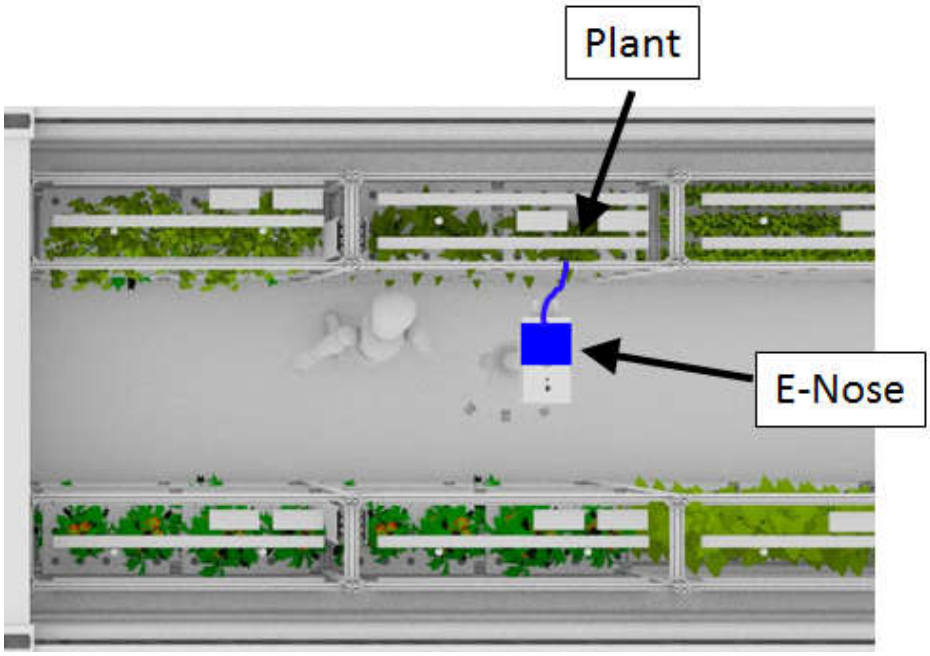


Figure 2-6: Sample taking inside the habitat.

2.2.2 Sample taking

Since the plants do not have flat surfaces, the following options have been considered:

2.2.2.1 Breaking off a leaf and measurement of the leaf with the available Sampler

In Figure 2-7 below the so-called Air-sampler can be seen. In this case the target is a piece of leaf. The sample is sucked in by the E-Nose. To protect the sensor array from particles like spores or dust, the particle filter is installed between the Air-sampler and the E-Nose itself. To generate a "Zero-Gas" a charcoal filter is installed at the inlet of the Air-sampler.

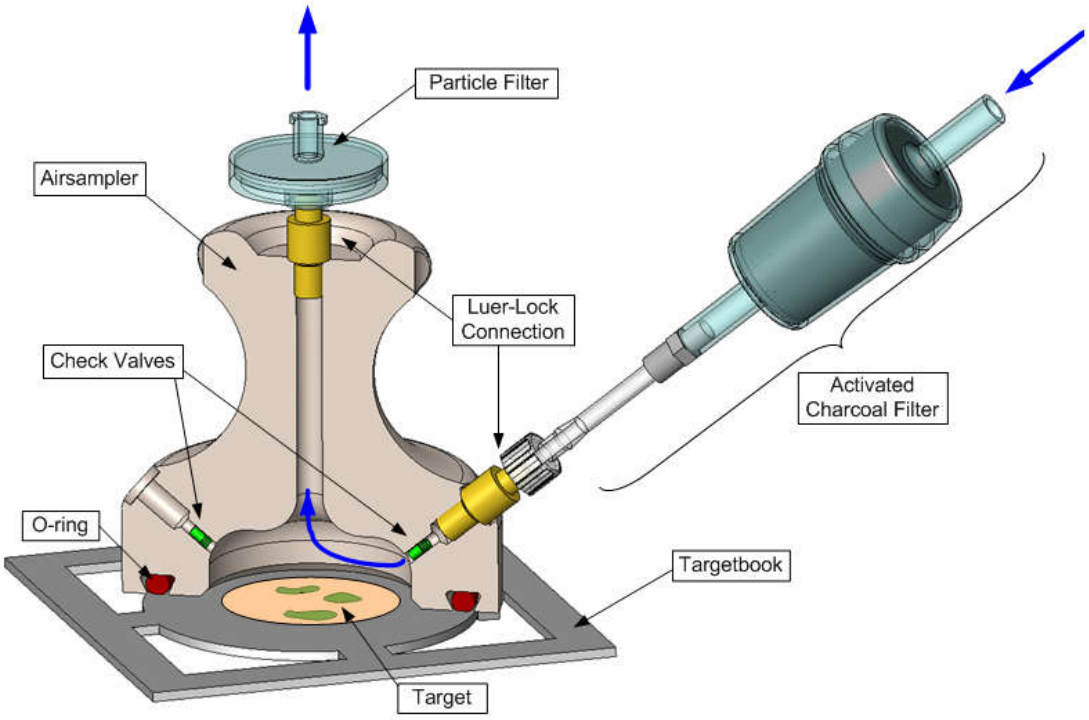


Figure 2-7: Sample Taking Unit.

From discussions with plant biologists it was learned that if the plant is injured (e.g. by cutting off leaves of pieces) it immediately starts to produce different compounds to close the "wound". These organic compounds are very volatile and would strongly affect the measurements. So cutting out samples of the plant for bio-detection was found to not be suitable for use with the E-nose.

2.2.2.2 Indirect Sample Taking on Plants with contact plate

With regards to the requirements of the Plant Health and the Microbial Investigation domain an indirect sample taking method was developed for Bio-Detection (see Table 1).

Table 1: Indirect Sample Taking Process.

Step 1: Contact plate is attached to the plant or surface, so a collection of the contamination on a defined area can be guaranteed	

Step 2: The Contact plate (Target) is now placed under the Air-sampler	
Step 3: The Contamination on the Contact Plate is now measured with the E-Nose	
Optional Step 4: Additionally the Contact Plate can be incubated for the classical counting of the CFUs (Colony Forming Units). Remark: Antarctica Protection issue has to be checked	

Figure 2-8 depicts the major drawback of this method. A test regarding the efficiency of this kind of sample taking was performed at Airbus DS and showed that only approx. 1 % of the defined contamination stuck and can be counted on the contact plate. The measuring result is only a subset of the original sample and this has to be taken into account.

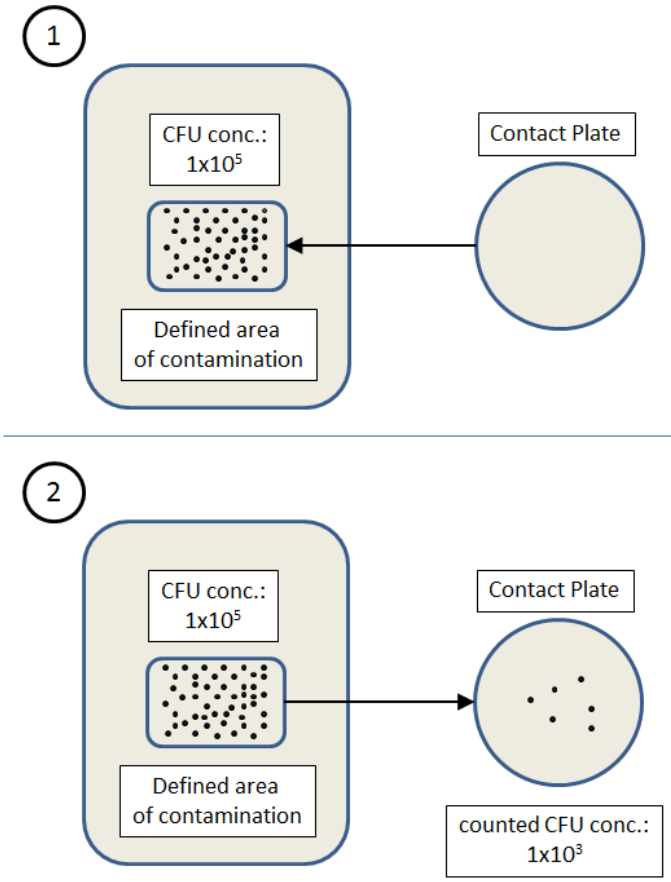


Figure 2-8: Indirect Sample Taking Method Schematic

2.2.2.3 Sample taking directly on the leave:

As another alternative, it is expected that bio-detection directly on a leaf (as seen in Figure below), without prior cutting of the leaf, should also be applicable for most of the selected plants. This kind of sample taking does not allow measurements on e.g. the plant trunk.

Figure 2-9: Sample taking directly on the leaf.

Figure 2-10: Possible use inside a habitat.

Since the Plant Sampler is modular and based on the already introduced Air-sampler, measurements on plants and on different surfaces can be performed (see Figure 2-11 below). The Air Sampler can be demounted from the Plant Sampler to perform measurements on different surfaces.

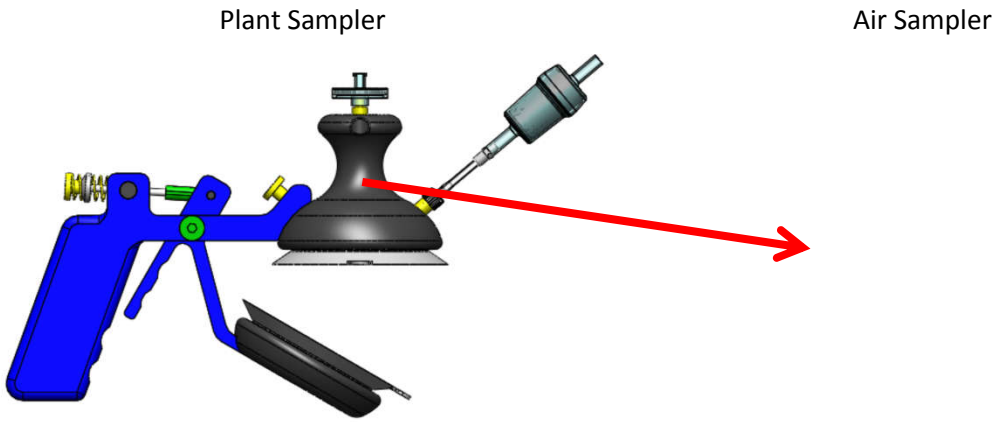


Figure 2-11: Possible use inside a habitat.

2.2.3 Training of the E-Nose

For the detection of the contamination the E-Nose needs a dedicated training. Training involves cultivation of a specific biological strain, measurement of the strain with the E-Nose and then including the measurement data in the electronic database for the system.

In the ISS E-Nose project some strains have been trained already. First of all it had to be checked if already trained strains are relevant for the EDEN project. The following cultures have been trained before:

- *Bacillus subtilis*
- *Staphylococcus warneri*

- *Aspergillus versicolor*
- *Penicillium expansum*
- *Pseudomonas geniculata*
- *Micrococcus luteus*

In Figure 2-12 below the so-called PCA (principal component analysis) of the E-Nose training with 4 different strains (PE = Penicilium Expansum, AV = Aspergillus Versicolor, BS = Bacillus Subtilis, SW = Staphilococcus Warneri) can be seen. If an additional strain will be trained, it will be included in the database in the same manner.

Figure 2-12: PCA of a E-Nose Training Including 4 Strains.

Possible considerations for additional strains to be detected within the EDEN ISS project were

- Pathogenic strains, which could affect plant or human health
- Additional strains which could affect materials
- Strains which were likely to be present in high quantities

After research and discussion with the dedicated disciplines regarding the relevant strains for the EDEN project, the following could be concluded:

- Only Pathogenic strains need to be detected
- The most important strains regarding Food Safety are:
  - *E. coli*
  - *Salmonella spp.*

2.2.4 Expression of the Results

For the expression of the results gained with the E-Nose, the thresholds for the maximum allowable concentration of biological contamination on the plants have to be considered. Since there is no expertise regarding regulations given by authorities, the requirements regarding the microbiological load are planned to be combined with the inputs given in the document shown in Figure 2-13 below.

COMMISSION REGULATION (EC) No 2073/2005

of 15 November 2005

on microbiological criteria for foodstuffs

(Text with EEA relevance)

Figure 2-13: Regulation Documentation

This document also includes thresholds for the control of foodstuff contaminations which are not applicable to the EDEN ISS project. In the document mentioned above there is only a requirement that in 25 g of the fruit the dedicated contaminants have to be absent (the thresholds are still to be clarified by the different disciplines). One common requirement in the EU regulations is the ALARA - as low as reasonable achievable. However, from the microbial investigations domain the requirement is given to express the amount of contamination in CFUs (Colony Forming Units). For that reason a new view for the expression of measurement data was introduced and agreed upon by the dedicated parties (see Figure 2-14 below).

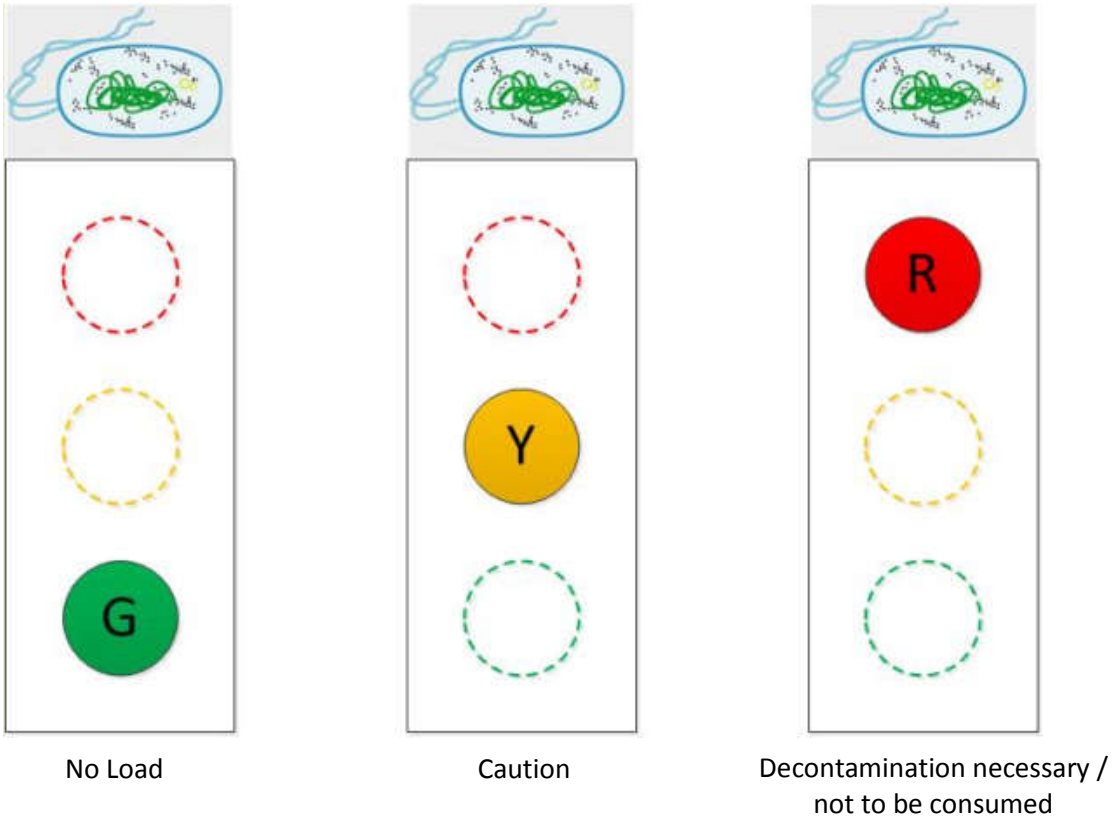


Figure 2-14: Expression of the Measurement Data.

- No Load: If either no contamination is detected or is below the requirement given by the regulation above or below the LOD (Limit of Detection), the measurement can be seen as without contamination

- Caution: If a contamination can be measured (e.g. in a range of 10e5 - 10e8 CFU) it has to be checked if the plants have to be further processed (cleaning, etc.).
- Decontamination necessary / not to be consumed: If a contamination above the maximum allowable limit (TBD) is measured (e.g. higher than 10e8 CFU tbc), the plants are not to be consumed or even the whole greenhouse must be decontaminated.

Remark: It is recommended to perform the measurements on the plants right before harvest (e.g. one day before).

2.2.5 Measurement Locations

Since the E-Nose measurements will be compared with the classical analysis, the measurement locations for both methods had to be determined prior to the testing and operations phase.

The classical microbial investigation method is performed by swabbing of the dedicated surfaces or plants with a Q-Tip / swab (see Figure 2-15 below).

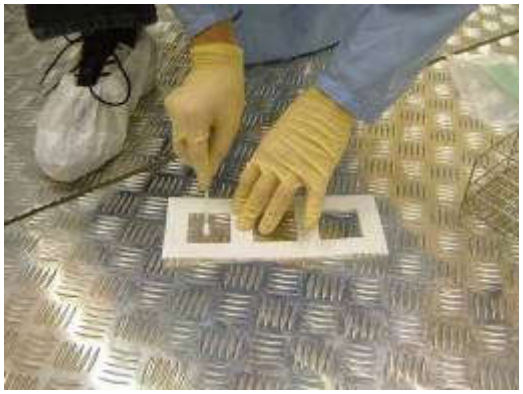


Figure 2-15: Sample Taking of a Surface.

For that reason, samples of the chosen surfaces and plants samples are taken with the Q-Tip / swab (see Figure 2-16) monthly on a predefined area (≤ 25 cm^2). The swab has a flocked Nylon head and is moistened with sterile pure H_2O.

Figure 2-16: Q-Tip / Swab for Classical Microbial Investigation.

A draft selection of the surfaces to be measured with the E-Nose and via the classical microbiological method can be seen in Figure 2-17. The sampling intervals and selected surfaces allow for both microbial contamination monitoring over time (e.g. ceiling, wall), as well as for assessment of the effectiveness of cleaning procedures (e.g. trays and tray covers).

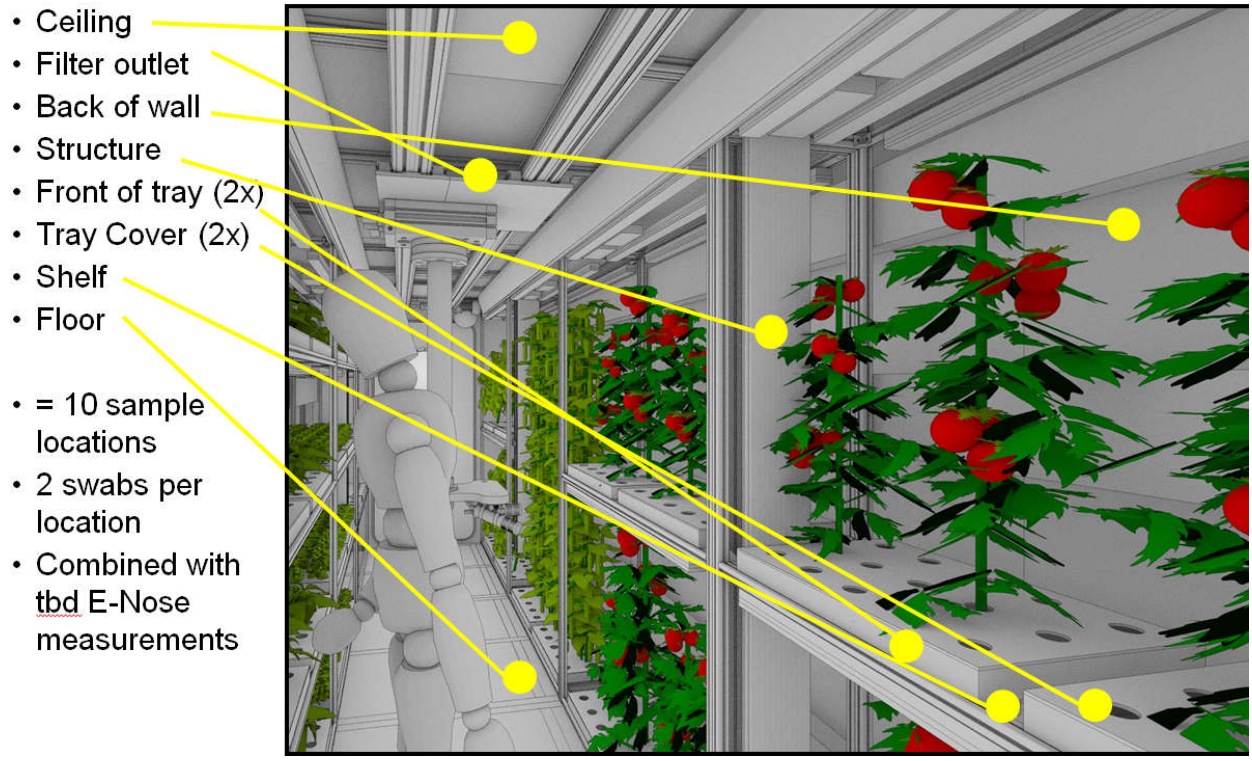
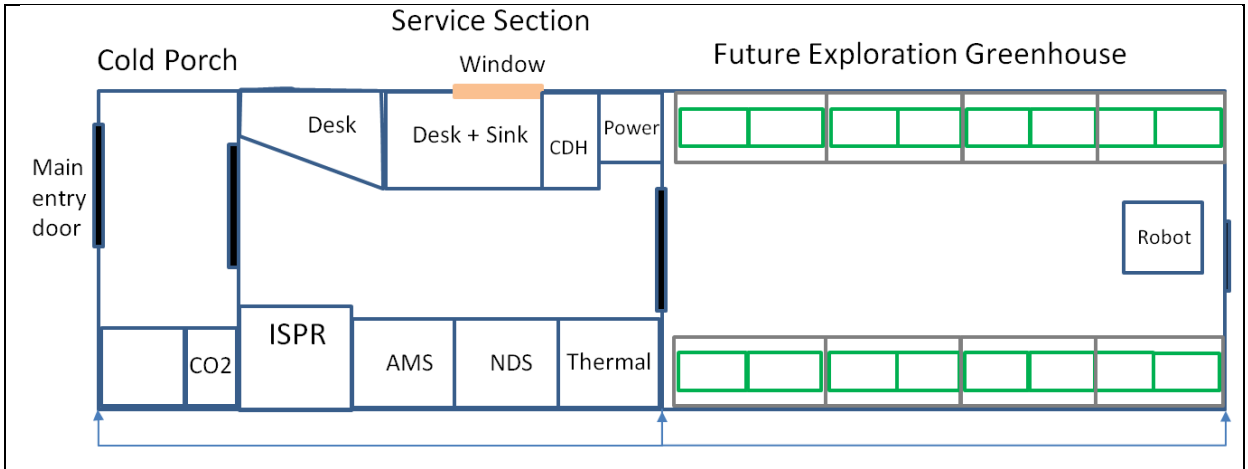


Figure 2-17: Selected Measurement Surfaces

It was agreed to perform microbial investigation (also with the E-Nose) in the Service Section as well as in the Future Exploration Greenhouse. In Figure 2-18 the draft distribution of the sample surfaces can be seen. It is planned to perform the classical method and the E-Nose measurements monthly.



Surfaces to be observed:	Surfaces to be observed:
• ISPR - 4 locations • SS - 6 locations	• FEG - 10 locations Plants to be observed: • FEG - 10 plants

Figure 2-18: Surfaces to be observed.

2.3 Decontamination

2.3.1 Baseline Design

If "dangerous" contamination were to be found with the microbial investigations mentioned above, it is planned to perform decontamination.

The already available decontamination system (TransMADDS … Trans*portable* M*odular* A*erosol based* D*econtamination* & D*isinfection* S*ystem*, see below) was judged as too bulky for the purposes within the Greenhouse. Furthermore, other requirements do not allow decontamination as initially planned (e. g. decontamination with Bacteriophages). The reasons can be found below in this chapter.

Figure 2-19: TransMADDS Decontamination.

First of all the question was raised if the decontamination system will be stationary installed (Figure 2-20) within the Future Exploration Greenhouse (FEG).

Figure 2-20: Stationary Installed Decontamination System.

Since it is not assumed that the decontamination of the whole FEG will be necessary during the exploitation period, it was decided not to install the decontamination system stationary within the FEG. The system will instead be stored within the Neumayer Station III and brought to the FEG if necessary. For that reason, a smaller, portable system has to be chosen for the decontamination of the FEG.

Also the decontamination agent has been discussed with the dedicated disciplines. For the decontamination agent the following requirements could be found:

- Material compatibility (if the agent is destructive to the materials)
- Plant compatibility (if the agent has an effect on the plants)
- Human compatibility (if the agent is dangerous for human beings)
- Antarctica compatibility (if the agent is harmful to Antarctica)

2.3.2 Selection of Decontamination Agent

Independently of the decontamination agent, the sequence of the whole process (Identification and Decontamination) can be seen in Figure 2-21 below. In this example case the decontamination agent is Bacteriophages.

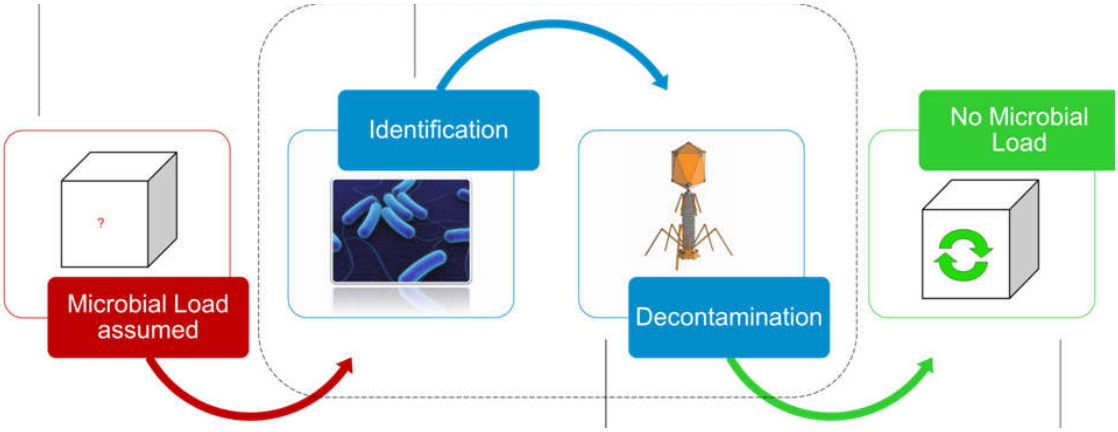


Figure 2-21: Decontamination Procedure with Phages.

Several decontamination agents were considered, taking into account the compatibility requirements listed previously. For that reason the following decision matrix (Figure 2-22) has been developed. The following four Agents have been compared:

- Peptide: since the effects of decontamination with Peptides are not clearly known, the Peptides have been judged as doubtful
- Bacteria-Phages: since the Bacteriophages are Viruses, the dedicated decontamination would not pass the Safety Review Team with regards to Antarctica Protection
- Ethanol: If the Ethanol is vaporized to a droplet size of up to 1 µm, an explosive gas mixture could be produced
- Hydrogen peroxide: H2O2 was chosen for the decontamination since it has no effect on the most involved systems.

Agent / Effect on	Peptide	Bacterio-Phage	Ethanol	Hydrogenperoxide
Material	OK	OK	OK	OK
Plant	???	OK	?	OK tbc
Human	???	?	OK	OK
Antarctica	???	NO	OK	OK
	Doubtful	Doubtful	EXPLOSIVE	

Figure 2-22: Decision Matrix on Decontamination Agent.

Attention:

It still has to be checked if there is an influence of H2O2 on the plants (regarding the µm droplet-size / stomata) and which concentration can be utilized within the FEG. A stoma is a pore on leafs, which is used to control gas exchange. It is possible that the small droplets can get in to the plant through the stomata and harm the plant.

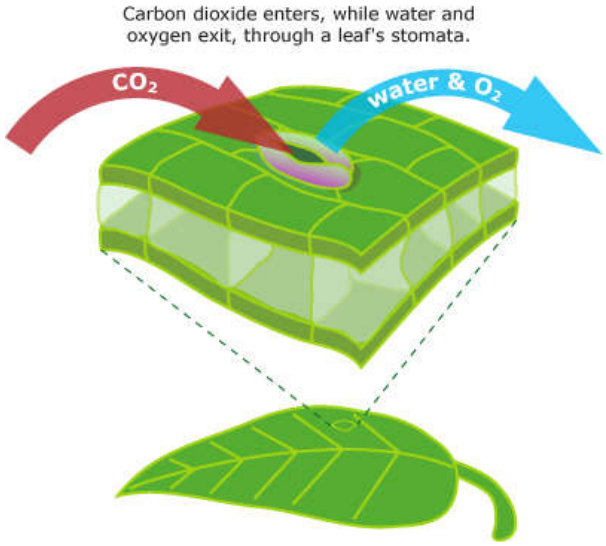


Figure 2-23: Stomata.

2.3.3 Decontamination System

As described in chapter 2.3.1 before, the decontamination system had to be adapted. The chosen system (see Figure 2-24 below) is fully automatic, mobile and has little labor costs. The system generates a very fine mist (2-5 µm) of Vaporized Hydrogen Peroxide (VHP). It eliminates bacteria and fungi which can be the most hazardous contaminations within the FEG. After completion of the decontamination process, the system will shut down automatically. The maximum volume, which can be disinfected, is accounted for 270 m^3.

Figure 2-24: Decontamination System.

2.3.3.1 Decontamination System inside the greenhouse

The decontamination system can be deployed inside the greenhouse. The system stays compact at one place.

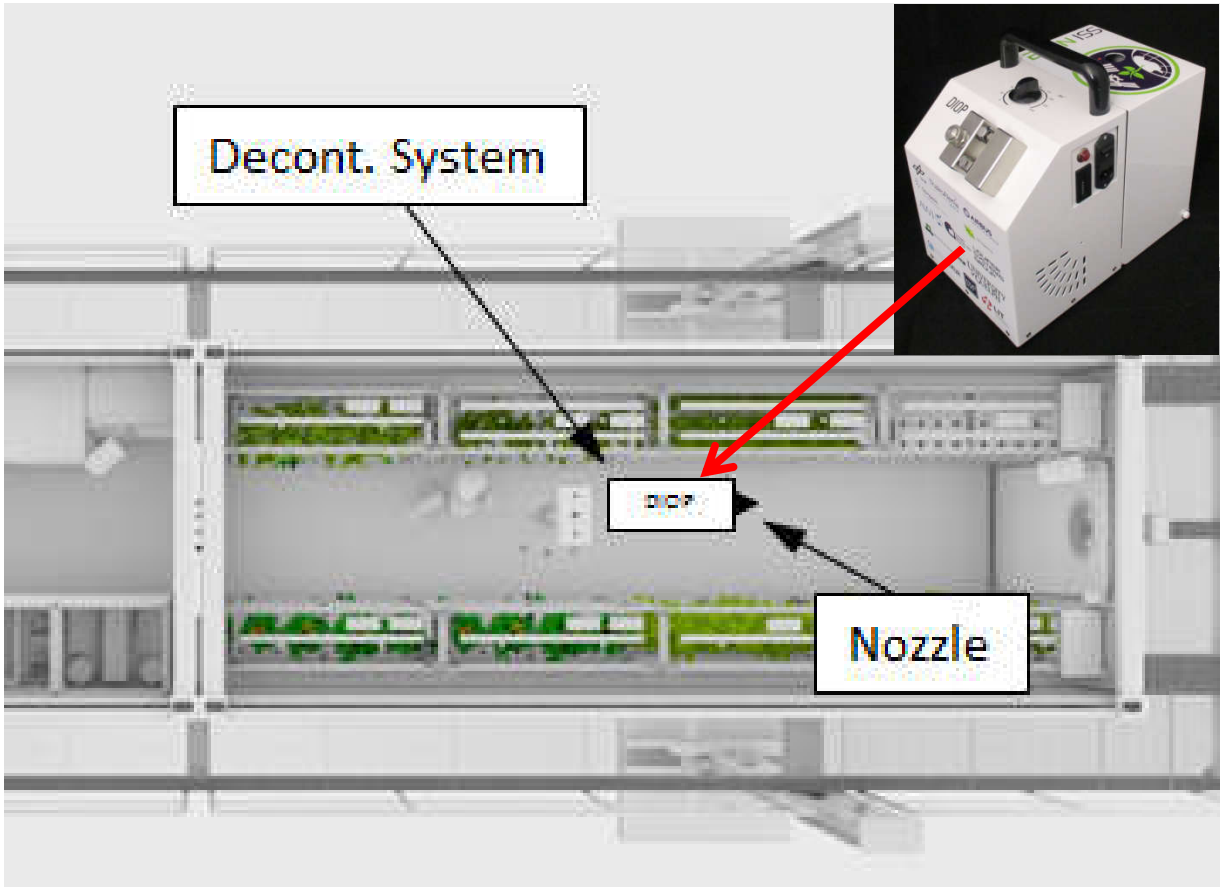


Figure 2-25: Decontamination System inside the FEG.

It has to be considered that during the decontamination the user has to leave the FEG.

2.3.3.2 Decontamination System outside the greenhouse, nozzle inside

The decontamination system can be split up into two pieces (see Figure 2-26 below).

Figure 2-26: Split Nozzle and Compressor.

The compressor can be placed outside the FEG and can be operated from there. The nozzle is deployed inside the greenhouse area and sprays the agent.

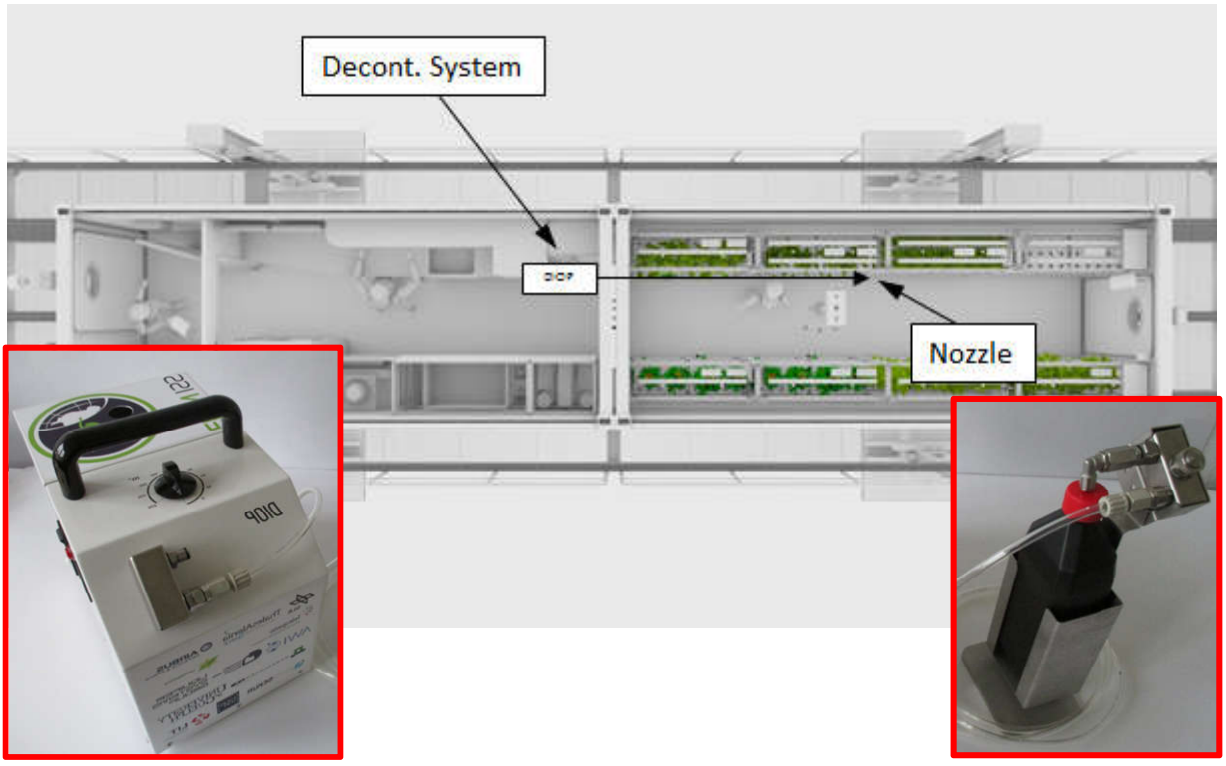


Figure 2-27: Decontamination System outside the FEG, nozzle inside.

To show the functionality of the decontamination system (even if there will be no need to decontaminate the FEG) a positive control experiment is planned to be performed with the MTF at DLR Bremen.

2.3.4 Brief Instruction

A short overview regarding handing of the decontamination system is given in Figure 2-28 below.

Figure 2-28: Brief Instruction - Decontamination System.

2.3.5 Decontamination Agent DIOSOL 3

Diosol-3 is dermatologically compliant and tested. It contains 3% hydrogen peroxide. It can be used for fumigation and eliminates phage, bacteria, fungi and spores. Its residence time is about 90 min. After 90 min, Diosol-3 is abreacted.

Figure 2-29: Decontamination Agent DIOSOL 3.

2.3.6 Test Setup for positive control experiment

Tests should verify the effectiveness of the decontamination system and detect potential side-effects to plants.

The test environment should simulate the greenhouse/habitat (e.g. as seen in Figure 2-30 below) environment.

Figure 2-30: Greenhouse.

Therefore e.g. a closed tent would be adequate (example can be seen in Figure 2-31).

The test setup shall include several samples of microorganisms (e.g. E. Coli) and the setup shall consist of:

- Ground samples
- Wall samples
- Non-line of sight samples
- Samples at different heights
- Samples in small sections
- Samples in back tapers

Also plants should be included within the habitat during the test to see if there are effects to H2O2. It can be considered also to include different material-samples and electronics.

The objectives of these tests are to verify clear reduction of colony forming units (CFUs) and to ensure there are no detectable negative effects on plants.

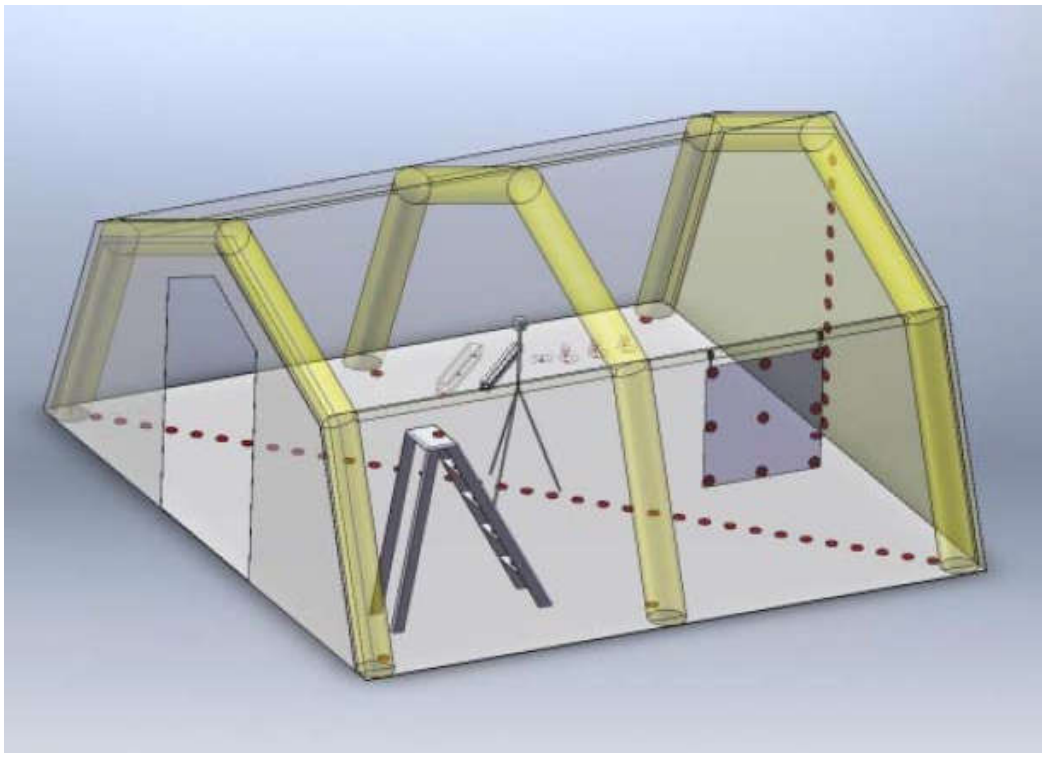


Figure 2-31: Test Setup within a tent.

During this test the H2O2 will be fogged within the closed habitat (see example in Figure 2-32 below). After the decontaminant is brought out, the effectiveness can be seen by counting the CFUs (no CFUs = microbial load successfully decontaminated). Test descriptions and results are detailed further in Annex E.

Figure 2-32: Fumigation via micro-fogging.

References

E. Heitz, H.-C. Flemming, W. Sand, Microbially influenced corrosion of materials, Springer-Verlag Berlin Heidelberg (1996)

S. Watkins Borenstein, Microbiologically influenced corrosion handbook, Industrial Press Inc. (1994)

Annex A: Camera Specifications

Table 1: Camera specification

Table 2: DS-2CD4026FWDA Camera specification

Table 3. Varifocal Lens Specification

Table 4: Verso Polar Datasheet

Annex B: TPZ SW application installation

This annex describes how to install the TPZ developed tools.

Step1. Download Python.

Go to http://www.python.org/, select the right version (windows 32 or 64 bit) and download it.

Step 2. Installing Python and "requests" module in c:\Python35

1) Launch the python-3.5.2.exe installer
2) Select add Python 3.5 to PATH e Customize installation (fig. 32)
3) Select all the optional features (fig. 33)
4) In Advanced options select install for all users c:\Python35 as installation location

then click on Install and wait until the process ends.

Fig.32: Phyton Installation Page

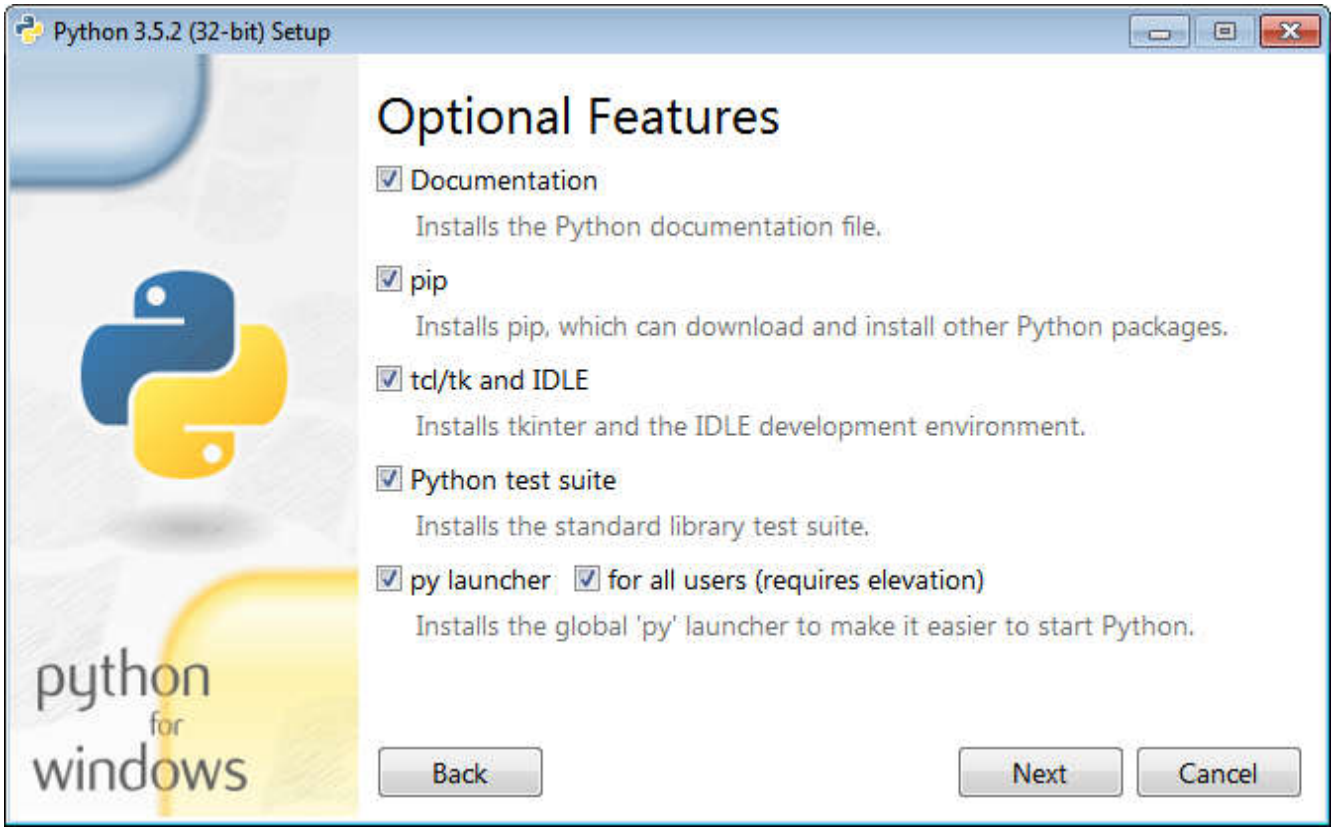


Fig.33: Phyton Installation – Features Definition

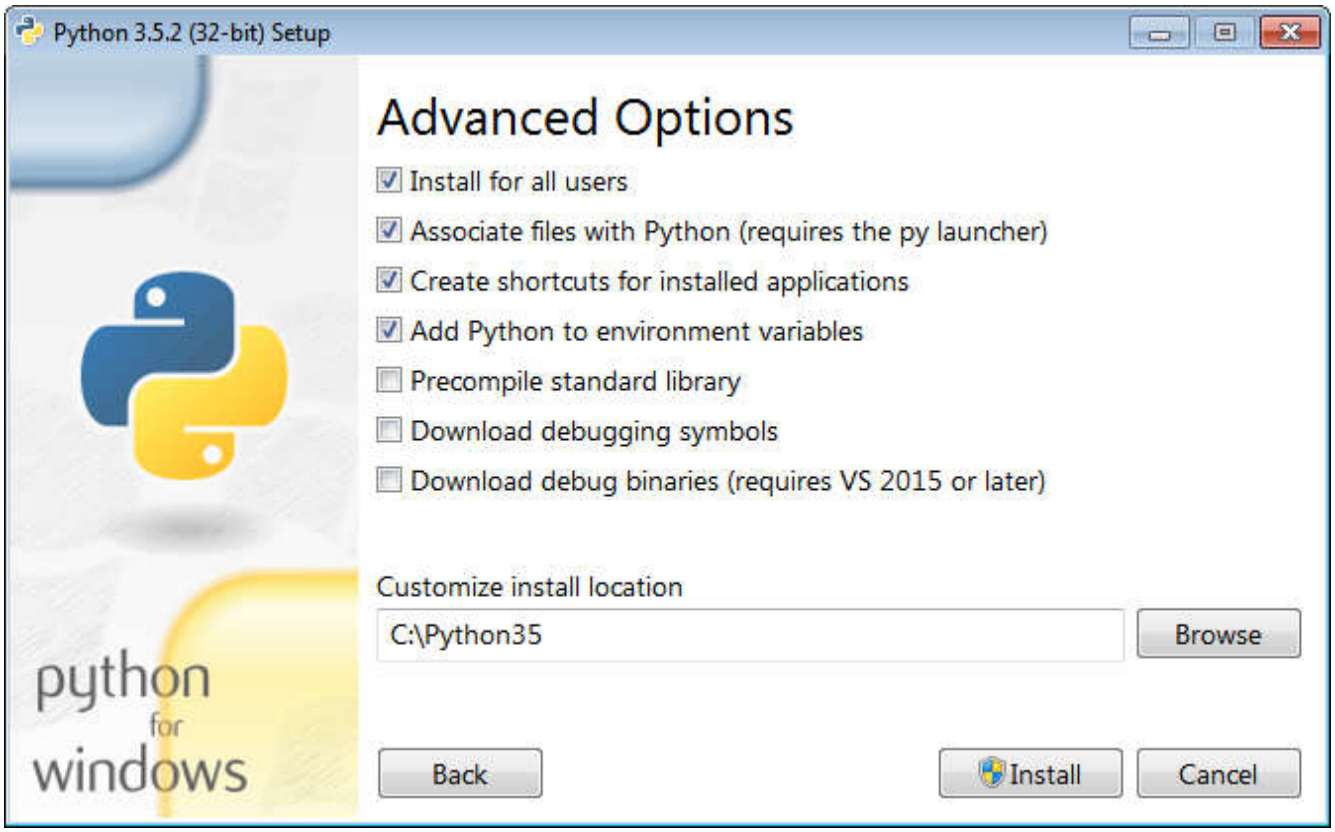


Fig. 34: Phyton Installation – Advanced Options

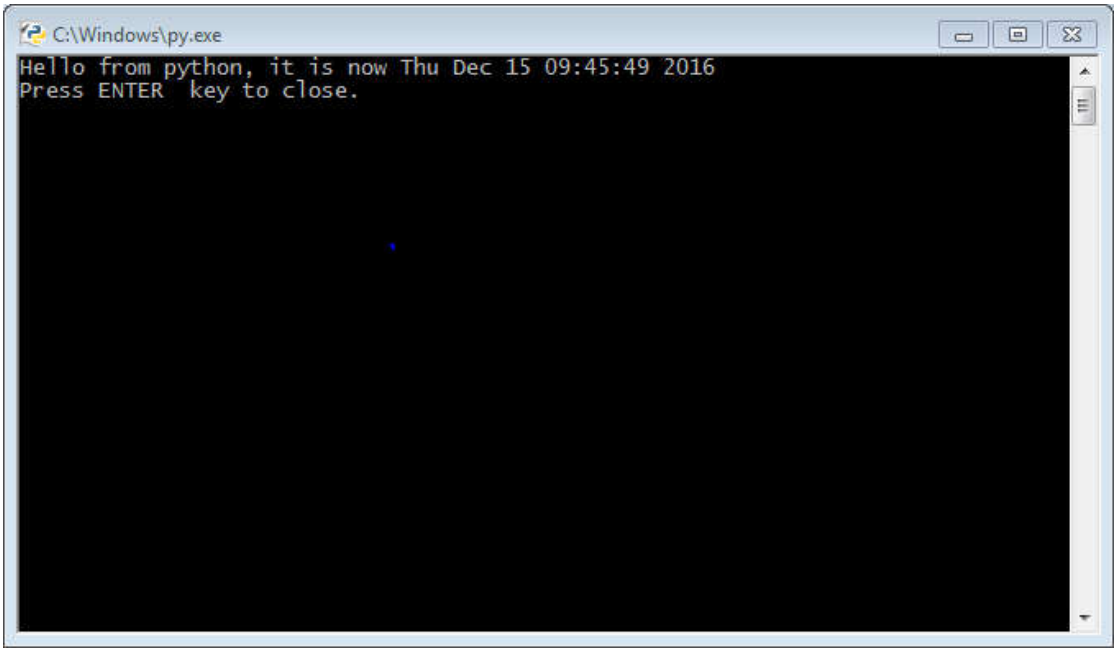


Fig.35: Check the correct installation of Phyton

5) Check the **correct python installation**: run the *python* command in a *Windows CMD command line.* If Python is correctly installed the Cmd prompt window will open with a welcome message (fig. 35).
6) Push enter to close the window
7) Install **Python Requests Module**
   a) If **you have** an internet connection available type (in a *Windows CMD command line)*
      - ***python -m pip install requests*** (V 8.1.1) or alternatively use ***pip install requests***
   b) If **you don't have** an internet connection available you need:
      - Download the tarball/zipball of the Python Requests Package from the link https://github.com/kennethreitz/requests
      - Unzip/Untar the files in your python directory
      - install it into your site-packages typing in a *Windows CMD command line*: ***python set-up.py install***

Step 3. Installation of the TPZ developed SW Application

1) Create a directory C:\Image_Acquisition\TPZ_SW
2) Copy the file **TPZ_SW_EDEN_yyyy_mm_dd.zip** in the folder just created
3) Extract the files from the zip folder. As results the following files are created
   - hikvision.py
   - camera_snapshot_robot.py
   - camera_ftp_robot.py
   - camera_ftp_robot_NMIII.py
   - edeniss_scheduler.bat
   - edeniss_scheduler_NMIII.bat
   - edeniss_mkdir.bat

If this sequence is executed without problems, python is correctly installed and working. The TPZ software application should be ready to be used (or to be customized for the EDEN ISS operations).

Remark: the editing of the python scripts requires a text/code editor with the capability to provide the 4-space indentation feature. For the development of the required applications the Geany text editor has been used. Alternatively the Notepad++ can be used. The following steps 4 and 5 describe how to install and configure them.

Step 4 (optional): Geany software installation and Setup – Windows platform.

Geany is a text editor using the GTK2 toolkit that is available in the full install package. Geany is an Integrated Development Environment or programming environment that is based around the KDE and GNOME Linux version, but the software was developed to run independently on Windows.

- Download Geany software installer for Windows from http://www.download3k.com/DownloadLink2-Geany.html
- Run the exe installer
- Remember to use 4-space indentation, and no tabs: from *Preferences -> Indentation*

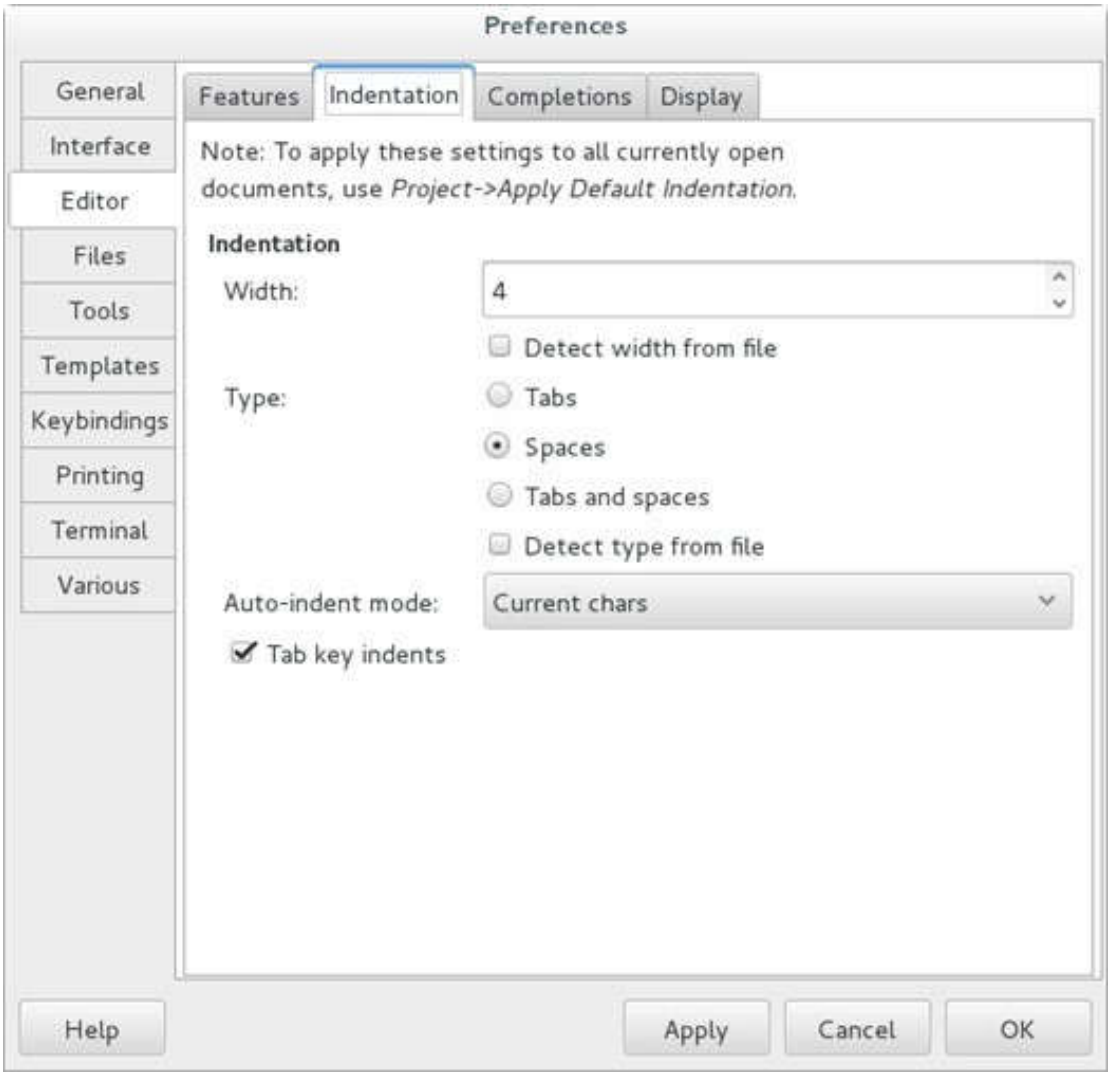


Fig. 36: Geany Editor Indentation preferences for Python files

Step 5 (optional): Notepad++ software installation and Setup – Windows platform.

- Download Notepad++ software installer for Windows from https://notepad-plus-plus.org/repository/7.x/7.3/npp.7.3.Installer.exe
- Run the exe installer

  Or

- Download Notepad++ software zip file for Windows from https://notepad-plus-plus.org/repository/7.x/7.3/npp.7.3.bin.zip
- Unzip the file
- Remember to use 4-space indentation, and no tabs: *Settings -> Preferences -> Language->Python*

Figure37 – Notepad++ Editor Indentation preferences for Python files

Annex C: Plant Health Monitoring System Configuration

As soon as the system is ready, i.e. all the physical connections have been installed, the switches and the camera's PC have been activated and configured in terms of SW installation and network parameters assignment, it is possible to set the camera's over the EDEN ISS LAN using the SADP or the iVMS software. It is worth underlining that the cameras are powered on as soon as they are connected to an active switch PoE port. Camera activation in this case concerns the setting of a (strong) password to make it available to the camera management tool. Once this job is done for each camera, it is possible to start using the PHM system and to configure it for plant monitoring purposes. Two main tasks are foreseen to be managed with two different software tools:

1. Accessing the camera for configuration management and for live video displaying
2. Planned picture taking, storing on the NMIII Camera PC and picture forwarding to the remote centers over satellite internet connection.

The first task is accomplished using the SADP and the iVMS-4200 SW provided with the cameras by HIKVISION, the second task is accomplished using the Telespazio developed SW. In the following paragraph the steps required to accomplish these tasks are described.

2.4 Activating the cameras

This activity can be done using either the camera PC in the MTF or the camera PC in NM-III. For sake of simplicity, the nominal procedures foresee that the first network parameters configuration is done using the SADP software. SADP (Search Active Devices Protocol) is a kind of user-friendly and installation-free online device search tool. It searches the active online devices within your subnet, displays the information of the devices, and allows the modification of the basic network information of the devices themselves.

As soon as the SADP software is loaded, it automatically searches the subnet where the computer is located for connected online devices every 15 seconds. It then displays the total number of devices and information of the found devices in the Online Devices Interface. Device information including the device type, IP address and port number, etc. will be displayed.

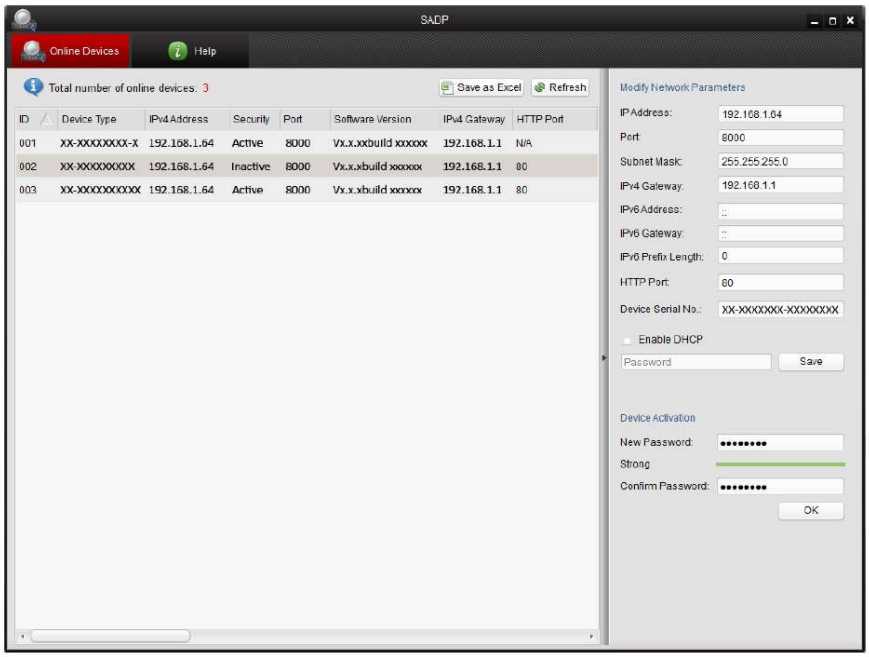


Fig. 10 Searching online devices

Devices can be found and displayed in the list in 15 seconds after it went online; the devices will be removed from the list within 45 seconds after going offline. It is also possible to search the online devices manually clicking the refresh button. The newly found devices will be added to the list.

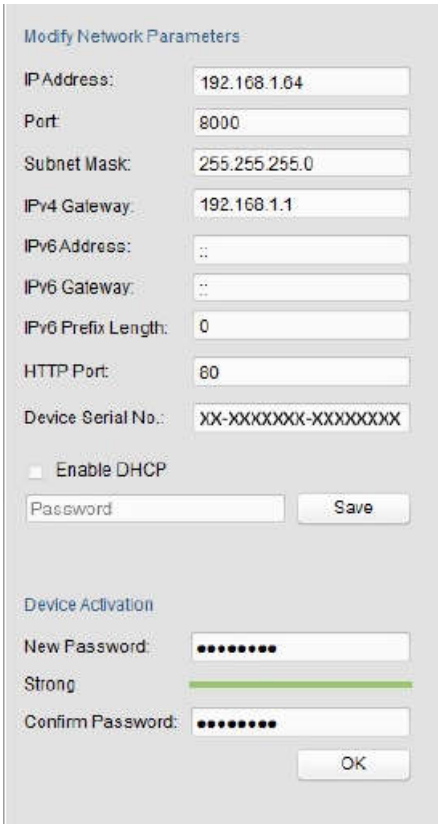


Fig. 11: Configuring Network Parameters

Once the devices are available in the Online Device Interface it is possible to modify the network parameters and activate the cameras as per the following steps:

1. Select the device to be modified in the device list and the network parameters of the device will be displayed in the Modify Network Parameters panel on the right side.
2. Edit the modifiable network parameters, e.g. IP address and port number.
3. Enter the password of the admin account of the device in the Password field and click to save the changes.

2.5 Accessing and configuring the cameras

Once the cameras have been correctly connected to the EDEN ISS network, it is possible to use the iVMS software to manage the configuration of the camera and to view the live video. The iVMS-4200 is an intelligent Video Management System provided by HIKVISION that offers an easy-to-use, highly reliable and feature-rich platform for security management, centralized video analytics, and open integration. The software offers several common basic configuration wizards including device adding, storage configuration, event configuration, and user configuration.

The control panel and live view interface of the iVMS-4200 are shown below.

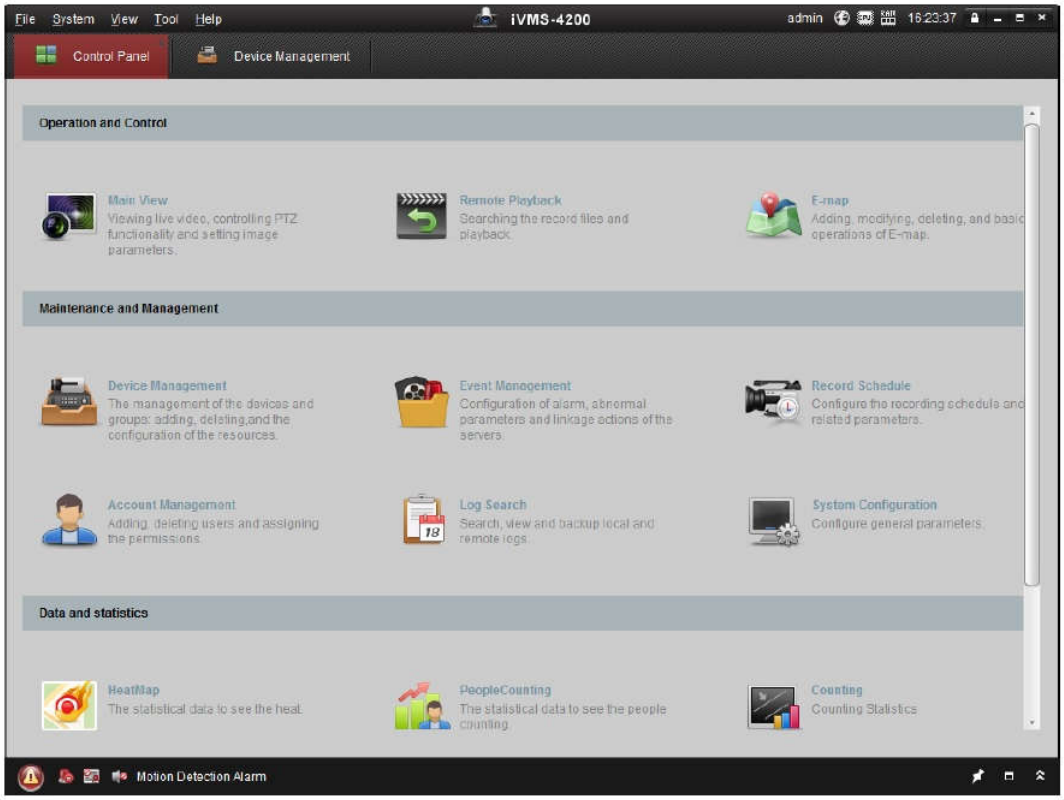


Fig. 12 iVMS -4200 Control Panel

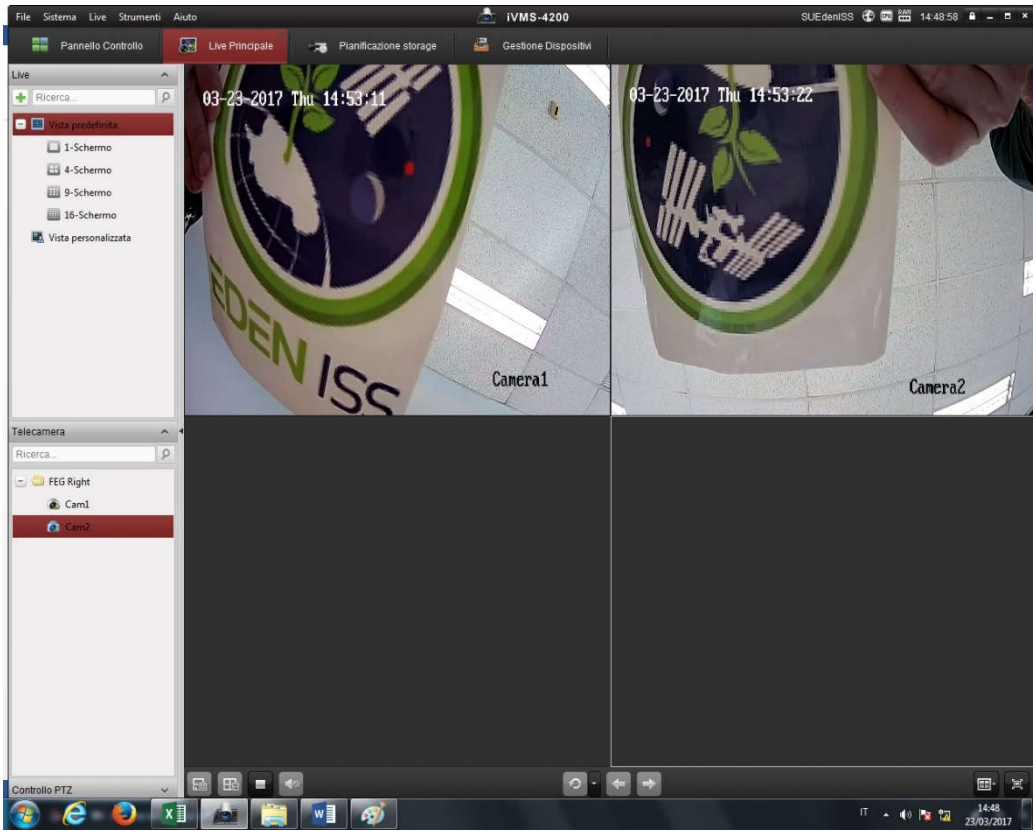


Fig. 13: iVMS Live View Page

2.5.1 Adding online cameras to the iVMS Server

After running the iVMS-4200, the first task to be completed is to add the cameras to the remote configuration and management client. That can be done via the Device Management page of the iVMS-4200 tool.

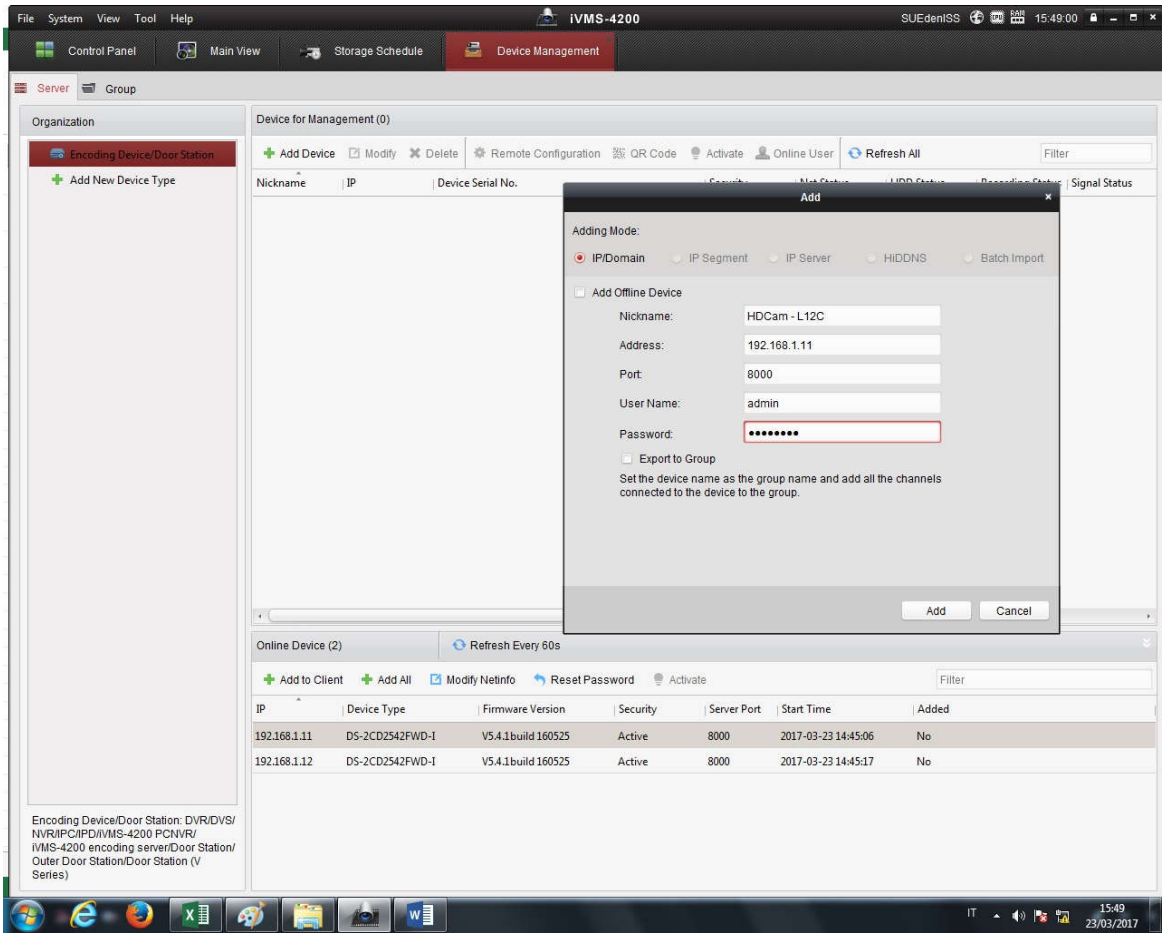


Fig. 14: Device Management Interface

The active online devices in the same local subnet as the computer with the client software will be displayed in the Online Device area. You can click the Refresh Every 60s button to refresh the information of the online devices. To add a camera to iVMS-4200 the following steps have to be executed (Remark: at this stage all the cameras should be in their active state).

1. Select the devices to be added from the list.
2. Click **Add to Client** to open the device adding dialog box.
3. Input the required information.

Nickname: Edit a name for the device as you want (it is recommended to have a name that refers to the position or the function of the camera).
Address: Input the device's IP address. The IP address of the device is obtained automatically in this adding mode.
Port: Input the device port number. The default value is *8000*.
User Name: Input the device user name. By default, the user name is *admin*.
Password: Input the device password.

Of course these steps have to be repeated for all the cameras of the network. The following figure shows the Device Management Panel after two cameras have been added to the iVMS Client.

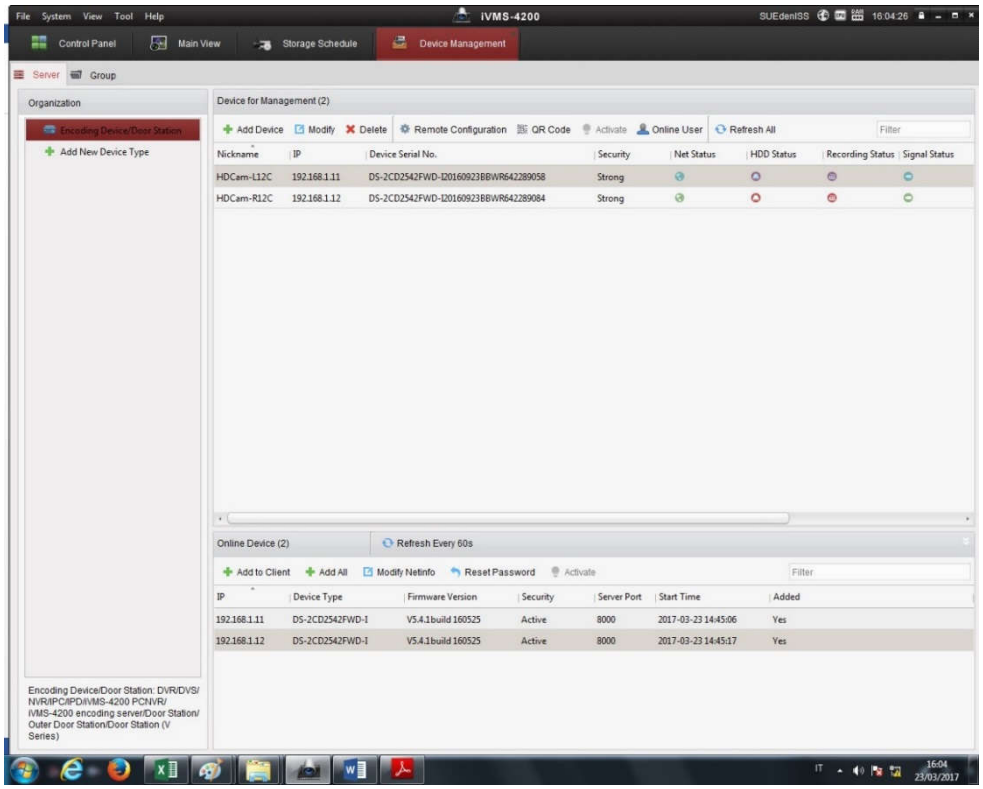


Fig. 15: Camera's added to the client

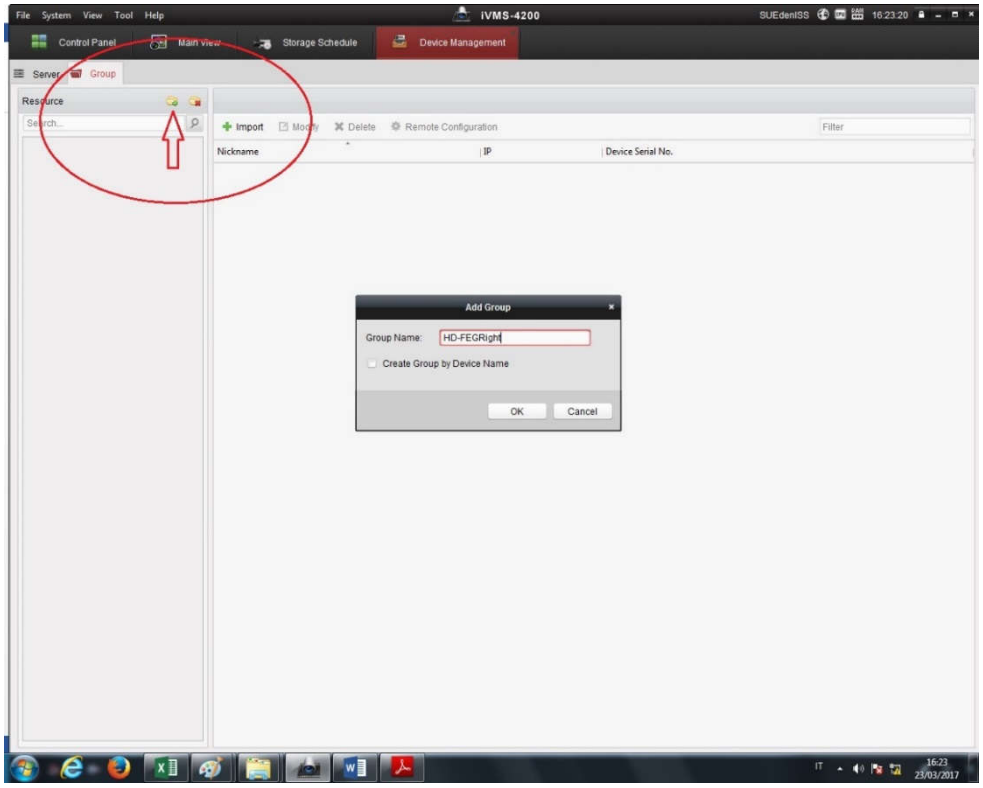


Fig 16: Add group

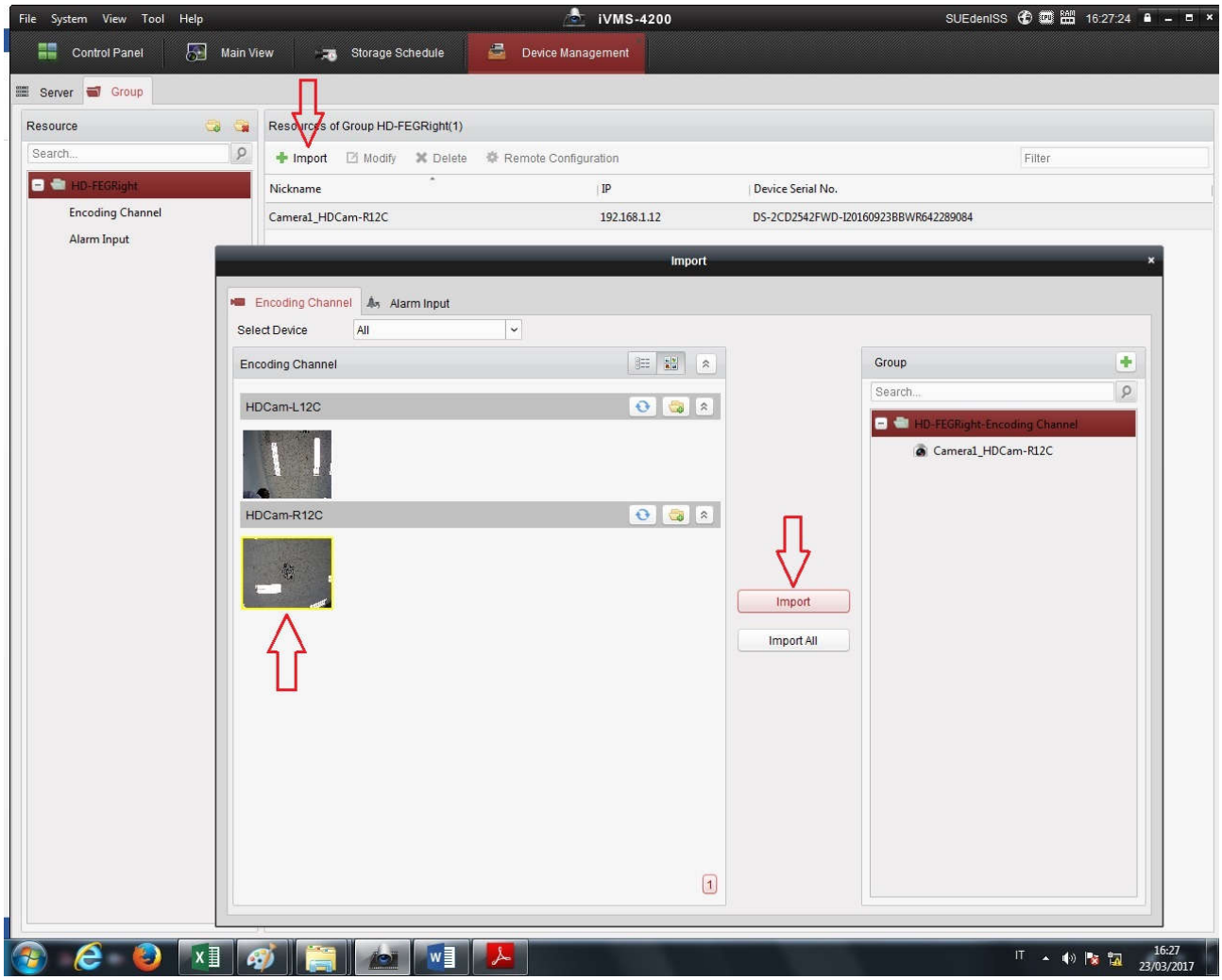


Fig. 17: Camera importing to group

Another important feature of the Device Management is the possibility to create groups of cameras. That is very important for the EDEN ISS project where we have a lot of cameras that can be organised by their position (e.g. right or left side of the FEG corridor), or by their function (Plant Health monitoring or ambient monitoring). The groups can be created as per the following steps:

1. Click **Group** in the Device Management page to open the group page
2. Click **Add Group** to create a new group
3. Insert the **Group Name**
4. Click **Import** on the tool bar of the Device Management to open the import page
5. Select the Image (it is possible to click on the image)
6. Click Import
7. Repeat for the other groups and cameras as desired

Once that activity is completed, the image viewer will show the cameras already organised by groups.

2.5.2 Configuring the cameras

Three actions are necessary before starting with the image/video acquisition:

1. The configuration of the camera parameters (like resolution, Frame Rate, etc)
2. The configuration of the video parameters (like the brightness, the contrast, etc)

3. The configuration of the display parameters (like camera name, time format, etc.)

These actions can be carried out as per the following steps:

1. Select the camera
2. Click **Remote Configuration** in the Device Management page to open the Remote Configuration page
3. Click Image to open a pop-up menu
4. Select **Video & Audio** for configuration of related camera parameters
5. Input the desired values
6. Select the **Image Setting** to define the video parameters of the cameras
7. Input the desired values
8. Select the **Video Display** to define how the video/images will be displayed and saved.
9. Input the desired values (Remark: It is important to define a camera name that refers to the position or the function of the camera. It is recommended to use the same nickname as defined previously)

The following picture show the software displays for the steps listed above.

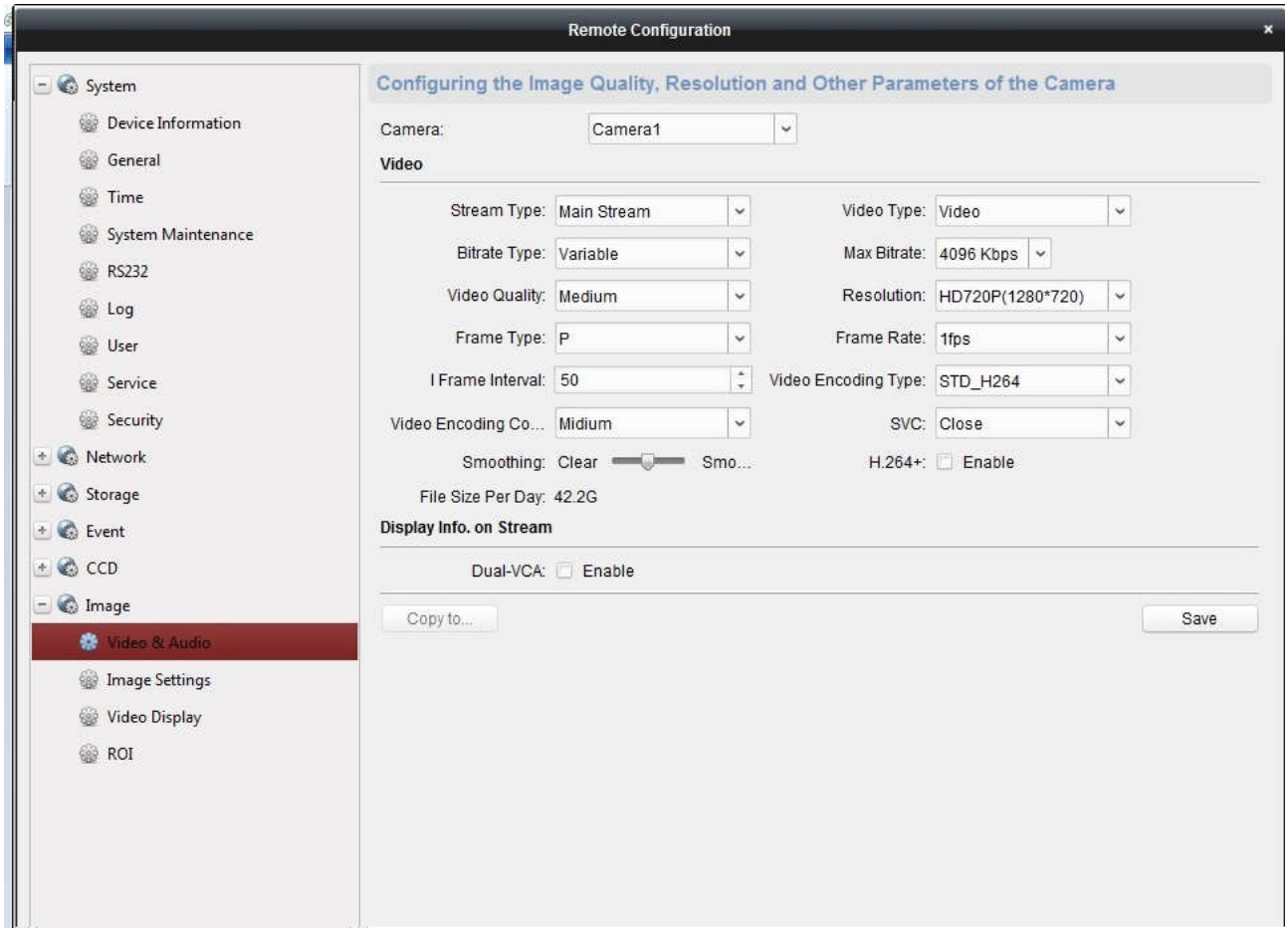


Fig. 18: Remote Configuration page- Video and Audio Configuration

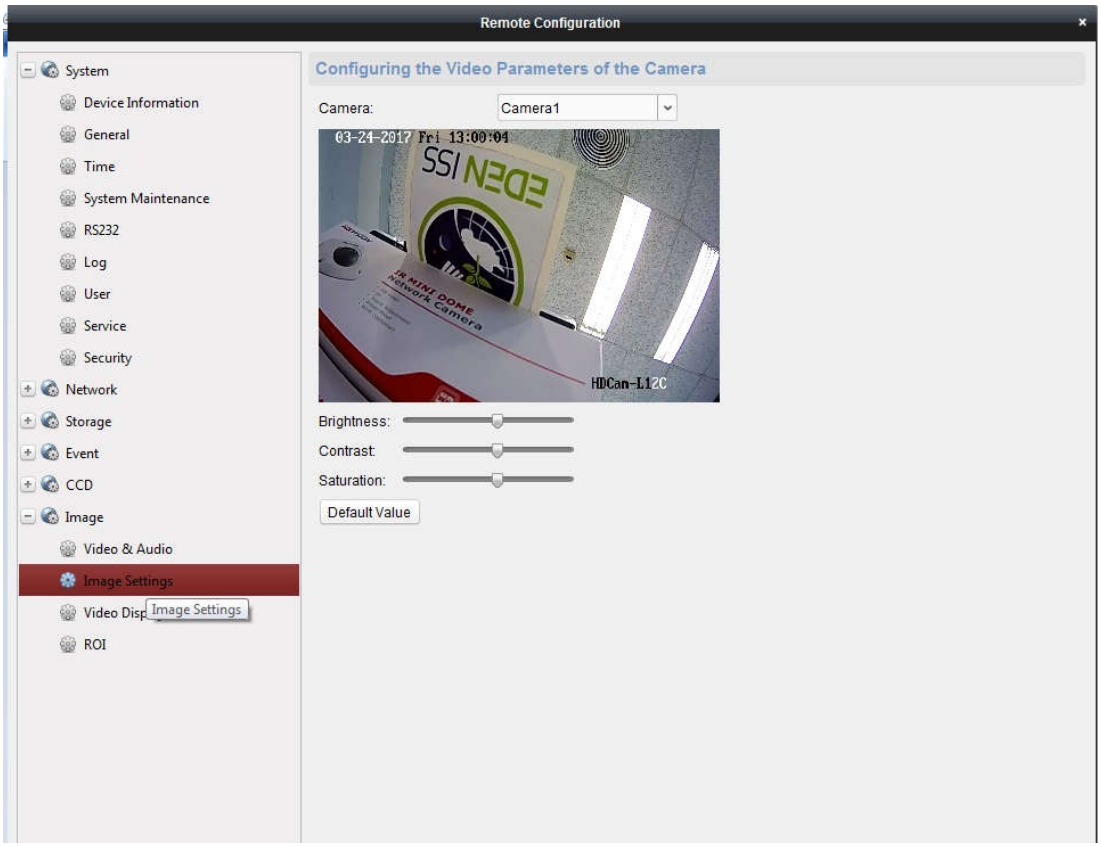


Fig. 19: Remote Configuration page- Image Configuration

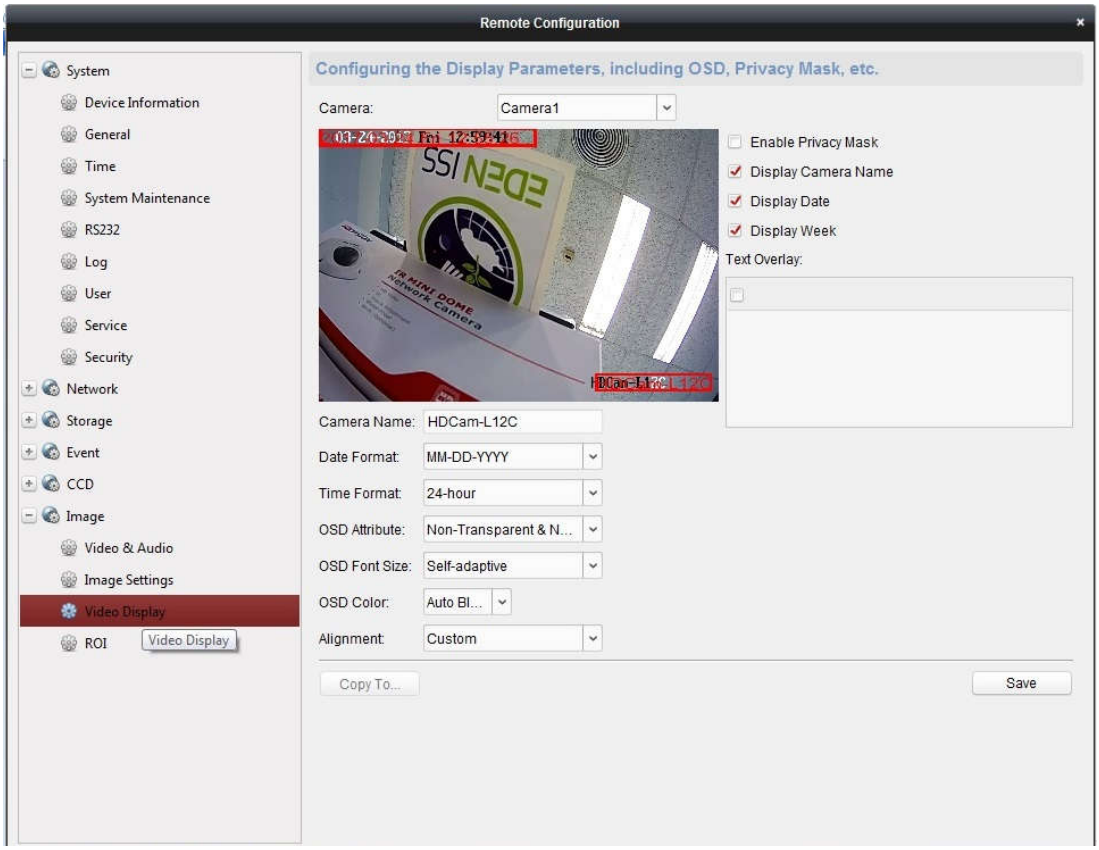


Fig. 20: Remote Configuration page- Video Display Configuration

2.6 Live View

The live view page allows the user to view the real time video, capture images, set/call pre-sets and configure video parameters. To access the Live View page select **Main View** on the toolbar or click **View → Main View**. The result is shown in the following figure. The yellow arrows indicate the areas for the screen layout selection. In fact the tool gives the possibility to manage up to 64 cameras in parallel, i.e. to display live video from 64 cameras at the same time. The blue arrow indicates the area where the active cameras are listed. Finally the central grey area is the area where the live video is displayed (in the figure a quad video is shown). To associate a camera to a window, it is necessary to select the window, by simply clicking on it, and then selecting the camera with a double click. A small green arrow close to the camera name indicates that the camera is sending a video to the window.

Remark: Apart from the live view status, the cameras can have the following status:

The camera is online and works properly.

The camera is in live view.

The camera is in recording status

The camera is offline.

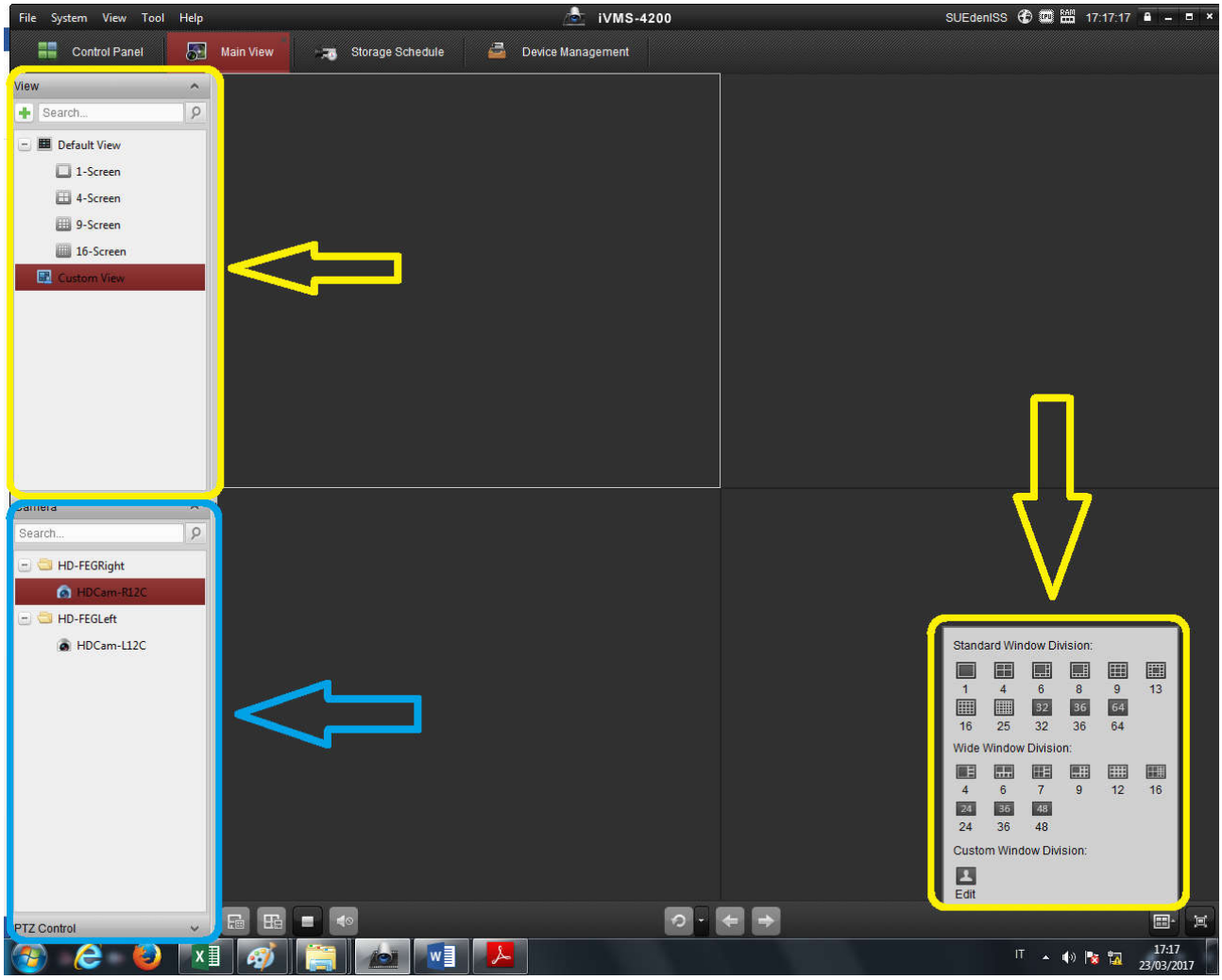


Fig 21: Live View Interface with four windows

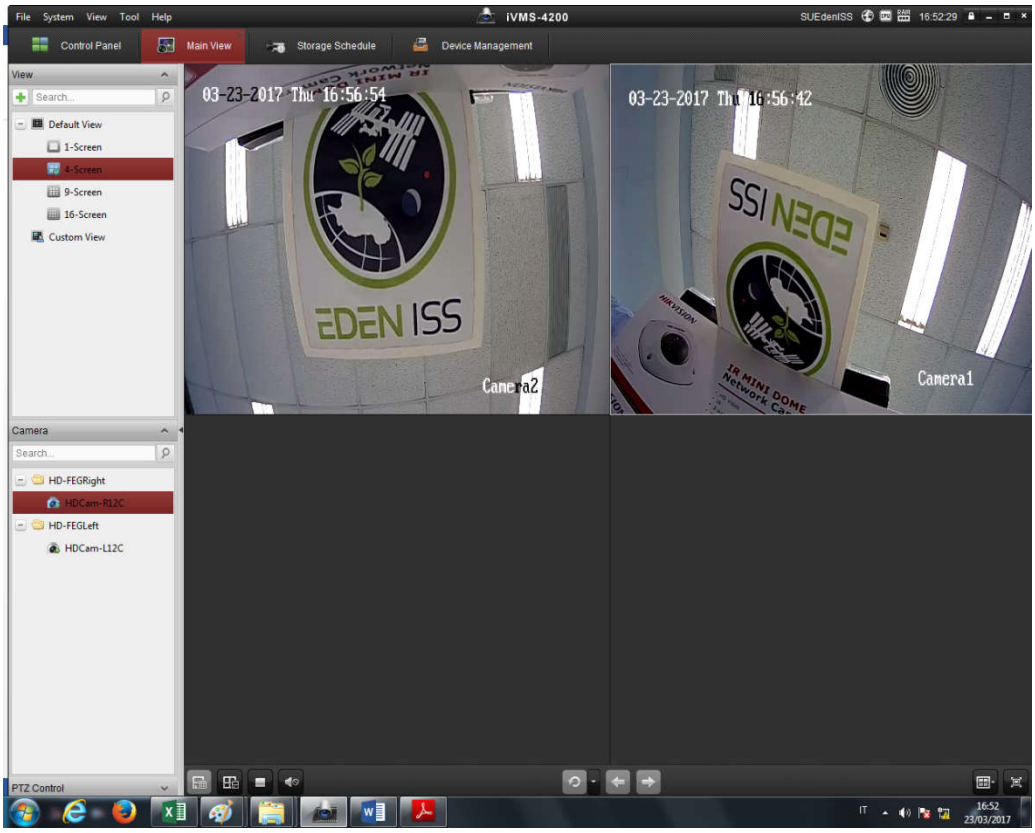


Fig. 22 – Live view

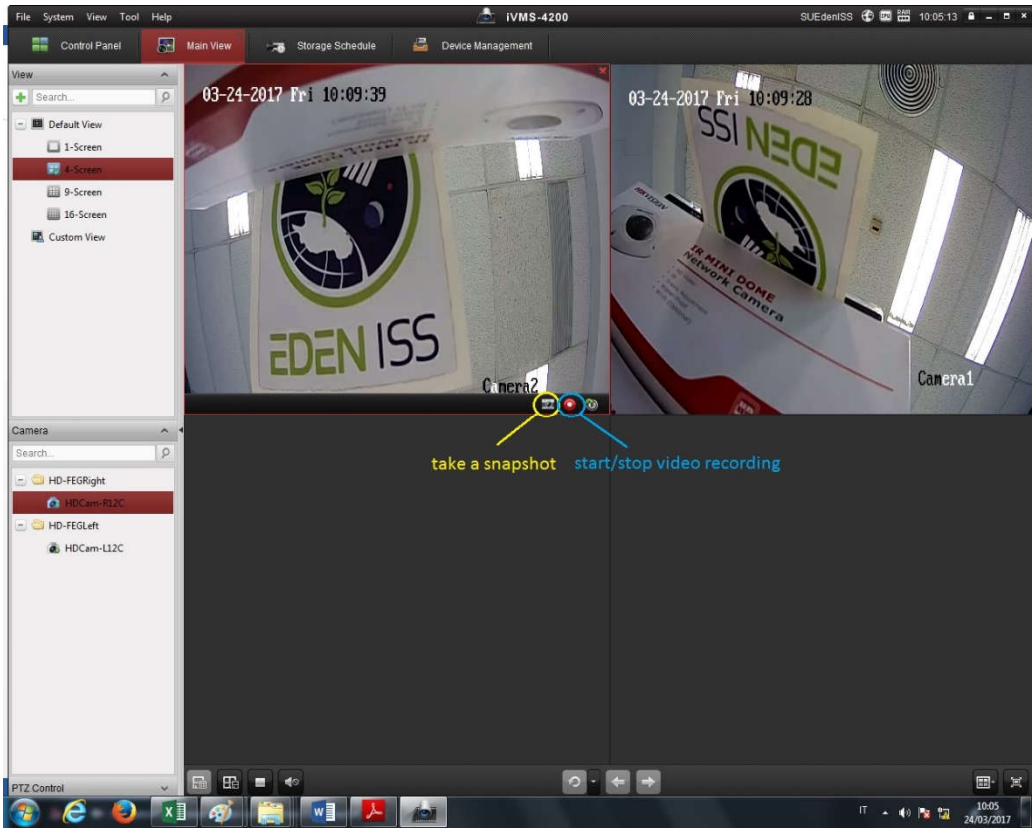


Fig. 23: Video recording/Snapshot Capture

At this stage the cameras are only sending video to the screen, i.e. no video and or pictures are recorded. As said, the daily planned automatic picture capture, storage and transmission process will be managed by a custom-developed script, but the iVMS-4200 SW gives other possibilities like a commanded recording of video and/or a snapshot capture by the operator whenever desired. That can be done in a very straightforward way by moving the mouse pointer on the selected image and then clicking on the button for video recording/snapshot acquisition as shown in fig. 23.

Remark: with this operation, the video and/or the snapshot are saved **locally,** i.e. on the computer used for the above described actions.

The iVMS-4200 SW has default definitions for the folders to store video recordings and/or snapshot acquisitions. For **recorded video** the path is: C:\iVMS-4200\video\Recordfile\yyyymmdd\IPaddress_port

The video is named according to the following format:

<date>_<datestarttime>_<datestoptime>_<counter>.mp4

For **snapshots**, the path is: C:\iVMS-4200\capture\yyyymmdd

The image is named according to the following format:

<groupname>_<cameraname>_<datetime>_<counter>.jpeg

Nevertheless, the iVMS-4200 does allow for custom path creation. That can be done in the following way:

1. Define a desired path (with folders and subfolders) for the video and/or snapshot recording. For example
    - C:\eden\ManualRecording\video
    - C:\eden\ManualRecording\snapshot
2. Click Tool→ System Configuration. A System Configuration windows opens
3. Select Files
4. Write the desired path in the dedicated field
5. Save the changes

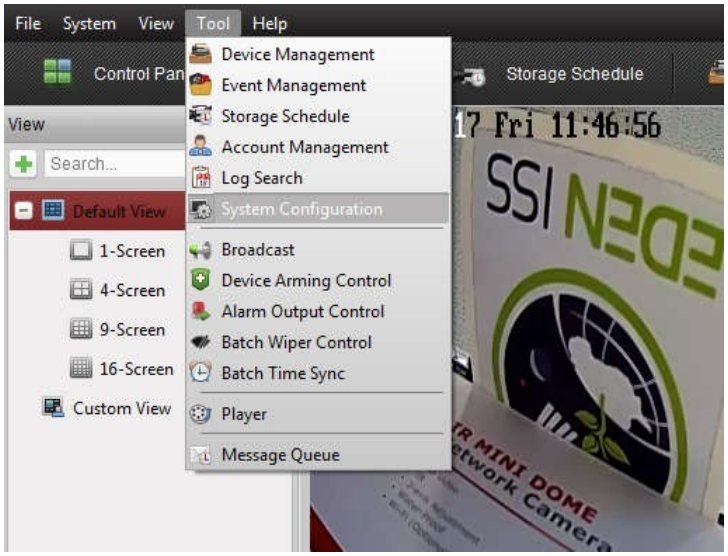


Fig. 24: Opening the System Configuration Window

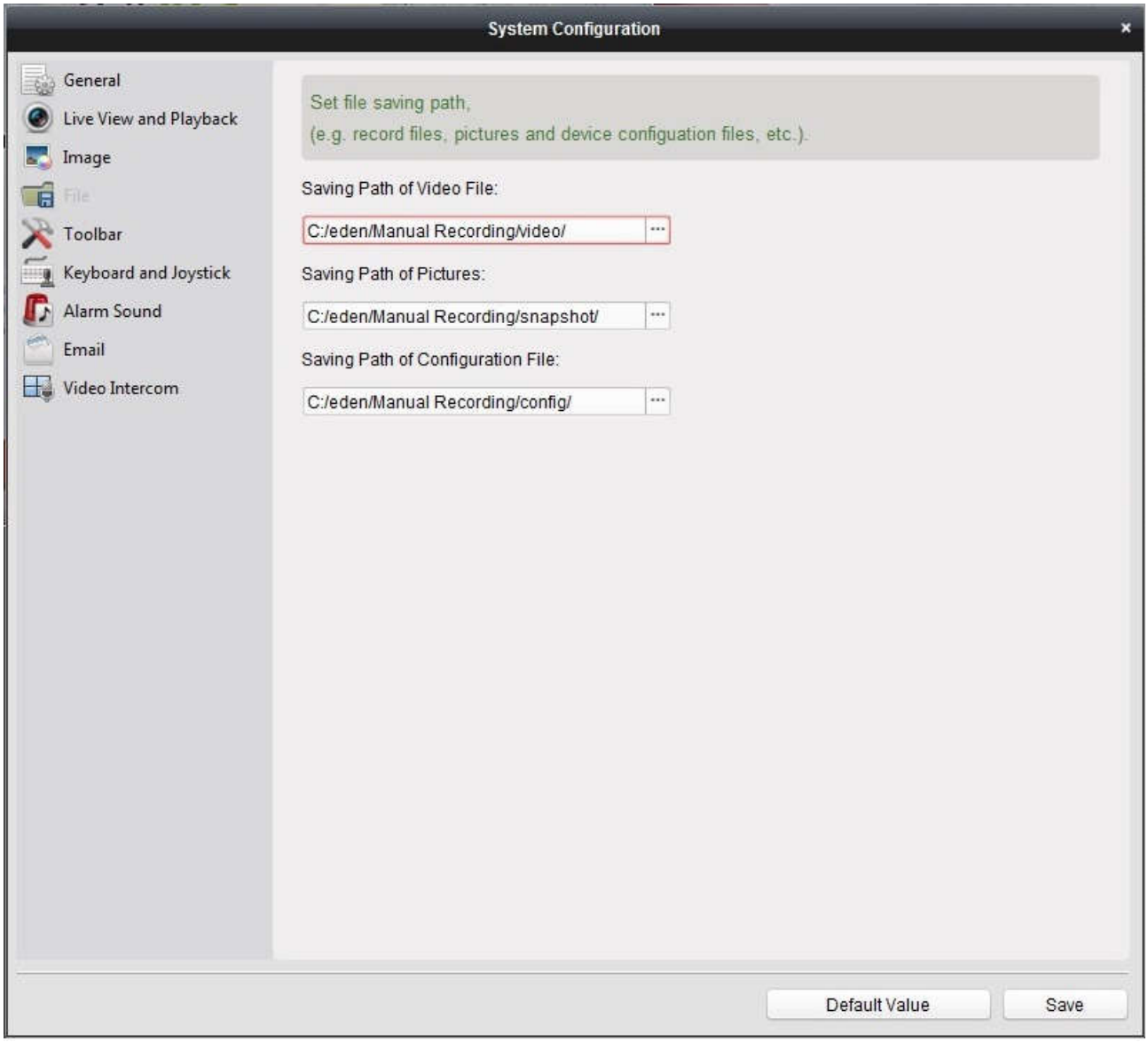


Fig. 25: System Configuration Window for File storage path definition

The iVMS-4200 provides a function to search and retrieve the manual recorded file. That can be done in the same way regardless of the path defined for file storage.

1. Click File -> Open Image File (or Open Video File). That operation opens the Image File (or the Video File) Search Window
2. Input the **Start Time** and the **End Time.** That defines the time interval of interest
3. Select the camera of interest (multiple selection is not possible)
4. Click on search. The images/video captured in the identified timeframe will be made available in the window

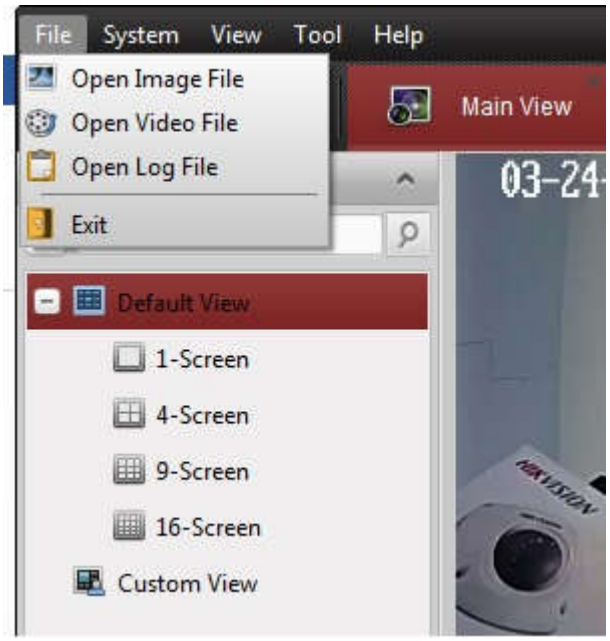


Fig. 26: Open Image/Video File

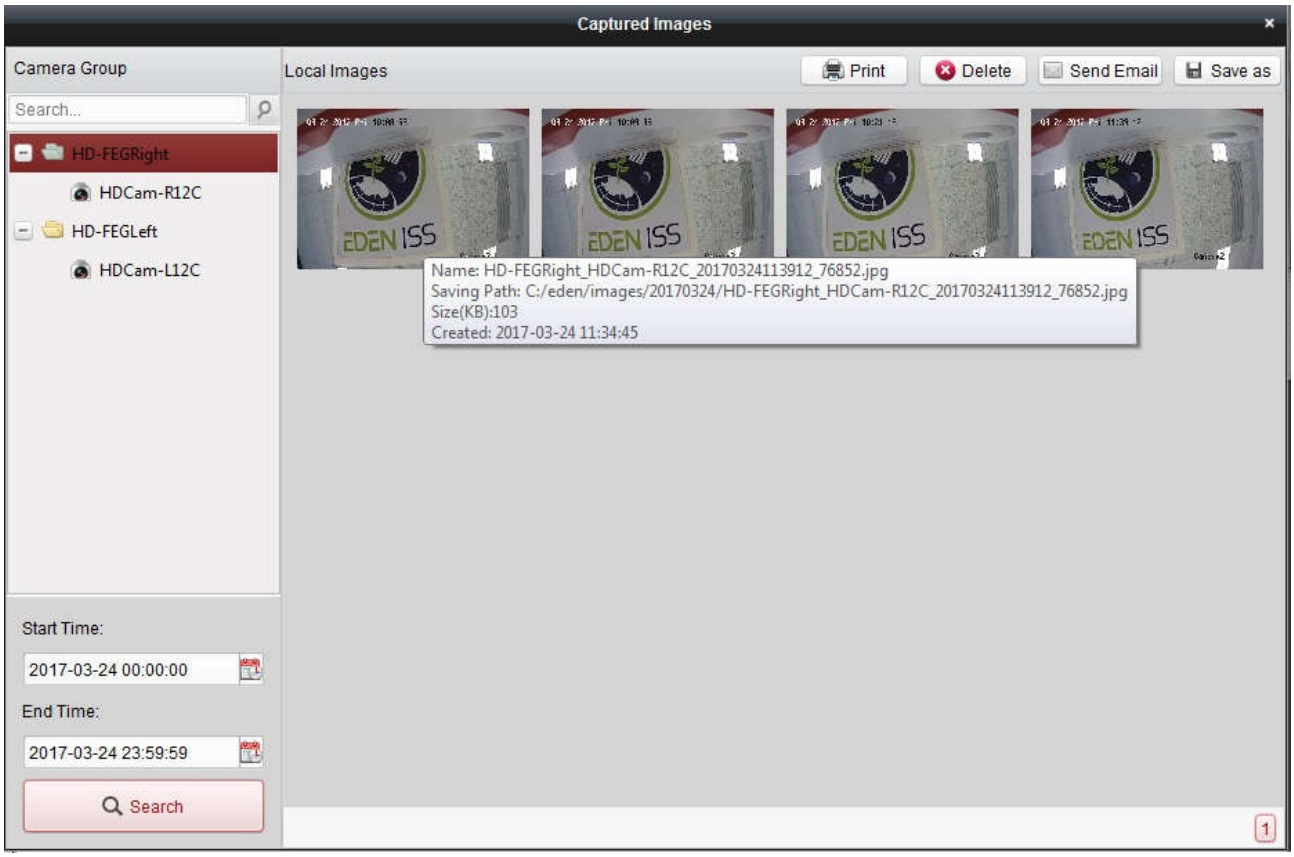


Fig. 27: Example of images search

2.7 Automatic Image Acquisition Scheduling

Beside the possibility for the EDEN ISS operator on site to have continuous streaming of data on the Camera PC, and the possibility to record videos and take snapshots whenever needed, the PHM system is required to:

- Schedule a daily picture acquisition and store them on the MTF Camera PC
- Forward the picture to the EDEN ISS Mission Control Center at DLR in Bremen, as well as to the NM-III PC.
- Forward the picture and data files (.csv files) to the EDEN ISS Mission Control Center at DLR in Bremen, as well as to the NM-III PC.

Ad-hoc software applications developed by TPZ will manage these tasks. These applications are based on the programming language **Python Version 3.5** (see Annex B for Python installation and configuration procedures); for that reason, and as already mentioned before, it is necessary that Python is installed on the camera PC in the MTF, on the DLR PC devoted to the data exchange with the remote users, and on the PC at NM-III. It is recommended that the editing of the Python codes is done using the Geany text editor (Notepad++ could be used instead) to correctly implement the line spacing and indentation in the code lines. Any other text editor can be used, but special attention needs to be paid to the code to ensure that Python's indentation rules (i.e. the four space rule for script indentation) are respected. Both tools are freeware. In annex B, step 4 and 5 contain the information on how to acquire, install and configure them.

The philosophy behind the application approach is that the system, once configured, is independent from the operator, who is then simply receiving the data and has to take actions only in case of problems in picture generation and/or transfer. In principle, the system configuration will be done as part of the system AIT, even if some remaining activities will be required as part of the MTF commissioning at the Antarctica site, such as the SW application start and verifying that the applications are correctly working and transferring data, over the satellite connection, to Europe.

The following TPZ developed applications are required to manage the above described tasks:

- hikvision.py

Application / subroutine defining several parameters, such as the camera network parameters, the storage path, the destination path, etc.

- camera_snapshot_robot.py

Application for automatic daily picture acquisition

- camera_ftp_robot.py

Application for the file transfer to DLR

- camera_ftp_robot_NMIII.py

Application for the image file transfer to the NM-III PC image repository for backup purpose.

- data_ftp_robot.py

Application for data file (.csv) transfer to DLR

- data_ftp_robot_NMIII.py

Application for data file (.csv) transfer to the NM-III PC data repository for backup purpose.

In addition, to automate the storage path creation, the acquisition of the images and their distribution to the DLR and NM-III, the following batch files have been developed:

- edeniss_mkdir.bat

- edeniss_scheduler.bat
- edeniss_scheduler_NMIII.bat
- edeniss_data_scheduler.bat
- edeniss_data_scheduler_NMIII.bat

In particular, the **edeniss_mkdir.bat** creates image directories following a predefined structure, while the **edeniss_scheduler.bat** and **edeniss_scheduler_NMIII.bat** are the applications that schedule the tasks for daily images acquisition and transfer to DLR and NM-III. The **edeniss_data_scheduler.bat** and **edeniss_data_scheduler_NMIII.bat** play a similar role for the csv data files.

2.7.1 Hikvision.py

This SW application defines three main kinds of parameters:

Camera parameters: the network parameters for camera access and the parameter (top or side view) to create the full path necessary for the FTP file transfer. The parameters are required in the following format:

cameras = (<cameraname>, <IP>,<username>,<password>,<top or side view>)

For example:

cameras = {

'camera1': Camera('camera1', 'http:// 192.168.39.111:80', 'admin', 'Apple-123', 'HDTOPVIEW'),

'camera2': Camera('camera2', 'http:// 192.168.39.112:80', 'admin', 'Apple-123', 'HDSIDEVIEW'),

…

'camera39': Camera('camera39', 'http:// 192.168.39.84:80', 'admin', ' Apple-123', 'HDTOPVIEW'),

}

4 lines were added in the cameras structure in order to send via FTP the image files obtained from the UF Imagers. These images are not acquired via the acquisition script. Those 4 lines, with fake IP addresses, are used to transfer all the files of the day that are present in the appropriate directories (file naming ***UFImager1_prefix*** or ***UFImager1_20170721***) using the *ftp transfer image file script.*

In the following, those 4 lines are shown:

'UFImager1': Camera('UFImager1', 'http://192.168.1.1:80', 'admin', 'Apple-123', 'UFIMAGERS'),

'UFImager2': Camera('UFImager2', 'http://192.168.1.1:80', 'admin', 'Apple-123', 'UFIMAGERS'),

'UFImager3': Camera('UFImager3', 'http://192.168.1.1:80', 'admin', 'Apple-123', 'UFIMAGERS'),

'UFImager4': Camera('UFImager4', 'http://192.168.1.1:80', 'admin', 'Apple-123', 'UFIMAGERS'),

Local Directory: This is the directory (on the MTF Camera PC) where the snapshots are stored.

This parameter has to be defined in the following format:

LOCAL_IMAGE_PATH = '<storage path>'

For example:

LOCAL_IMAGE_PATH = 'D:/FTP_EDEN-ISS/CropImages/'

Remote FTP host: Define the path for remote image destination. The parameters are defined as follows:

FTP_HOST = '<hostIPaddress>'

FTP DIRECTORY = '<storage path>'

For example

FTP_HOST = should be that of the OPS PC @DLR IP address 192.168.39.105

FTP_HOST_NMII for Camera-PC (NM-III) IP address 192.168.39.104

FTP DIRECTORY = "D:/FTP_EDEN-ISS/CropImages/"

2.7.2 Camera_snapshot_robot.py

This application gets camera snapshots using HTTP requests. It implements an HTTP client that, for each webcam, gets the device info (including the definition of the storing path) from the hikvision.py script, then obtains and stores snapshot(s) in the defined directory.

In the following the file naming for images acquired: the pattern for the **image snapshot** is ***HDCam_{}_{}.{}*** that is ***HDCam_position_prefix.fileextention***, position in {*L1-2C, L1-4C, L2-1C, L2-2C, L2-3C, L2-4C, L3-1C, L3-2C, L3-3C, L3-4C, L4-1L, L4-2L, L4-3L, L4-1R, L4-2R, L4-3R, L4-4C, R1-2C, R1-4C, R2-2C, R2-4C, R3-4C, R4-2C, R4-4C* for HDSIDEVIEW cameras. *L12-1S, L12-3S, L34-1S, L34-3S, R12-1S, R12-3S, R34-1S, R34-3S* for HDTOPVIEW cameras. *UFImager1, UFImager2, UFImager3, UFImager4* for UFIMAGERS cameras}, *prefix* is *YYYYMMDD_hhmm* where YYYYMMDD is the GMT year-month-day and hhmm is the hour-second (GMT) in local daytime*, fileextention=jpeg.* Example *HDCam_L1-2C_20171124_0730.jpeg* where position=*L1-2C*, prefix=*20171124_0730*, fileextention=*jpeg*.

In the following the usage of the script (*python.exe camera_snapshot_robot.py –h*) is shown:

usage: camera_snapshot_robot.py [-h] [--logfile LOGFILE]

optional arguments:

-h, --help show this help message and exit

--logfile LOGFILE

You could run **manually** the Python script typing "*python.exe camera_snapshot_robot.py*" in a *Windows CMD command line*, if you want to acquire the image file at this moment.

In addition, the script has been designed in order to log the activities and to maintain a log history in the system. The maximum dimension of a log file is 10 MB, and therefore at any time the log reaches the value, it is closed in favour of the new one. A maximum of 6 logs are maintained in the system at the same time.

The first is the current log file, the others are the old logs and have a suffix from 1 to 5 in the name (the log with 5 is the older log).

When a current log is closed it is renamed with the suffix .1, the other logs are renamed to increment the suffix and the log that should increment its suffix from 5 to 6 is erased. Therefore in the system we will have at the same time:

Camera_snapshot.log < 10MB

Camera_snapshot.log.1 = 10MB

Camera_snapshot.log.2 = 10MB

Camera_snapshot.log.3 = 10MB

Camera_snapshot.log.4 = 10MB

Camera_snapshot.log.5 = 10MB

2.7.3 Camera_ftp_robot.py

This application sends camera snapshots over ftp. It implements an FTP client that, for each webcam, acquire the basic network camera's information and the destination path from the hikvision.py script, then open an ftp session and send all images acquired in a defined timeframe to the remote site @DLR.

In the following the file naming for file transfer: The pattern for the FTP image file transfer is *{}_{}*.{} that is ***position_prefix*.fileextention**, for position, prefix and fileextention and example see above paragraph for camera snapshot.

In the following the usage of the script with the arguments (*python.exe camera_ftp_robot.py –h*):

usage: camera_ftp_robot.py [-h] [--logfile LOGFILE] [--userid USERID]
[--password PASSWORD] [--offset OFFSET]

optional arguments:

-h, --help show this help message and exit

--logfile LOGFILE

--userid USERID

--password PASSWORD

--offset OFFSET

The following optional arguments could be defined in this script:

userid of the remote server (the default value is *ftp_edeniss_dlr)*

password to access the remote server (the default value is *12345)*

offset to define the timeframe of the acquisitions (the default value is *86400* seconds in a day, means yesterday)

logfile (the default value is *camera_ftp.log)*

You could change the default parameters inside the script. Otherwise, you could run manually the Python script using different parameters in a *Windows CMD command line*.

Example, if you run "*python camera_ftp_robot.py*", you are using the default parameters that you find in the script file: *--logfile camera_ftp.log --userid ftp_edeniss_dlr --password 12345 --offset 86400* (means yesterday), that allows the FTP files transfer of all the acquisition files, to DLR site, with the yesterday date.

Otherwise, you could run **manually** "*python.exe camera_ftp_robot.py --userid ftp_edeniss_dlr --password 12345 --offset 0*" (means today), that allows the FTP files transfer of all the acquisition files, to DLR site, with the today date, in case you want to use different parameters.

The script saves all the activities done in a log file named "*camera_ftp.log*" of 10MB with 5 old backups. This log is generated and managed in the same way described for camera_snapshot_robot.py

2.7.4 Camera_ftp_robot_NMIII.py

This application is the same as the one described in the previous paragraph with the only exception that it sends all the images acquired in a defined timeframe to the remote site at NM-III.

Usage and default parameters are described above.

Example, if you run "*python camera_ftp_robot_NMIII.py*", you are using the default parameters that you find in the script file: *--logfile camera_ftp.log --userid ftp_edeniss_dlr --password 12345 --offset 86400* (means yesterday), that allows the FTP files transfer of all the acquisition files, to NMIII site, with the yesterday date.

Otherwise, you could run **manually** "*python.exe camera_ftp_robot.py --userid ftp_edeniss_dlr --password 12345 --offset 0*" (means today), that allows the FTP files transfer of all the acquisition files with the today date, to NMIII site, in case you want to use different parameters.

The script saves all the activities done in a log file named "*camera_ftp_NMIII.log*", default parameter, of 10MB with 5 old backups. This log is generated and is managed in the same way described for camera_snapshot_robot.py.

2.7.5 Data_ftp_robot.py

This application is the same as the one described in the previous paragraph with the only exception that send all the **csv data file** acquired in a defined timeframe to the remote site @DLR.

In the following the file naming for csv files: the pattern is ***{}*.{}** that is ***prefix*.csvfileextention**, *prefix* is *YYYY-mm-dd*, *csvfileextention=csv*.

Example **2017-08-28*.csv* where prefix=*2017-08-28*, csvfileextention=*csv*. In this case all the files in the directory that will match with **2017-08-28*.csv* pattern, they will be considered for the ftp DATA FILES transfer, for example *datafile1_2017-08-28_telemetry.csv* and so on.

Usage and default parameters are described above.

Example, if you run "*python data_ftp_robot.py*", you are using the default parameters that you find in the script file: *--logfile data_ftp.log --userid ftp_edeniss_dlr --password 12345 --offset 86400* (means yesterday), that allows the FTP files transfer of all the csv data files, to DLR site, with the yesterday date.

Otherwise, you could run **manually** "*python.exe data_ftp_robot.py --userid ftp_edeniss_dlr --password 12345 --offset 0*" (means today), that allows the FTP files transfer of all the csv data files with the today date, to DLR site, in case you want to use different parameters.

The script saves all the activities done in a log file named "*data_ftp.log*", default parameter, of 10MB with 5 old backups. This log is generated and is managed in the same way described for camera_snapshot_robot.py.

2.7.6 Data_ftp_robot_NMIII.py

This application is the same as the one described in the previous paragraph with the only exception that send all the **csv data file** acquired in a defined timeframe to the remote site @NMIII.

In the following the file naming for csv files: the pattern is ***{}*.{}** that is ***prefix*.csvfileextention**, *prefix* is *YYYY-mm-dd*, *csvfileextention=csv*.

Example **2017-08-28*.csv* where prefix=*2017-08-28*, csvfileextention=*csv*. In this case all the files in the directory that will match with **2017-08-28*.csv* pattern, they will be considered for the ftp DATA FILES transfer, for example *datafile1_2017-08-28_telemetry.csv* and so on.

Usage and default parameters are described above.

Example, if you run "*python data_ftp_robot_NMIII.py*", you are using the default parameters that you find in the script file: *--logfile data_ftp.log --userid ftp_edeniss_dlr --password 12345 --offset 86400* (means yesterday), that allows the FTP files transfer of all the csv data files, to NMIII site, with the yesterday date.

Otherwise, you could run **manually** "*python.exe data_ftp_robot_NMIII.py --userid ftp_edeniss_dlr --password 12345 --offset 0*" (means today), that allows the FTP files transfer of all the csv data files with the today date, to NMIII site, in case you want to use different parameters.

The script saves all the activities done in a log file named "*data_ftp_NMIII.log*", default parameter, of 10MB with 5 old backups. This log is generated and is managed in the same way described for camera_snapshot_robot.py.

2.7.7 System Configuration for Automatic Images Acquisition and Transfer to DLR

This chapter deals with the description of the procedure to be used to configure the systems for the scheduling of an automatic daily images acquisition and the subsequent automatic FTP file transfer to DLR.

Prerequisites

The following files/scripts need to be copied on the MTF Camera PC, for example in C:\Image_Acquisition\TPZ_SW

- *hikvision.py*
- *edeniss_mkdir.bat*
- *camera_snapshot_robot.py*
- *camera_ftp_robot.py*
- *edeniss_scheduler.bat*
- *camera_ftp_robot_NMIII.py*
- *edeniss_scheduler_NMIII.bat*
- *data_ftp_robot.py*
- *edeniss_data_scheduler.bat*
- *data_ftp_robot_NMIII.py*
- *edeniss_data_scheduler_NMIII.bat*

The following file/application needs to be installed on the DLR OPS PC and NMIII in C:\Image_Acquisition\TPZ_SW

- *edeniss_mkdir.bat*

In addition an ftp Server (for example filezilla) is required to permit the file transfer. This application has to be installed on the DLR OPS PC and NM-III PC.

Step 1: On DLR OPS PC create the folder for image storage.

- Launch the ***edeniss_mkdir.bat*** batch file in a *Windows CMD command line*.
- Verify the following folders have been created:
  - *D:\FTP_EDEN-ISS\CropImages\HDTOPVIEW\<camera position1>*

    Where camera position1 is:
    - L1-2C, L1-4C
    - L2-1C, L2-2C, L2-3C, L2-4C
    - L3-1C, L3-2C, L3-3C, L3-4C
    - L4-1L, L4-2L, L4-3L
    - L4-1R, L4-2R, L4-3R, L4-4C
    - R1-2C, R1-4C
    - R2-2C, R2-4C
    - R3-4C
    - R4-2C, R4-4C
  - *D:\FTP_EDEN-ISS\CropImages\HDSIDEVIEW\<camera position2>*

    Where camera position2 is:
    - L12-1S, L12-3S, L34-1S, L34-3S
    - R12-1S, R12-3S, R34-1S, R34-3S
  - *D:\FTP_EDEN-ISS\CropImages\UFIMAGERS\<ufimager camera position2>*

    Where ufimager camera position is:
    - UFImager1, UFImager2, UFImager3, UFImager4
  - *D:\FTP_EDEN-ISS\DATA*

Remark: If the folder structure already exist, the command is not executed and a message will appear in the command window stating that the folders already exist.

Step 2: On DLR OPS PC and NMIII configure the ftp server. You need an ftp account on the FTP server with user and password specified below. In the following, the steps describing the ftp configuration using the *Filezilla Server software*:

- Open Filezilla Server Interface
- In the Menu Bar/Edit→Users
- In the Users Page/Users →Click on Add Button
- In the Add User Account window→ type the name of the ftp user Account: ***ftp_edeniss_dlr***
- In the account settings → click on Password and then enter: ***12345*** (if another is desired the edeniss_scheduler.bat has to be updated)
- In Page Select Shared Folders → Click Add and then select D:\FTP_EDEN-ISS\CropImages → Click OK
- Select the privileges (in Files select Read, Write, Delete, Append; in Directories select Create, Delete, List, Subdirs) →Click on set as home dir →Click Ok
- In FileZilla Server Interface verify the operations has succeeded.

Step 3: On MTF Camera PC create the folder for image storage:

- Launch the ***edeniss_mkdir.bat***
- Verify the following folders have been created:
  - *D:\FTP_EDEN-ISS\CropImages\HDTOPVIEW\<camera position1>*

    Where camera position1 is:
    - L1-2C, L1-4C
    - L2-1C, L2-2C, L2-3C, L2-4C
    - L3-1C, L3-2C, L3-3C, L3-4C
    - L4-1L, L4-2L, L4-3L
    - L4-1R, L4-2R, L4-3R, L4-4C
    - R1-2C, R1-4C
    - R2-2C, R2-4C
    - R3-4C
    - R4-2C, R4-4C
  - *D:\FTP_EDEN-ISS\CropImages\HDSIDEVIEW\<camera position2>*

    Where camera position2 is:
    - L12-1S, L12-3S, L34-1S, L34-3S
    - R12-1S, R12-3S, R34-1S, R34-3S
  - *D:\FTP_EDEN-ISS\CropImages\UFIMAGERS\<ufimager camera position2>*

    Where ufimager camera position is:
    - UFImager1, UFImager2, UFImager3, UFImager4
  - *D:\FTP_EDEN-ISS\DATA*

Step 4: Automatic image acquisition and image ftp transfer scheduling:

On MTF Camera PC, in a *Windows CMD command line*, launch **edeniss_scheduler.bat** batch file for automatic picture acquisition files(It is worth to underline that this application allows for one single automatic acquisition per day). The following window appears.

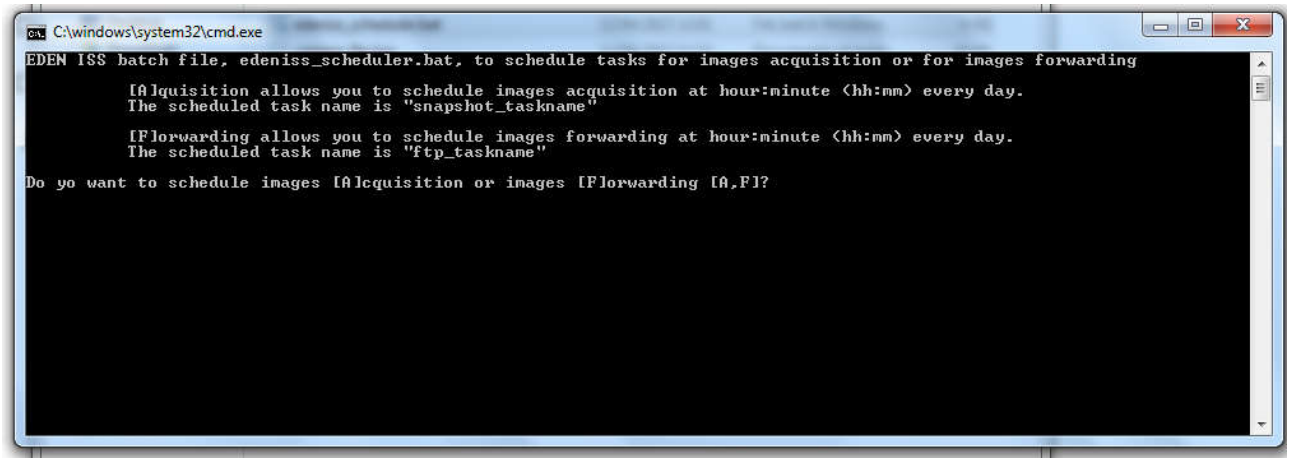


Figure 29: Scheduler Dialog Window

For Images automatic acquisition scheduling, type ***A***, then type the hour and then the minutes by when the images has to be acquired automatically every day. A message will provide a feedback on the result of the operation (**Remark**: The scheduling time is local time as the acquisition time in the name of the image)

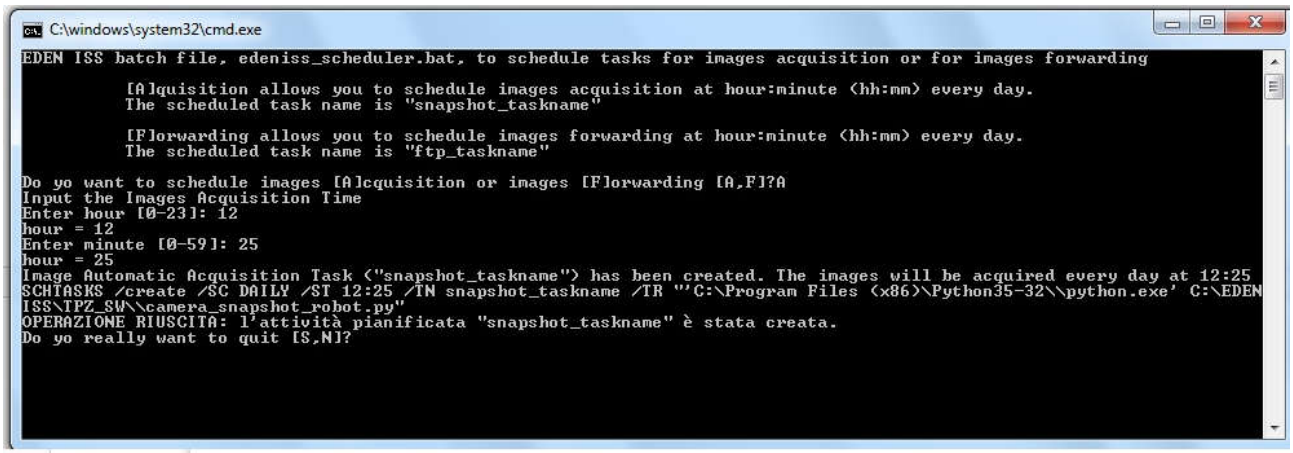


Figure 30: Scheduler Dialog Window – Feedback Message (acquisition task)

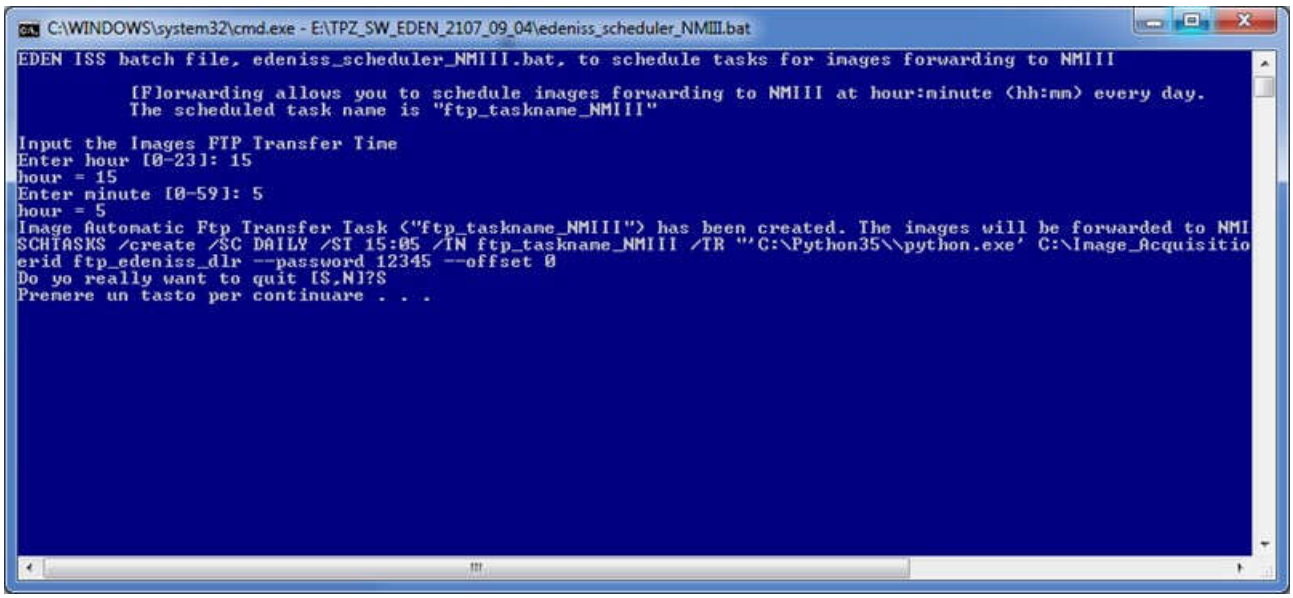


Figure 30: Scheduler Dialog Window – Feedback Message (ftp transfer task – to NMIII)

If you want to set the automatic transfer scheduling, type ***N*** when the message "Do you really want to exit [S/N]" appears on the screen, then repeat the above steps, but typing ***F*** in the first window.

After you have scheduled the tasks using *schtasks* command line as showed before, you have to check that the task run regardless of the connection of users who are connected. In other words, it is necessary **to check whether the** "**Run whether user is logged on or not" radio button is selected** in the Run Task Scheduler using the Windows interface.

In the following is shown how to start the Task Scheduler Windows interface:

Step 4a) To Run Task Scheduler using the Windows interface

1. Click the **Start** button.
2. Click **Control Panel** .
3. Click **System and Maintenance** .
4. Click **Administrative Tools** .
5. Double-click **Task Scheduler** .

Alternatively, to Run Task Scheduler from the Command Line

1. Open a command prompt. To open a command prompt, click **Start**, click **All Programs**, click **Accessories**, and then click **Command Prompt**.
2. At the command prompt, type **Taskschd.msc**.

Step 4b) Please, open the window property of the task scheduled (snapshot_taskname or ftp_taskname), in the "General" tab. Please, check whether the "**Run whether user is logged on or not"** radio button is selected.

Remark: The log files (*camera_ftp.log* and *camera_snapshot.log*) for the automated task scheduled are generated in *C:\Windows\System32* system directory.

To verify that the above scheduling tasks are correctly running, type the following commands in a *Windows CMD command line:*

- *schtasks /query | findstr /B /I "snapshot_taskname",* to verify that the automatic image acquisition is correctly running
- *schtasks /query | findstr /B /I "ftp_taskname",* to verify that automatic images transfer, to DLR site, process is correctly running.
- *schtasks /query | findstr /B /I "ftp_taskname_NMIII",* to verify that automatic images transfer, to NMIII site, process is correctly running.
- *schtasks /query | findstr /B /I "ftp_taskname_data",* to verify that automatic csv data files transfer, to DLR site, process is correctly running.
- *schtasks /query | findstr /B /I "ftp_taskname_data_NMIII",* to verify that automatic csv data files transfer, to NMIII site, process is correctly running.

As result, a message containing the schedule name, the next execution date and the status of the scheduled task will appear on the screen

To stop the schedule, type the following commands in a *Windows CMD command line:*

- *schtasks /delete /TN "snapshot_taskname",* to stop the image acquisition process
- *schtasks /delete /TN "ftp_taskname",* to stop the image transfer process to DLR
- *schtasks /delete /TN "ftp_taskname_NMIII",* to stop the image transfer process
- *schtasks /delete /TN "ftp_taskname_data",* to stop the csv data files transfer process to DLR
- *schtasks /delete /TN "ftp_taskname_data_NMIII",* to stop the csv data files transfer process to NMIII

2.7.8 Manual use of the Python developed SW Applications

In case an additional acquisition and transfer of images is required the Python SW applications can be used manually and can be launched from the DOS prompt commands, in a *Windows CMD command line*, as shown in the example in fig. 31.

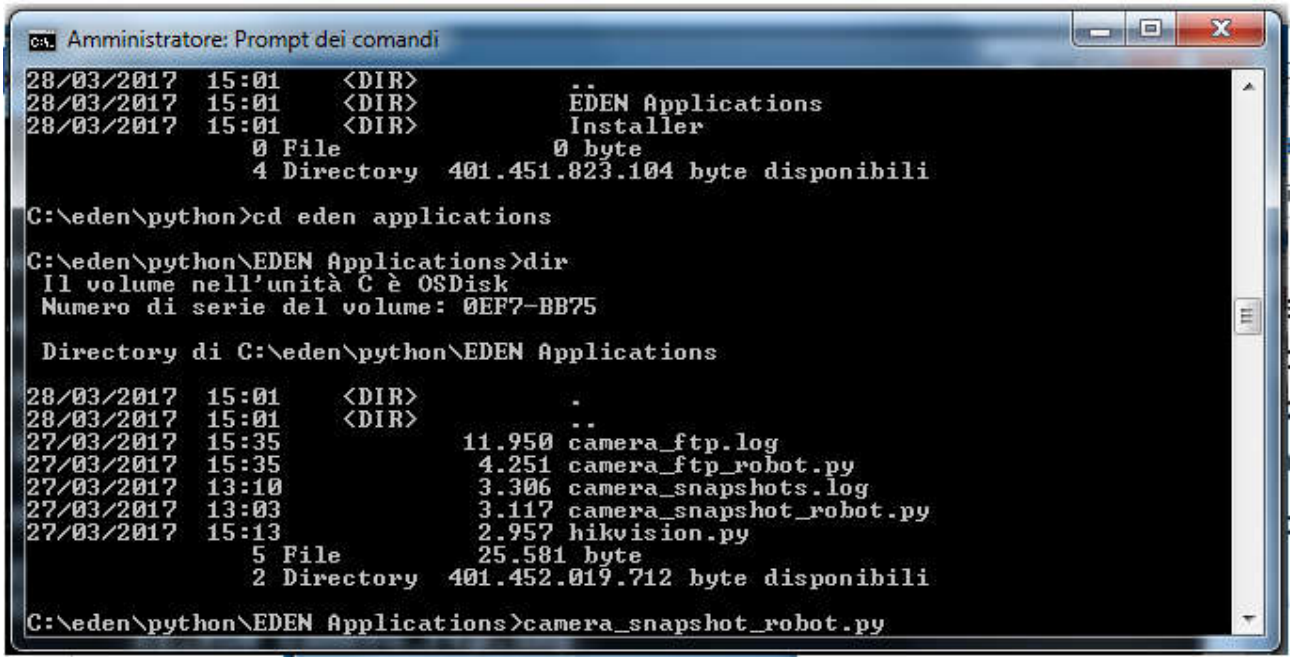


Fig. 31: camera_snapshot_robot

In particular the Python script *camera_snapshot_robot.py* can be run **manually** by typing "*python.exe camera_snapshot_robot.py*" in a *Windows CMD command line*, providing the possibility to immediately start the images acquisition

In the same way the Python script *camera_ftp_robot.py* can be run **manually** by typing "*python.exe camera_ftp_robot.py --userid ftp_edeniss_dlr --password 12345 --offset 0*" in a *Windows CMD command line*, to transfer all the image files acquired from the beginning of the day.

For manual operations a nominal sequence foresees that the operator first launch the *camera_snapshot_robot.py*, in order to acquire images, and then the *camera_ftp_robot.py*, in order to transfer the images via FTP.

Remark: In this case, the log files (*camera_ftp.log* and *camera_snapshot.log*) are generated in the same folders where the application are stored.

2.7.9 Telespazio SW Applications testing: Manually and Automatically

Steps to test TPZ software applications Manually:

1) Start a *Windows CMD command line*

2) PROMPT> ***cd C:\Image_Acquisition\TPZ_SW***

3) PROMPT> ***python.exe camera_snapshot_robot.py***

This creates the file *camera_snapshots.log* in the same directory of the software (*C:\Image_Acquisition\TPZ_SW*). Have a look inside that file for the results.

4) Check if the files image are acquired in the directories, example file

D:LOCAL_IMAGE_PATH\cameraposition\cameraname\HDCam_cameraname_20170721_hhss.jpeg

PROMPT> ***cd D:\FTP_EDEN-ISS\CropImages***

PROMPT> ***dir *_20170721_*.jpeg /s /p***

The images list is shown one page at a time.

5) PROMPT> ***python.exe camera_ftp_robot.py --userid ftp_edeniss_dlr --password 12345 --offset 0***

All the camera images acquired in the day are transferred to DLR site. This create the file *camera_ftp.log* in the same directory of the software (*C:\Image_Acquisition\TPZ_SW*). Have a look inside that file for the results.

PROMPT> ***python.exe camera_ftp_robot_NMIII.py --userid ftp_edeniss_dlr --password 12345 --offset 0***

All the camera images acquired in the day are transferred to NMIII site. Check the log file *camera_ftp.log* in the same directory of the software (*C:\Image_Acquisition\TPZ_SW*).

6) Check the files at FTP destination directories, on the remote host.

Steps to test TPZ software applications Automatically:

1) Check if the taskname for automated snapshot is scheduled

PROMPT> ***schtasks /query | findstr /B /I "snapshot_taskname"***

2) If the task exist, delete it

PROMPT> ***schtasks /DELETE /TN "snapshot_taskname" /F***

The task for automated snapshot snapshot_taskname is deleted

3) Check if the taskname for automated ftp file transfer is scheduled

PROMPT> ***schtasks /query | findstr /B /I "ftp_taskname"***

4) If the task exist, delete it

PROMPT> ***schtasks /DELETE /TN ftp_taskname" /F***

The task automated ftp file transfer ftp_taskname is deleted

5) Start the *edeniss_scheduler.bat* to schedule the tasks:

PROMPT> ***edeniss_scheduler.bat***

[A] automated snapshot task, *Enter hour* [0-23]: , *Enter minute* [0-59]: , then **[F]** automated FTP file transfer task, *Enter hour* [0-23]: , *Enter minute* [0-59]:

6) Important, check whether the "Run whether user is logged on or not" radio button is selected in the Run Task Scheduler using the Windows interface.

Step 6a) To Run Task Scheduler using the Windows interface

1. Click the **Start** button.
2. Click **Control Panel** .
3. Click **System and Maintenance** .

4. Click **Administrative Tools** .
5. Double-click **Task Scheduler** .

Alternatively, to Run Task Scheduler from the Command Line

1. Open a command prompt. To open a command prompt, click **Start** , click **All Programs** , click **Accessories** , and then click **Command Prompt** .
2. At the command prompt, type **Taskschd.msc** .

Step 6b) Please, open the window property of the task scheduled (snapshot_taskname or ftp_taskname), in the tab General checks whether the "**Run whether user is logged on or not"** radio button is selected.

7) Wait until the scheduled task *"snapshot_taskname"* is executed at the exact time.

8) Check if the files image are acquired in the directories, example file

D:LOCAL_IMAGE_PATH\cameraposition\cameraname\HDCam_cameraname_20170721_hhss.jpeg

PROMPT> ***cd D:\FTP_EDEN-ISS\CropImages***

PROMPT> ***dir *_20170721_*.jpeg /s /p***

The images list is shown one page at a time.

9) Wait until the scheduled task *"ftp_taskname"* is executed at the exact time.

10) Check the files at FTP destination directories, on the remote host.

11) Check the log files, *camera_snapshots.log* and *camera_ftp.log*, in the directory C:\Windows\System32

2.8 Automatic forwarding of the images to the UHB's

The same approach and tools described above could be used. For example the *camera_ftp_robot.py* could be customized to send the images to the four UHB ftp addresses, installed on the DLR camera PC, and scheduled to activate after the reception of the images at DLR.

This is not currently foreseen for implementation.

Annex D: Multi-Wavelength Imaging System Guide Manual

General Component List

- Go Pro Hero 4 Black Camera w/Back-Bone System
- NDVI-7 Filter
- M12 Lens
- USB Power Supply (5.2V/2.5A)
- CamDo Blink Controller (two versions, white and black)
- CamDo Remote Switch
- iPad Control Tablet
- **128 GB Micro SD Cards (MUST BE U3 rated)**
- Stand or Mounting arms /beams

Network Connections

The BLINK system requires the following network connections:
The SSID must not have any spaces, and we need to have the MAC Address. There must not be a password on the SSID.

SSID of the access point is: WLAN_MTF_EDEN-ISS
BSSID of the access point is: 94:05:B6:5C:EC:EC
MAC-address of the access point is: 94:05:B6:5C:EC:EB
IP-address of the access point is: 192.168.39.21
Wifi: 802.11n

Direct Photo Access

Photos can be accessed through the following methods:

- GoPro App
- BLINK Photo transfer to FTP
- Removal of the SD card

NEVER TRY TO ACCESS PHOTOS BY USING USB CONNECTION. The USB port should only ever be used to power the camera, never for data transfer.

Usual EDEN-ISS functionality is by FTP transfers from the UFImager GoPros through WiFi.

FTP Access

ftp://212.201.100.47/

- userid: UF
- PW: alp+rf85

Passwords

BLINK (all)	Wifi	1234567890	EDEN ISS 2018
BLINK /camdo	Webpage	192.168.1.1	
1 - NDVI Blink	Hero4	password	
UFSPL	Hero3	password	
2 - ALP NDVI*	Hero4	password	UFImager2

4 - EDEN IrPro	Hero4	Password	
3 - RJF NDVI*	Hero4	password	UFImager3
5 – GatorNDVI*	Hero4	password	UFImager 4
6 – AllyNDVI*	Hero4	password	

* Denotes Deployed to Antarctic Units

SD Card

We have found that the most common compatibility problems are due to the type of SD card being used. You are strongly advised to use an SD card from the recommended list on GoPro's website, http://gopro.com/help/articles/Block/microSD-Card-Considerations.

Use of cards not on the list, especially *SanDisk Ultra* cards, will greatly increase your chance of trouble with your setup. Some SD cards have the same product name as the cards on the list but are an older version that only has a U1 rating. **Make sure your SD card has a U3 rating.** Underperforming SD cards can prevent proper functioning of your GoPro camera's features.

Camera set up

Scheduled data collection is controlled by the Blink Hardware modification (see section 3.5). However, before using the Blink to control automation, the camera must be pre-equipped with the NDVI-7 filter, and configured and tested for focus and view. The camera can be connected to WIFI for live viewing of image (low definition option) or connected to a computer display screen via HDMI for hi-definition live viewing. For accurate focus, it is best to use HDMI. If HDMI connection not an option, focus through WiFi connection, but then further calibrate and adjust by reviewing test images taken at several focal planes.

GoPro connection and configuration

The first step is to download the application to the controlling tablet, and then follow the on screen instructions to add a new device. The first time the wireless connection is established, the camera and the tablet must be paired via Bluetooth. The tablet will thereafter be able to recognize the camera (Figure 4). This step is only necessary the first time.

1. Turn on GoPro camera, scroll to Setup and enable GoPro Wi-Fi
2. Exit Setup screen on Camera
3. Using your phone or tablet device, connect to GoPro Wi-Fi. – The device is connecting to the Camera's Wi-Fi, rather than the camera connecting to the device.
4. Open the App →
5. Select "Connect Your Camera"
6. Press "Control" under the device to be used
7. Check the field of view in the Live Preview screen
8. The "settings" tool (wrench icon) enables the manipulation of all camera options

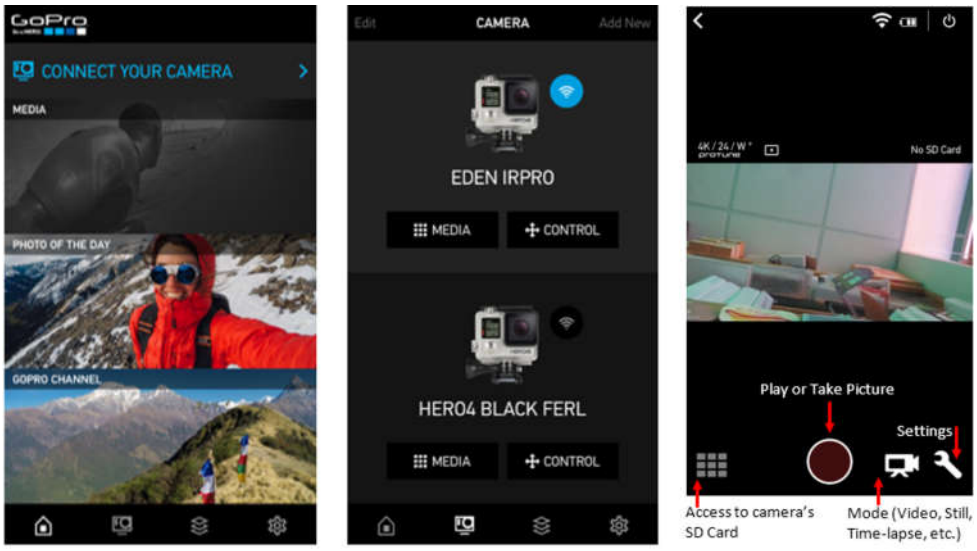
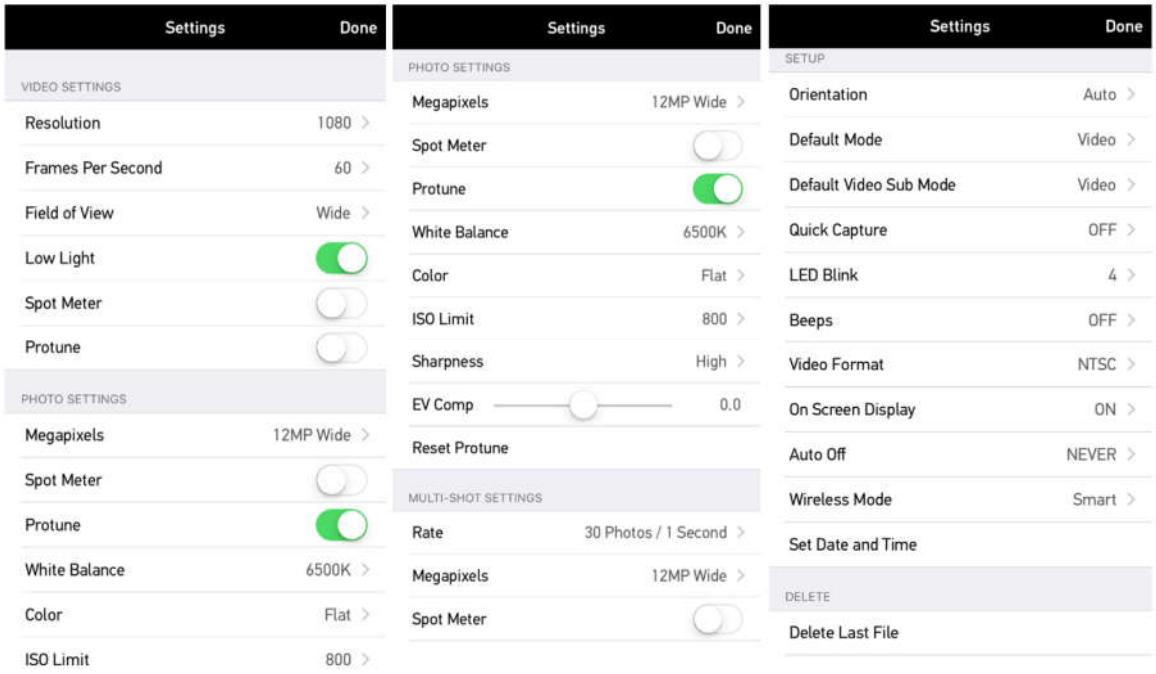


Figure 33. Screen shots of the pairing process (left and middle) and the Live Preview screen (right) on the tablet or phone.

Camera settings

The GoPro settings have been found to work best for initial setup under the Heliospectra light bank are as follow. Figure 5 provides screen captures of the camera selections under "Settings".

- Megapixels; various choices
  - There are wide frame and "zoomed" frame settings
  - Retest focus after selecting different MP
  - Note that irrespective of the size chosen to collect, when the image is saved, it will be slightly smaller (e.g. 12MP file will be about 4.5-6MP)
- Spot Meter: OFF
- Protune : ON
  - protune enhances quality of video by increasing bit rate from 30 to 45 megabits per second; not so important for capturing individual images, however, enabling protune unlocks features of interest, such as white balance, color adjustment, ISO, sharpness and EV compensation.
    - White Balance: 6500k
- Color: Flat
- ISO Limit: 800
- Sharpness: High
- EV Comp: 0.0



Camdo BLINK set up

The instructions below are adapted from the on-line guides provided on the Camdo web documents (https://cam-do.com/pages/blink-quick-start-guide). NOTE: 2018 Field Season Quick Notes are below, the following examples are for background and reference.

Blink assembly and activation

1. Turn on the camera and adjust settings as outlined in 3.4, but set the AutoOff to 1 minute to prevent the camera battery from draining in the event of an error that fails to turn off the camera after an imaging session. Also confirm that the camera firmware is updated to the latest version; either through the GoPro App for iOS, or on the GoPro web site (v05.00 was the latest HERO4 version at April 2017). NOTE: for the 2018 season, all firmware updates are in place.
2. Connect the Blink the HERO4 camera. This is as simple as snapping it onto the back of the GoPro (Figure 6).

3. Wait 10 seconds. After this time, Blink will tur

n on the camera, read the camera's time, and then turn off. The camera's time will be inaccurate, but will be set it later in the process.

4. Plug the Wired Remote into the Blink's 2.5mm port. Blink's WiFi can be turned on and off by holding down the button of the Wired Remote for 3 seconds. Blink's LED will flash cyan three times to indicate WiFi has been turned on and twice to indicate WiFi has been turned off. The LED will continue to flash blue every 5 seconds while Blink's WiFi is active and WiFi will automatically turn off after 15minutes to conserve power. The Wired Remote can be unplugged after activating Blink's WiFi. Or you can leave it attached and remove the Wired Remote after turning off the WiFi at the end of the setup.
5. Connect to the Blink wireless network "CamDo-Blink_XXXX", where XXXX is the MAC address of Blink. Unless you have multiple Blinks you will only see one Blink wireless network to connect to. The password to connect is "1234567890". NOTE: If the LED of Blink is not flashing blue to signify WiFi is on, connect the wired remote and hold the button for 3 seconds to turn it on.
6. Open a web browser and navigate to http://192.168.1.1 in the search bar. (If a shortcut is not present on the iPad, one should be created). A screen capture of the Blink interface is shown in Figure 8.

Blink settings

Note: 2018 Field season has white blinks that use this example below. UFImager4 uses a black blink that has a slightly different setup procedure. See 2018 Quick Notes below.

The first step is to sync camera time with local time by clicking on the "Sync Camera Time" button. Then the schedule can be modified for the capture requirements of the experiment. In the example shown in Figure 8, a schedule was created to take an image every 20 minutes, Monday through Friday 10am-4pm.

- Tap the days of the week to enable the schedule for that day. As can be seen in the image below, M, T, W, T, F are highlighted in blue indicating they are active.

- Set the Start Time and End Time
- Select “Intervalometer” under “Select a mode”
- Set to desired interval (20 mins in this example)
- “Select an action” - in this case a Photo during the Day is selected.
- Click the “Enabled” button (if enabled, the schedule in the list will be dark blue).
- Click “Save All”. The page will refresh and your modified schedule should be shown in the appropriate drop-down box. Clicking “Clear All” will reset all schedules to their default values and disable.

Note. The same procedure can be followed to set additional Schedules. Schedule conflicts will be resolved by priority in descending order. For example, schedule 1 has priority over schedule 2.

STEP 8 - ONLY USE ONE SCHEDULE WITH FTP Software

Modify a schedule to suit your capture requirements. In this example, we will create a schedule to take an image every 20 minutes, Monday through Friday, 10am till 4pm.

To create the schedule, complete the following:

- Tap the days of the week to enable the schedule for that day. As can be seen in the image below, M, T, W, T, F are highlighted in blue indicating they are active.
- Set the Start Time and End Time
- Select “Intervalometer” under “Select a mode”
- Set to your desired interval (20 mins in this example)
- “Select an action” - in this case a Photo during the Day is selected.

That’s it! Follow the same procedure to set additional Schedules as needed.

NOTE: for the black Blink, you must Select and Action, choose photo, set for 0 (zero) seconds.

STEP 9

Once the schedule has been modified, be sure to click the “Enable” button. A quick way to tell if a schedule is enabled is by the dark blue colour of the drop-down box. Schedule conflicts will be resolved by priority in descending order. For example, schedule 1 has priority over schedule 2.

STEP 10

ENABLE SCRIPTING!

NOTE: for the black Blink, there is no enable scripting box, nothing to worry about.

STEP 11

Click “Save All”. The page will refresh and your modified schedule should be shown in the appropriate drop-down box. Clicking “Clear All” will reset all schedules to their default values and disable.

System Reset / System Set Up

If complete meltdown happens and you need to redo the whole system.

1. Update Hero 4 firmware to camera specific version (v5.00)

   a. This camera specific update is provided in each file for an imager. This will update the camera to GoPro v5.00 firmware. The files are not interchangeable as they are created specifically for individual serial numbers.
   b. Find and move the UPDATE file to your microSD card. For the camera to find the file, it should be the ONLY folder on the card and should be titled “UPDATE”
   c. Make sure the camera is off
   d. Insert the SD card into the camera
   e. Power the camera on
   f. Camera will cycle through a number of beeps and turning itself on and off as it updates.
   g. As long as you don’t get a “CAMERA UPDATE FAILED” message when it is done, then your update is successful.
2. Update Blink firmware to latest version (v2.00 currently)
   a. Download the .bin update file and transfer it to a USB flash drive. The USB flash drive must be using the FAT32 file saving format (default format for PC).
   b. Connect the flash drive to one end of the USB OTG Cable and the other end to Blink. Blink should not be connected to the GoPro camera during the firmware update process.
   c. Finally connect the last end of the cable to a power source such as a mobile phone USB charger/USB battery pack/Laptop, etc.
   d. Blink will start the update immediately. During the firmware update, the LED will follow the pattern below.
   e. Magenta flash continuously every 0.25 seconds: Firmware from the USB flash drive is transferring to Blink storage.
   f. Green flash twice: Firmware successfully stored.
   g. Magenta flash continuously every 0.25 seconds: Firmware from internal Blink storage is installing.
   h. Green flash three times: Firmware successfully installed.
   i. Red flash: Any number of red flashes indicates an error has occurred. Remove power and try again. If red LED flashes continue to occur during the firmware update process, please contact CamDo customer support.
   j. To verify the update, turn WiFi on with the remote and check the top right corner of the UI.
   k. Note: The USB flash drive must be using the FAT32 file format to be able to perform the Blink firmware update and Blink does not support partitioned drives or other formats. The FAT32 file saving format is the default format for PC and used with most USB flash drives under 32GB. Large capacity flash drives might be using the exFAT format which is not compatible with Blink. If you are using a MAC, the following link explains how to format a USB flash drive to FAT32.
3. Update Hero 4 firmware to CamDo version (from Camdo)
   a. This version of the firmware for the Hero4 is the same on each camera (not camera specific.)
   b. Use the UPDATE folder as in step one – but the version from Cam Do in the Updates from CamDo after V5.00 update
4. Set-Up camera through app (make sure photo settings and focus are correct)
   a. See Camera set up above
5. Set-Up Blink through WiFi connection
   a. See BLINK set up above
6. Set-up SD card with autoexec and CSIController files
   a. Remember each camera has its own individual CsiController file, but the autoexec
7. Plug in – and get rolling.

Image nomenclature

- The pattern for the **UFIMAGERS image snapshot FTP transfer** is {}*.{} that is ***UFICamPosition*.fileextention***, UFICamPosition in {*UFImager1, UFImager2, UFImager3, UFImager4* for UFIMAGERS cameras}, *fileextention=jpg*. Example: source UFI picture name UFImager2_1502722694_102GOPRO_GOPR3299.jpg UFICamPosition=*UFImager2*, fileextention=*jpg*. As you can see, in the image snapshot file name an Unix time stamp is present.

  Before transfer of the images, the script does a conversion of time stamp in local GMT daytime so that the destination picture name is ***UFICamPosition_prefix_*.fileextention***, UFICamPosition in {*UFImager1, UFImager2, UFImager3, UFImager4* for UFIMAGERS cameras}, *prefix* is *YYYYMMDD_hhmm* where YYYYMMDD is the GMT year-month-day and hhmm is the hour-second (GMT) in local daytime, *fileextention=jpg*.

  Example:

  - Source UFIMAGER picture name **UFImager2_1502722694_102GOPRO_GOPR3299.jpg** where **1502722694** is the Unix time stamp;
  - Destination UFIMAGER picture name **UFImager2_20170814_1658_102GOPRO_GOPR3299.jpg** where **20170814_1658** is the GMT local daytime in the form *YYYYMMDD_hhmm* where *YYYYMMDD* is the GMT year-month-day and *hhmm* is the hour-second (GMT) in local daytime.

Troubleshooting

Computer connection error

If a GoPro is accidently connected to a computer via USB, it will need to be reset before image transfer via FTP will work. To reset:

- Remove the battery and the Blink from the back of the camera (wait a few minutes)
- Replace the battery and reattach the Blink to the back of the camera
- After the Blink has cycled, you can turn off the camera and plug it in (to 5v power, not to a computer).
- It should now work normally; the Blink should turn on the camera and take pictures according to the schedule programmed in the Blink.

Photo lighting – unexpected changes

If a noticeable change in photo lighting is seen, with no adjustment to the lighting schedule, the camera clock should be checked. By accessing the BLINK on the back of the camera (see BLINK setup) the camera clock can be resynced using the “Sync Camera Time” button.

Adjusting Focus

Because of the coding located on the SD card mounted in the camera, focusing the imagers can be tricky. Camera focus can be tested by using an HDMI connection of a WiFi connection to the GoPro application. Unless there is an HDMI cord and screen handy, WiFi is the most likely path.

1. Remove SD Card
2. Turn on GoPro WiFi
3. Connect tablet
4. Open app
5. Do a rough focus using the “live” view
6. Test focus by taking a picture and viewing it on the tablet.

The “live” view quality on the camera app should not be trusted for high quality focus. An image should be captured and viewed as the “live” view is not meant for focus, only frame adjustment. Fine focus should be done by making adjustments and checking them by taking a picture.

Imager Storage

If the imagers are to be stored, it is best to remove the Blink from the camera to avoid unnecessary photos being taken. This will stop the battery from being drained when being stored for short periods of time. It is also important to make sure that the WiFi signal isn't on (no blue flashing light.)

Multiple Schedules

Do to the use of beta code on the imagers, it is best not to run multiple schedules over extended time periods. Once the first schedule is rolled to the second, it will not go back to the first schedule. There is also a chance of failure in the second schedule. DON'T USE MULTIPLE SCHEDULES.

General Problem with imagers

One of the first things to check if the imagers aren't working as expected is the CSIlog on the SD card. This log can tell us where the issue is happening. To do this, you will need to remove the SD card from the camera and open on a computer. The file can be read as txt, but is easier in a coding program such as Notepad++

Pictures taken, but not transferred

Make sure that **Enabled Scripting** is checked in the Blink set up for the white Blinks. For the black Blink be sure that Select an Action has Photo for 0 (zero) seconds is set.

UFImagers EDEN-ISS 2018 Field Operations Quick Notes

UFImager GoPro Pelican – stored in Service Section

This pelican is used to store and transport the UFImager GoPro cameras. It also contains switches and parts for use during the season whenever repositioning or refocusing of the UFImagers is necessary, or during troubleshooting of UFImager functionalities. Pelican contents:

1. Ziploc bag with GoPro Imager Lens caps (3)
2. Fisheye Lens w/NDVI filter (1) in box
3. Component and tool bag (1)
    a. CamDo USB
    b. Micro SD cards and SD adapters
    c. Small Allen wrenches
    d. Filters
4. Hardware Zipper Bag (1) lower level
    a. GoPro Power USB cord (1)
    b. CamDo USB Reset cord (1)
    c. Arm / Beam adapter nuts (5)
5. Blink Zipper Bag (1) lower level
    a. CamDo Blink Wifi Remote Switches (2)
6. CamDo sticker (1)

UFImager Operations Quick Ref

WiFi password for all UFImager GoPros = **password**

WiFi password for all CamDo Blink adapters = **1234567890**

GoPro App for focusing and checking settings on UFImagers:

The GoPro app is used for focusing and altering the settings of the UFImagers. This is done the apps in either the Tablet or the iPad and requires connection to the GoPro WiFi

1. Remove SD card from the GoPro, OK also to remove power cable, but not necessary

2. Start GoPro App on iPad or Tablet
3. Turn on GoPro WiFi
    a. Press and hold the settings button on the side, or
    b. Press the front panel skull button and navigate to settings
4. Navigate to Settings iPad or Tablet and connect to the GoPro WiFi
    a. All GoPro WiFi passwords should be stored in the iPad and Tablet but if not enter the password = password to connect
    b. Return to the GoPro App
    c. Use Live Preview to focus
    d. Use Settings to check or change imaging parameters (consult ops document)
5. Turn off WiFi
6. Turn off GoPro by pressing and holding the Skull front button till the display clears.
7. Replace SD card. Be sure that it is seated properly
8. Replace power connection

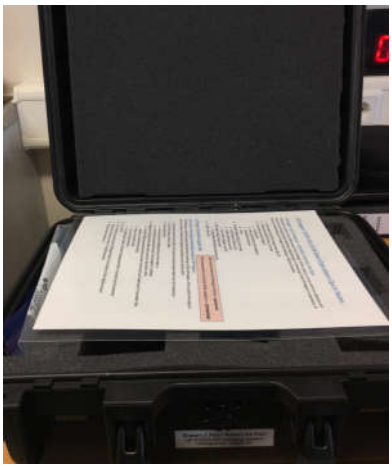
CamDo Blink for settings on UFImager timings

The Blink adapters control the times that images are taken by the UFImager GoPros. All settings for time of images are managed by WiFi connection to the Blink Adapter. The Blink adapter also syncs the camera time to the station time. UFImagers 2 and 3 are controlled by white Blink adapters. UFImager4 is controlled by a black Blink. Connect to only one Blink at a time.

1. Recover a Blink Remote switch from the UFImager Pelican case
2. Connect a Blink remote switch to Port 1 of the Blink adapter on the back of the GoPro
    a. OK to leave power connected to GoPro
    b. Must leave SD card in GoPro
3. Turn on Blink WiFi by holding the button on the remote for ~5 seconds
4. Open Settings on the iPad or Tablet and Connect to Blink WiFi
    a. If necessary enter the password = 1234567890 to connect
5. Open Safari or other web browser, navigate to http://192.168.1.1
    a. Site should already be stored in the browser
    b. Shortcut should be on the iPad
    c. If connection to the Blink cuts out, sometimes reconnecting the power cord helps
6. Touch Sync Camera time
7. Modify the imager schedule as necessary (see full ops document)
8. Save and close
    a. White Blinks-
        i. Be sure to check Enable Scripting
        ii. Touch Save All
    b. Black Blink
        i. No enable scripting required
        ii. Go to Select an Action
            1. Select Camera On
            2. Set 0 (zero) seconds
        iii. Touch Save All
9. Use the Remote Switch to turn off the Blink WiFi
10. Ensure that Blink Wifi is off using the iPad or Tablet
11. Disconnect the remote switch and replace it to the UFImager GoPro pelican case

Inside the UFImager GoPro Pelican case is a laminated copy of the 2018 Field Ops Quick Notes


PELICAN
GoPro
Be a HERO.
EDEN ISS
Field Ops Quick Notes INSIDE



Annex E: Decontamination Test Reports

AIRBUS DEFENCE & SPACE

EDEN ISS

Dok.Nr./Doc.No.:	EN-TR-AST-0066			
Ausgabe/Issue:	01	Datum/Date:	23.02.2016	
Seite/Page:	1	von/of:	50	

Titel: / **Title:** **E-Nose2 BG Dilution Series**

Dokumenten Typ: Document Type:	Test Report	**Konfigurations-Nr.:** Configuration Item No.:	
Referenz- Nr.: Reference No.:		**Klassifikations-Nr.:** Classification No.:	
Lieferbedingungs-Nr.: DRL/DRD No.:		**Freigabe Nr.:** Release No.:	
Gruppierung (Dok.): Group (Doc.-related):		**Gruppierung (Version):** Group (Version-related):	
Thema: Subject:	EDEN – Examination of different H_2O_2 solutions		
Kurzbeschreibung: Abstract:	The results demonstrate the offensive sanitizing effects of 12% H_2O_2 solutions on *E. coli*. 3% solutions of H_2O_2 do not deliver the desired results. This is the reason why further testing is performed with 6% H_2O_2 solutions.		

Autor: Prepared by:	Alexander Gärtner Author	**Org. Einh.:** Organ. Unit:	TSIE3	**Unternehmen:** Company:	Airbus DS GmbH
Geprüft: Agreed by:	Friedrich Menzel Product Assurance	**Org. Einh.:** Organ. Unit:	TSIE3	**Unternehmen:** Company:	Airbus DS GmbH
Genehmigt: Approved by:	Viktor Fetter Project Manager	**Org. Einh.:** Organ. Unit:	TSIE3	**Unternehmen:** Company:	Airbus DS GmbH
Freigegeben: Released by:	Ulrike Schafheutle Configuration Control	**Org. Einh.:** Organ. Unit:	TSIE3	**Unternehmen:** Company:	Airbus DS GmbH

Daten/Dokument-Änderungsnachweis / *Data/Document Change Record (DCR)*

Ausgabe *Issue*	Datum *Date*	Betroffener Abschnitt/Paragraph/Seite *Affected Section/Paragraph/Page*	Änderungsgrund/Kurze Änderungsbeschreibung *Reason for Change/Brief Description of Change*
1	22.08.2016	all	Initial issue

AIRBUS DEFENCE & SPACE

EDEN ISS

Dok.Nr./Doc.No.:	EN-TR-AST-0066		
Ausgabe/Issue:	01	Datum/Date:	23.02.2016
Seite/Page:	3	von/of:	50

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TABLE OF CONTENTS

1 INTRODUCTION

Objective of the EDEN Diosol Exposure Test on *Escherichia coli* is to prove, if the vaporization of hydrogen peroxide provides an adequate decontamination of bacteria.
Therefore, the decontamination efficiency of two H_2O_2 solutions (3% and 12%) is investigated on the test bacterium *Escherichia coli* DSM 613. Besides the investigation on the different H_2O_2 solutions, it evaluates the Decontamination system and the Hydrogen Peroxide Vapor (HPV) treatment on its efficiency.

This document presents the following content:

- Test Articles
- Test Description
- Results
- Discussion
- Conclusion

The results of this test serve as inputs for the EDEN H_2O_2 Decontamination Test, as it helps to determine the solution of H_2O_2, which is used for further testing.

2 APPLICABLE AND REFERENCE DOCUMENTS

2.1 Applicable Documents

The following table shows the top level applicable documents.

AD	Doc. Number	Issue	Date	Title	Applicability
AD0	-	1	2016-10-20	EDEN ISS-ABDS WP2.3-D2.5-Design Report-v1.0	all
ADD1	-	1	2016-02-08	EDEN ISS-ADS-WP3.5-CDR E-Nose and Decontamination System-v0.2	all

2.2 Reference Documents

The following table shows the reference documents.

RD	Doc. Number	Issue	Date	Title	Applicability
RDD1	-	1	2016-07-28	Gefährdungsbeurteilung ArbSchG und BetrSichV_V1.0	all

3 TEST ARTICLE

The following articles were used:

No.	Article	Part Number	Model	S/N	Number of Items	CIDL/ABCL or Drawing:
1.	Diosol 3 (without Ag-complex)					
2.	Diosol 12 (without Ag-complex)					
3.	*E. coli* DSM 613					
4.	EDEN Decontamination System					
5.	Consumable lab. Material (see TP)					
6.	Lab. Equipment (see TP)					
7.						
8.						
9.						
10.						
11.						

4 TEST DESCRIPTION

First of all, a graphical overview is given, since several steps are performed in scope of the EDEN Diosol Exposure Test.

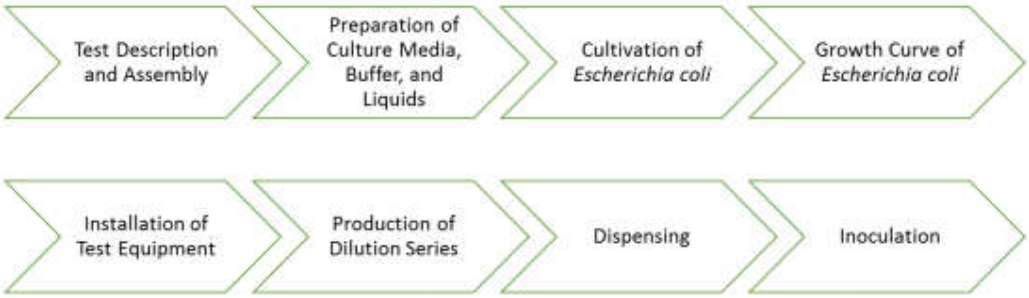

Figure 4-1: Test overview

Figure 4-1 provides an overview over the EDEN Diosol Exposure Test:

Chapter 3.2.1 gives a short description of the test methodology and assembly. Additionally, a schedule is prepared, according to which the following test scenario is performed.

Chapter 3.2.2, describes the preparation of culture media, buffer, and liquids as well as the adjustment of the pH-value. Furthermore, information about each used chemical is given.

Chapters 3.2.3 and 3.2.4 explain the cultivation of *E. coli* and demonstrate the dedicated growth curve. In addition, a short review about considered sterile working conditions is given.

The subsequent chapter 3.2.5 shows the installation of the test equipment and the assembly under the cleanbench. In this chapter, it is important to realize, which samples are placed at which positions. Positioning of the samples is illustrated by X-marks. The positioning and nomenclature of the samples is important, since in further evaluations of the results the references to the dedicated labeling of the samples is created.

Chapter 3.2.6 shows how the dilution series are produced.

Next, chapter 3.2.7 demonstrates how dispensing of the dilution series into the petri dishes is performed.

Finally, chapter 3.2.8 provides information about the settings of the incubation chamber for the petri dishes.

4.1 Test Description and Scheduling

All in all, four test runs are performed in scope of the EDEN Diosol Exposure Test. Two test runs are executed with Diosol 3 and two test runs with Diosol 12. Therefore, the test strain *E. coli* is cultivated and inoculated on glass slides, which are put into sterile petri dishes in order to prevent colonization on the glass slides from the room air.

The test habitat is placed under a cleanbench and five samples are used in each test run. Two test samples are placed under the cleanbench outside the test habitat and three test samples are placed inside the test habitat. After arranging the samples, the decontamination process is initiated by turning on the Diosol generator. The test habitat has to be kept closed for 90 minutes after turning on the Diosol generator. Then, the glass slides

are removed from the cleanbench and the test habitat in order to evaluate the results. Therefore, each glass slide is placed in a vortex mixer and the colonies are washed down. For further evaluation, the parental solutions are prepared from these solutions in order to produce a 6x dilution series. After diluting, a small share is taken out of the dilution series and pipetted into petri dishes. Subsequently, the petri dishes are incubated in the incubation chamber and the colony forming units (CFU) are counted.

Figure 4-2 demonstrates the test assembly and the sample arrangement under the cleanbench and inside the test habitat. Concerning the test habitat, the nozzle, which is connected to the Diosol generator is placed inside as well as three test samples. Two of these three samples are inoculated with *E. coli*. Under the cleanbench, two further samples are placed next to the test habitat, of which one is inoculated with *E. coli*. The inoculated negative control sample outside the decontamination chamber is important for further evaluations since it provides a reference of the colony growth with the impact of the decontamination agent.

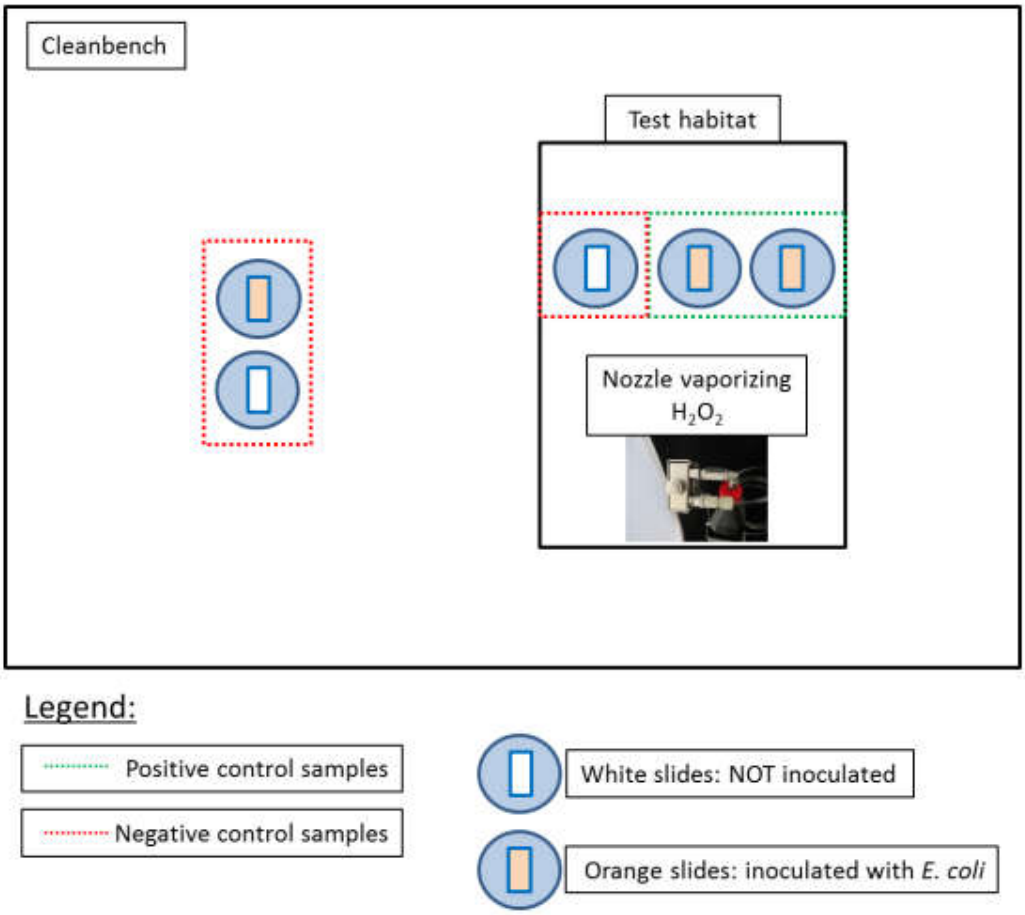

Figure 4-2: Sample arrangement

The test samples, which are inoculated with *E. coli* and placed inside the test habitat serve as positive control samples. All in all, eight positive control samples are used (PC.1 – PC.8), as four test runs are performed, in which two positive control samples are used in each. Positive control samples prove the efficiency of the agent

and system, which are used in scope of the EDEN Diosol Exposure Test. Furthermore, three negative control samples are used in each test run, which leads to a sum of twelve negative control samples (NC.1 – NC.12). Negative control samples exclude extrinsic influencing factors as contamination of surfaces or the room air.

Nomenclature of glass slides, petri dishes, and reaction tubes is a crucial issue in microbiological experiments. Therefore, nomenclature is specified in the tables, which are listed in the appendix allowing a clear assignment of the test samples.

Since several test runs and the respective dilution series have to be produced, a reasonable schedule is necessary and given in the following table.

Table 4-1: EDEN Diosol Exposure Test schedule

Monday, 22.08.2016			
Actionee	Dr. Reidt, Ulrich	Braun, Robert	Gärtner, Alexander
Actions	Safety instruction	Safety instruction	Safety instruction
	Preparation of Pre-culture	Nomenclature	Nomenclature
		Preparation of Pre-culture	Preparation of Pre-culture
Tuesday, 23.08.2016			
Actionee	Dr. Reidt, Ulrich	Braun, Robert	Gärtner, Alexander
Actions	Inoculation of slides #1,2	Inoculation of slides #1,2	Inoculation of slides #1,2
		Decontamination #1,2	Decontamination #1,2
		Sample Evaluation: Dilution #1,2	Sample Evaluation: Dilution #1,2
Wednesday, 24.08.2016			
Actionee	Dr. Reidt, Ulrich	Braun, Robert	Gärtner, Alexander
Actions	Sample Evaluation: Counting #1,2	Inoculation of slides #3,4	Inoculation of slides #3,4
		Decontamination #3,4	Decontamination #3,4
		Sample Evaluation: Dilution #3,4	Sample Evaluation: Dilution #3,4
		Sample Evaluation: Counting #1,2	Sample Evaluation: Counting #1,2
Thursday, 25.08.2016			
Actionee	Dr. Reidt, Ulrich	Braun, Robert	Gärtner, Alexander
Actions		Sample Evaluation: Counting #3,4	Sample Evaluation: Counting #3,4

Monday, 22.08.2016:

First of all, a safety instruction is given by the laboratory supervisor Dr. U. Reidt. Next, Schott bottles, glass slides, petri dishes, falcon tubes, and reaction tubes are labeled according to the tables listed in Appendix B. Then culture media, buffer, liquids and the pre-culture of the test strain *E. coli* are prepared.

Tuesday, 23.08.2016:

On Tuesday, the glass slides for the decontamination test runs #1 and #2 are inoculated and the respective test runs are executed. After finishing the decontamination procedures, the dilution series of batches #1 and #2 is produced and the petri dishes are placed into an incubation chamber at consistent conditions. Besides, a growth curve of *E. coli* is measured photometrically.

Wednesday, 24.08.2016:

On Wednesday, the glass slides for the decontamination test runs #3 and #4 are inoculated and the respective test runs are executed. Then, the dilution series of batches #3 and #4 is produced. Furthermore, petri dishes from dilution series #1 and #2 are removed from the incubation chamber and the samples are evaluated by counting the CFU.

Thursday, 25.08.2016:

On Thursday, the counting of batches #3 and #4 is performed and the results are discussed among the participants.

4.2 Preparation of Culture Media, Buffer, and Liquids

First of all, Lysogeny Broth – Agar (LBA) nutrient media is produced, which is considered to be a favorable growth medium for the test strain *E. coli*. Therefore, 13 Schott bottles (size: 500mL) are prepared and 10.5g LBA (Lennox) is weight and filled into each Schott bottle.

Next, the Schott bottles are filled with 300mL deionized water, labeled, stirred, and autoclaved at a temperature of at least 121°C and 2,2bar for 20min. After autoclaving, the LBA medium is placed on a magnetic stirrer to guarantee its liquidity. After it is cooled down, the liquid LBA is cast into the petri dishes.

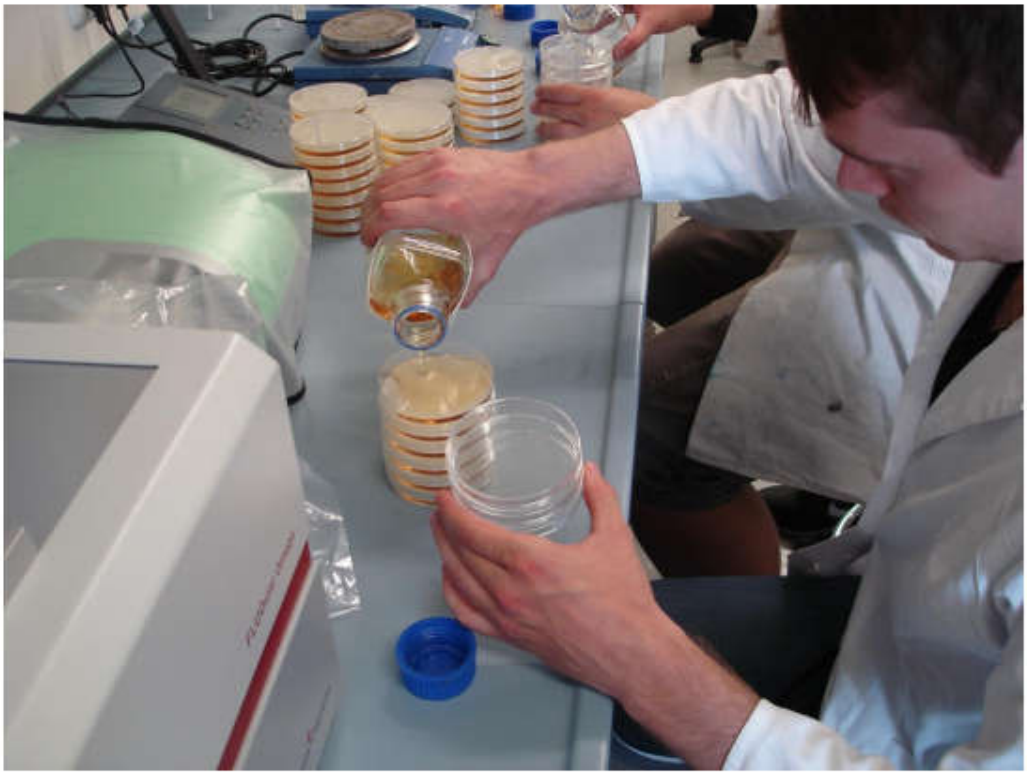

Figure 4-3: Casting of petri dishes

In order to reach 121°C, the autoclave needs approximately one hour to heat up and cool down, which results in an absolute time of 180 minutes per autoclaving cycle.

After the preparation of LBA, Lysogeny Broth Broth (LBB) is prepared. Therefore, 6g LB – Broth (Lennox) is given into each Schott bottle (size: 500mL) and filled with 300mL deionized water. All in all, two Schott bottles with LBB are prepared, stirred, and autoclaved at the same settings as specified above.

Next, phosphate buffered saline (PBS) is prepared, which is used to wash inoculated *E. coli* off the glass slides. The PBS is made out of four chemicals: Sodium chloride (NaCl), dibasic anhydrous sodium phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), and potassium chloride (KCl). As 500mL PBS are produced, the amount of each chemical is weight accurately according to the receipt introduced by the supervisor U. Reidt, which is similar to the receipt predefined by DLR:

The molar mass (M) of NaCl is 58.44g/mol. According to the predefined receipt, 140mmol are needed for the PBS. From this information, the amount (A_x) of the chemicals listed in the section above, is calculated for the PBS as follows:

$$A_{\mathrm{NaCl}} = \frac{140mmol * 58.44g}{1000mmol} = 8.18g \quad (4.1)$$

As a result of equation (4.1), 8.18g NaCl are needed for the production of 1L PBS. Since only 500mL are produced, 4.09g are weight and given into a Schott bottle (size: 500mL).

The molar mass (M) of Na_2HPO_4 is 141.96g/mol. According to the receipt, 10mmol are needed for the PBS. Therefore, the amount of Na_2HPO_4 is calculated as follows:

$$A_{\mathrm{Na2HPO4}} = \frac{10mmol * 141.96g}{1000mmol} = 1.42g \quad (4.2)$$

Equally, the calculated mass of 1.42g in equation (4.2) accounts for 1L PBS. Since only 500mL PBS are produced, 0.71g are weight and put into the Schott bottle.

The molar mass (M) of KH_2PO_4 is 136.09g/mol. According to the given receipt, 1.8mmol are needed for the PBS. The necessary mass is calculated in the following equation:

$$A_{\mathrm{KH2PO4}} = \frac{1.8mmol * 136.09g}{1000mmol} = 0.24g \quad (4.3)$$

Again, only half the value of equation (4.3) is necessary. Therefore, 0.12g are weight and put into the Schott bottle.

The molar mass (M) of KCl is 74.56mol/g. According to receipt, 2.7mmol are need to produce 1L PBS. The required mass is calculated in the following equation (4.4):

$$A_{KCl} = \frac{2.7mmol * 74.56g}{1000mmol} = 0.20g \quad (4.4)$$

0.20g KCl are needed to produce 1L PBS. Again, only half of the value is weight and put into the Schott bottle since only 500mL PBS are produced.

After weighting all chemicals with a maximum permissible deviation of ±0.01g with a fine scale, 500mL deionized water is given into the Schott bottle.

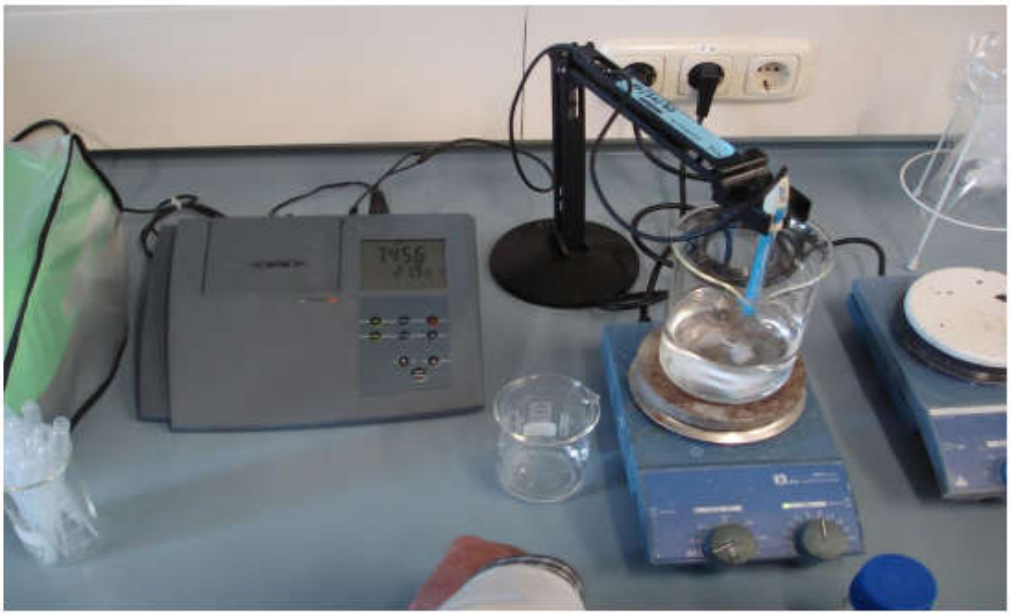

Figure 4-4: pH-value adjustment

As demonstrated in figure 4-4, the Schott bottle is placed on a magnetic stirrer and pH-value is adjusted to 7.4 (±0.2). The first pH-measurement indicates a pH-value of 9.2. Therefore, the pH-value is adjusted with 37%-hydrochloric acid (HCl) in order to reduce the pH-value. By using 37%-HCl, the pH-value is reduced to 7.36, which complies with the determined thresholds.

4.3 Cultivation of *Escherichia coli*

Generally, before transferring bacteria, liquids, and chemicals, several precautions are taken, which ensure a sterile working process in the laboratory:

1. The hands are washed with antiseptic soap and warm water before leaving or entering the laboratory.
2. Likewise, the hands are washed before initiating any sterilization procedures or working with chemicals and bacteria.

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3. 99%-Isopropyl alcohol (IPA) is used to sterilize surfaces and benches. Therefore, IPA is applied generously on the surfaces and dispensed with a disposable wipe.
4. Additionally, a Bunsen burner is always ignited while working with chemicals, liquids or bacteria. The flame is adjusted until a blue cone is visible in the midst of the flame. This provides a sterile area around the flame, as it produces an updraft, which prevents volatile compounds and microorganisms from entering the area around the Bunsen burner. Nevertheless, precipitant movements have to be avoided to preserve the sterile area around the Bunsen burner.

After the preparation of culture media, buffer, and liquids, the cultivation of *E. coli* is performed. Therefore, 50mL LBB are decanted from the Schott bottles to an Erlenmeyer flask (size: 100mL). The flasks are breamed carefully before decanting. Next, *E. coli* is scraped off a petri dish prepared with LBA nutrient medium, which was inoculated and incubated on the previous days by the laboratory supervisor.

To scrape *E. coli* off the petri dish, an inoculation loop is used. The inoculation loop is breamed to ensure sterility that only *E. coli* is scraped off the petri dish. The colonies, which are detached from the petri dish and stuck in the inoculation loop are given into an Erlenmeyer flask (size: 100mL) filled with 50mL liquid LBB medium.

Figure 4-5: Pure LBB Medium and LBB with *E. coli* after incubation

On the left, figure 4-5 shows pure LBB medium before adding *E. coli* and on the right, the figure shows LBB medium after adding *E. coli* and incubating. The Erlenmeyer flasks are fixed into an incubation shaker and incubated at 37°C and 200 rotations per minute (rpm) for a period of approximately 12h. Comparing the two solutions, a significant turbidity is recognized, which indicates the growth of *E. coli*.

The following section describes the methodology, how the dilution of the turbid medium is performed:

First, the optical density (OD) of the turbid medium is adjusted to $OD_{(\lambda=600)}$ = 1.4 (±0.1) as specified in the test procedure. Therefore, in order to determine the zero-point, 1mL pure LBB medium is pipetted into a cuvette and the optical density of the reference medium (pure LBB) is measured at a wavelength of λ=600nm:

$$OD_{zero} = 0.028 \quad (4.5)$$

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Next, the optical density of the turbid medium is measured photometrically. Again, 1mL of the medium is pipetted into a cuvette and the optical density is measured with the following result:

$$OD_{\text{culture}} = 2.001 \quad (4.6)$$

Before diluting the culture in the Erlenmeyer flask, the necessary amount of autoclaved deionized water, which is needed for an appropriate dilution is identified by diluting the culture in the cuvettes.

By diluting 700µL culture with 900µL autoclaved deionized water, an optical density of 1.452 is achieved. Consequentially, the dilution factor (df_1) for the culture (3.6) is calculated as demonstrated in equation (4.7):

$$df_1 = \frac{900\mu L}{700\mu L} = 1.29 \quad (4.7)$$

As a result of equation (4.7), (4.8) proves that 50mL culture have to be diluted (D_A) with 64.5mL autoclaved deionized water to achieve an optical density of 1.452:

$$D_A = 50mL * 1.29 = 64.5mL \quad (4.8)$$

After diluting the culture in the Erlenmeyer flask, a final photometric measurement is performed:

$$OD_{\text{Diluted culture}} = 1.442 \quad (4.9)$$

Finally, the primary culture (OD=2.001) is diluted to an optical density (OD=1.442). Subsequently, the solution with an optical density of 1.442 is further used in the test runs #1 and #2.

Since test runs #3 and #4 are executed on the following day, a new culture is set up and diluted to the specified optical density of 1.4. The photometric measurements are performed as described previously.

Again, the pure LBB medium provides the zero-point as reference for the following measurements:

$$OD_{\text{zero 2}} = 0.028 \quad (4.10)$$

1mL of the culture is pipetted into a cuvette and placed in the photometer with the following result:

$$OD_{\text{culture 2}} = 1.804 \quad (4.11)$$

Subsequently, the culture is diluted to a specified value of 1.4. Therefore, 700µL culture are diluted with 400µL autoclaved deionized water in a cuvette. Thereby, an optical density of 1.449 is achieved. The dilution factor is calculated as follows:

$$df_2 = \frac{400\mu L}{700\mu L} = 0.57 \quad (4.12)$$

From this dilution factor in the small cuvette, the dilution of the culture in the Erlenmeyer flask is performed:

$$D_{A2} = 50mL * 0.57 = 28.5mL \quad (4.13)$$

Equation (4.13) provides information about the amount of the LBB medium that is added to 50mL culture in order to reach an optical density of 1.449.

A final photometric measurement is performed after diluting the culture with 28.5mL LBB medium. This diluted culture is used for the inoculation of the glass slides for test runs #3 and #4:

$$OD_{\text{Diluted culture 2}} = 1.442 \quad (4.14)$$

After diluting the culture to the specified optical density, the solution is pipetted on the previously autoclaved glass slides, as shown in 4-6. The glass slides are 76mm long, 26mm wide, and 1mm thick (Carl Roth: Microscope slides, ground edges 90°). After autoclaving, the glass slides are placed with breamed tweezers into a sterile petri dish.

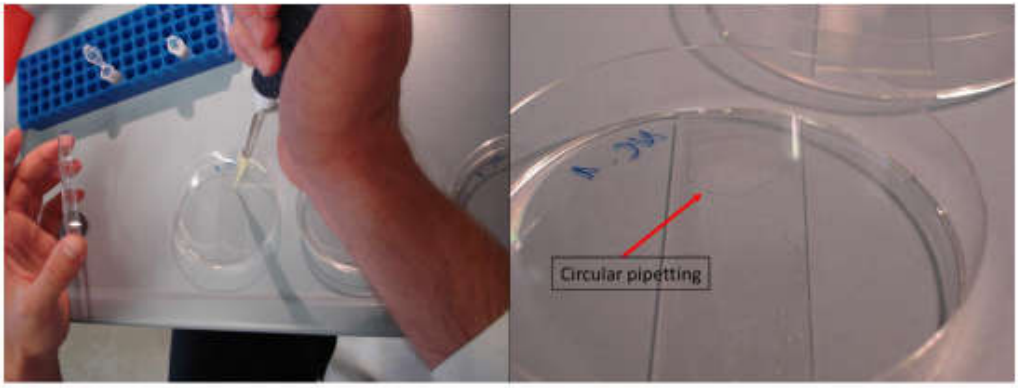

Figure 4-6: Circular pipetting onto the glass slides

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Then, 50µL of the diluted culture have been pipetted on the glass slides, which were previously put into the petri dishes. The culture is inoculated circularly on one side of the glass slide, at which the labeling is visible. It is necessary to ensure that the pipetted culture does not drain off the glass slide. After pipetting, the glass slides are dried before initiating the decontamination procedure.

4.4 Growth curve of *Escherichia coli*

Besides, a growth curve of E. coli is created. Therefore, 1mL undiluted culture (OD = 2.001) is mixed into an Erlenmeyer flask (size: 300mL) filled with 200mL pure LBB. As already mentioned in equation (4.5), pure LBB is determined as zero-point with an optical density of 0.028 and serves as reference for the following measurements. The Erlenmeyer flask is placed into an incubation shaker incubating at 37°C and shaking with approximately 200rpm. From this point, the optical density of the culture is measured every 30min at a wavelength of λ=600nm by pipetting 1mL of the diluted culture into a cuvette and executing the photometric measurement. Table 4.2 lists the photometrically measured values at a wavelength of λ=600nm. In the left column, the time is given in minutes and in the right column, the measured values of the optical density are listed.

Table 1.2: Growth curve - values

t [min]	OD $_{[\lambda=600nm]}$
0	0.028
30	0.025
60	0.049
90	0.078
120	0.169
150	0.374
180	0.565
210	0.838
240	0.936
270	1.085
300	1.193
330	1.196
360	1.301
390	1.347
420	1.407
450	1.416
480	1.558

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A growth curve of *E. coli* is classified into four phases:

1. Lag phase
2. Log phase
3. Stationary phase
4. Death phase

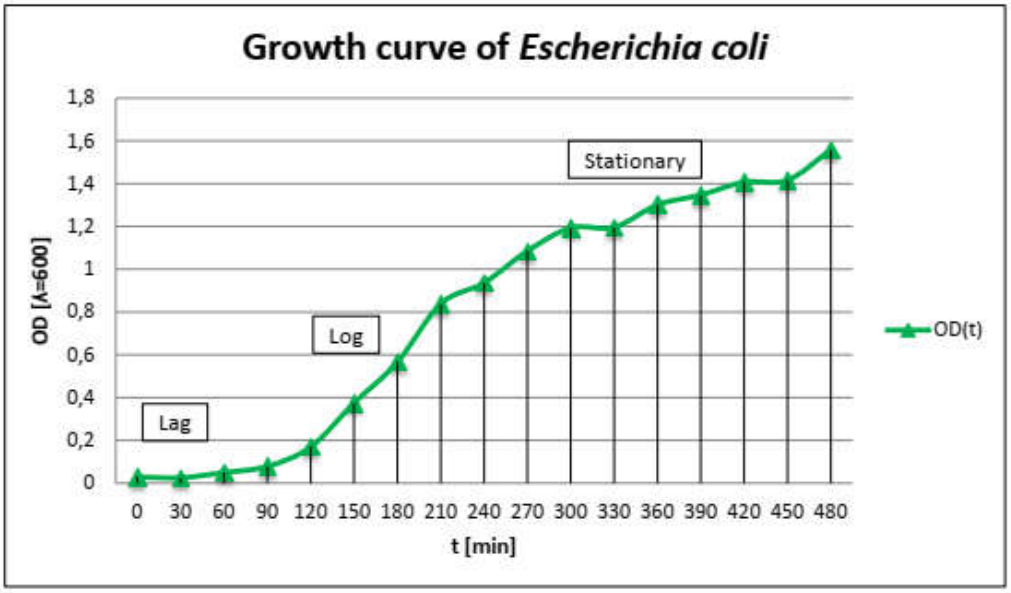

Figure 4-7: Growth curve of E. coli

Figure 4-7 illustrates the growth curve of *E. coli* measured over eight hours at an optical density of λ=600nm. The growth curve demonstrates the phases Lag, Log, and Stationary. The growth curve enters the final Death phase after a delay of several days, which cannot be measured in scope of the EDEN Diosol Exposure Test.

First, during the lag phase, the figure shows that within 60min after pipetting bacteria into the LBB medium, almost no bacterial growth is realized. Actually, after 30min the optical density is even lower than the reference value, which indicates that some E. coli did not survive the transfer from the culture to the LBB medium. Furthermore, the inhibition of bacterial growth arises from several influencing factors. As the bacteria are removed from their familiar environment, they first have to adapt to the new growth conditions and environment. Additionally, the Lag phase is considered to be a period of time, in which bacteria mature and are thereby not able to perform cell division. In 1991, Buchanan and Klawitter described the Lag phase as follows: "The Lag phase is an adjustment period, during which cells modify themselves in order to take advantage of the new environment and initiate exponential growth".

Second, after adapting to the new growth conditions and the new environment, *E. coli* enters a phase of logarithmic growth – the Log phase. Referring to figure 4-7, the Log phase initiates after a delay at approximately 60min. The Log phase is characterized by rapid colony growth due to cell doubling of the present colony. During the Log phase, cell doubling progresses continuously if not disturbed by limiting factors (limiting factors are explained in the following section). In figure 4-7, the Log phase starts after approximately 30min and ends after roughly 210min.

Third, the growth curve enters the Stationary phase, which is induced by influencing or limiting factors. Cell growth is disrupted by changing growth conditions and environments. As *E. coli* doubles in a closed system (Erlenmeyer flask), at some point the colony reaches a limit due to a lack of nutrients. Furthermore, *E. coli* maintains metabolic processes, through which the formation of acids that serve as inhibitory factors for the colony is promoted. Eventually, the Stationary phase leads to a horizontal line in the diagram, which results from an equilibrium state of cell growth and cell death. Referring to figure 4-7, cell growth is reduced continuously from 210min to the final point.

Fourth, after the Stationary phase is over, the growth curve enters a final period of time – the Death phase. The Death phase is not illustrated in figure 4-7. Therefore, observations for a longer period of time are necessary. However, there are several reasons for a decline of the colony. In most of the cases, metabolic processes of *E. coli* lead to a total absence of nutrients, which causes death just as in any other species. Likewise, *E. coli* undergoes an aging process, which causes death after a certain period of time.

4.5 Installation of the test equipment

After the preparation and the methods described in the previous chapters, the installation of the test equipment is installed subsequently.

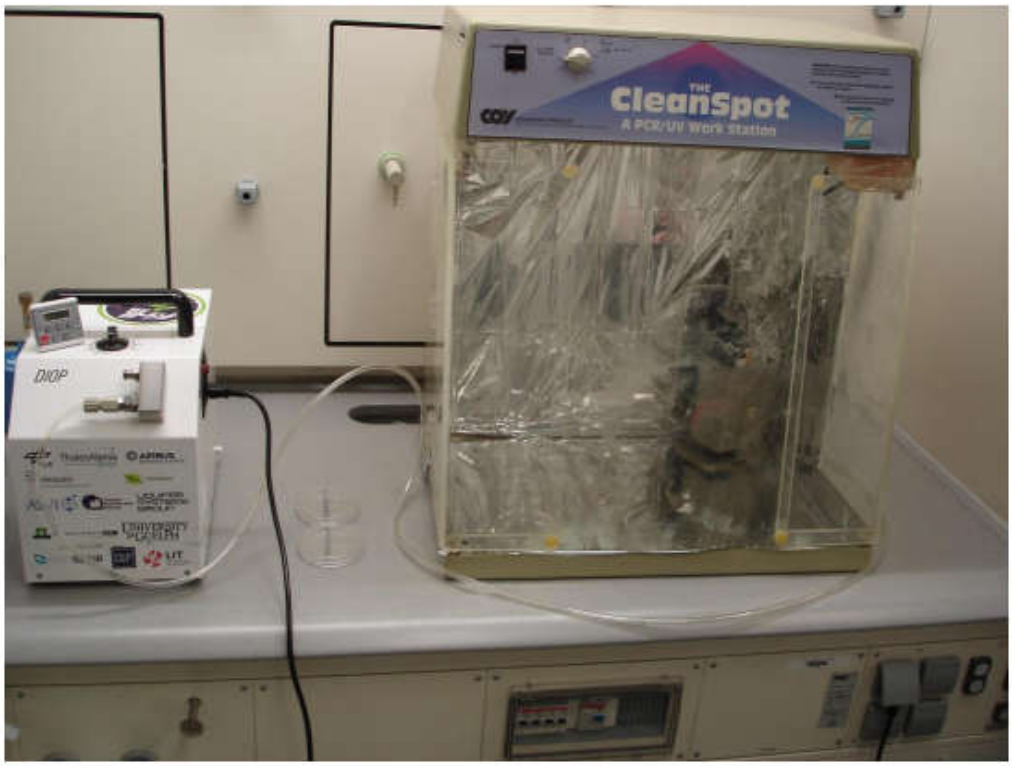

Figure 4-8: Test assembly under the cleanbench

Figure 4-8 and 4-9 demonstrate the actual test assembly as described in the previous chapter 2.2.1 (Test Description and Assembly). As mentioned, the EDEN Diosol Exposure Test is, due to safety reasons, executed under a cleanbench. In scope of this test, a hazard assessment is performed preventing the occurrence of possible hazards and the actions that have to be taken in case of an emergency. The decontamination chamber is placed on the right and the Diosol generator is placed on the left under the cleanbench.

Figure 4-9: X-marking of sample positions

Between, a gap is left, which allows the arrangement of the negative control samples. Furthermore, in order to guarantee equal sample arrangement in every test run, the positions of each petri dish inside and outside the decontamination chamber are marked with an X as highlighted in figure 4-9.

The hose pipe from the Diosol generator is conducted via a small inlet into the decontamination chamber and connected to the nozzle, which is mounted in the flask holder. The flask holder ensures stable positioning of the bottle, in which the decontamination agent is contained. On top of the flask holder, a mechanical attachment is fixed, which mounts the nozzle. This mechanical attachment also allows the alignment of the nozzle.

Figure 4-9 on the following presents the positioning of the flask holder and the alignment of the nozzle. The nozzle is aligned to the top center of the decontamination chamber. Thereby, a fast and equal distribution of the decontamination agent is assured. Likewise, the figure shows the jet of the vaporized decontamination agent during the first test run.

Figure 4-10: Alignment of the nozzle

4.6 Dilution series

After initiating the test runs, the decontamination chamber is closed for the following 90min. As next step, a dilution series of each glass slide is produced in order to count the results.

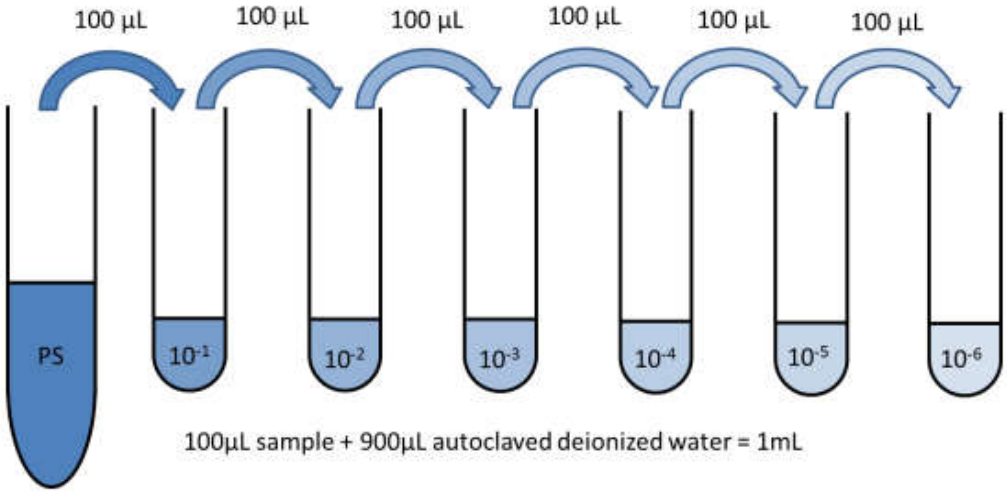

Figure 4-11: Schematic diagram of a dilution series

Figure 4-11 illustrates a schematic diagram, how a dilution series of a maximum dilution of 1:1.000.000 (10^{-6}) is produced. Therefore, reaction tubes (size: 1.5mL) are filled with 900µL autoclaved deionized water and 100µL of the sample. The first dilution is filled with 100µL of the parental solution (PS). Continuing the dilution series, 100µL of the first reaction tube (10^{-1}) are pipetted into the second reaction tube (10^{-2}) and thereof 100µL into the third reaction tube (10^{-3}). This reaction chain is proceeded until the maximum dilution is reached.

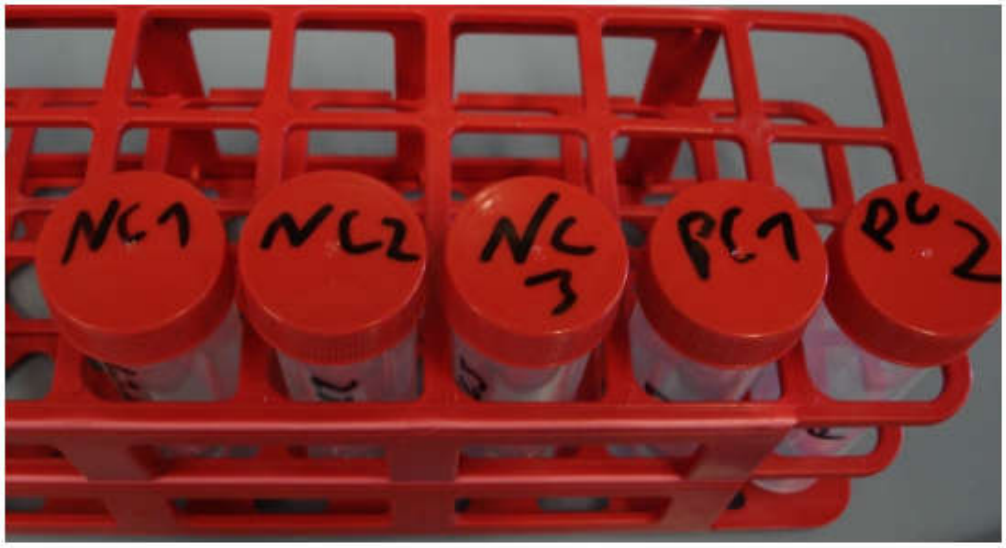

Figure 4-12: Falcon tubes filled with PBS and glass slides

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However, several precautions have to be considered so that no external contamination occurs and the dilution series provides the intended results. Therefore, pipette tips always have to be changed after every pipetting operation. Another important issue is an accurate use of the vortex mixer. Since bacteria are heavier than deionized water, they deposit on the bottom of the reaction tube after a certain time, which leads to a heterogeneous solution. In order to preserve the solution homogeneously, conscientious vortexing before every pipetting operation is a crucial issue.

Concerning the EDEN Diosol Exposure Test, a dilution series of 1:1.000.000 (10^{-6}) is produced. In order to set up a dilution series, the bacteria have to be washed down the glass slide. Therefore, tweezers are breamed and the glass slide is carefully removed from the petri dish and placed into a falcon tube (size: 50mL).

The falcon tubes are previously filled with 15mL PBS. After placing each glass slide into the dedicated falcon tube, the flacon tubes are vortexed thoroughly to wash down the bacteria:

Every tube is vortexed two times for one minute with an intermediate period of five minutes. Besides, the reaction tubes are prepared and filled with 900µL autoclaved deionized water to set up the dilution series. Before removing any liquid from a tube, the tube is always vortexed to guarantee pipetting and transferring a homogenous solution.

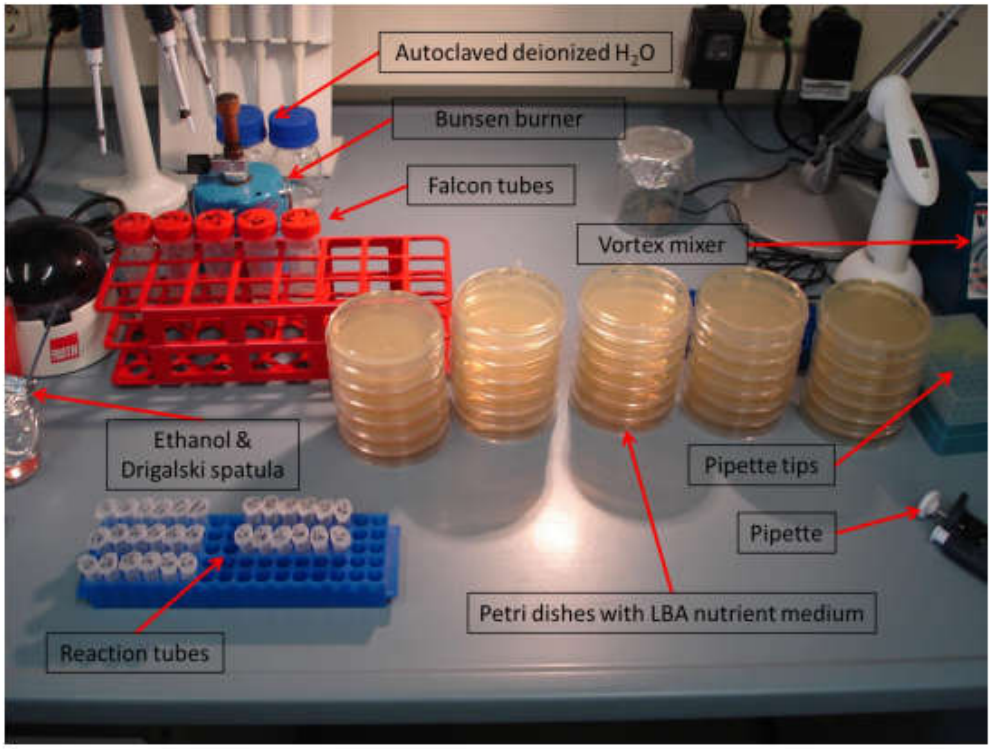

Figure 4-13: Preparation of dilution series

Figure 4-13 illustrates the workplace, at which the pipetting of the dilution series and the dispensing into the prepared petri dishes is performed. The petri dishes, which are casted with LBA nutrient medium are used for the dispensing the content of the falcon and reaction tubes.

As shown in the schematic diagram, 100µL of the PS, which is contained in the flacon tubes is transferred to the first dedicated reaction tube. Thereof, the reaction chain is continued by transferring 100µL to the next reaction tube in order to perform appropriate dilutions from 10^{-1} to 10^{-6}.

4.7 Dispensing

As a dilution series of each glass slide is performed, the content of each falcon and reaction tube is pipetted into the petri dishes, which were casted with LBA nutrient medium at the beginning of the EDEN Diosol Exposure Test.

Besides the dilution series, the content of the falcon tubes, the water, which is deployed for the production of the dilution series and the undiluted culture are dispensed into petri dishes.

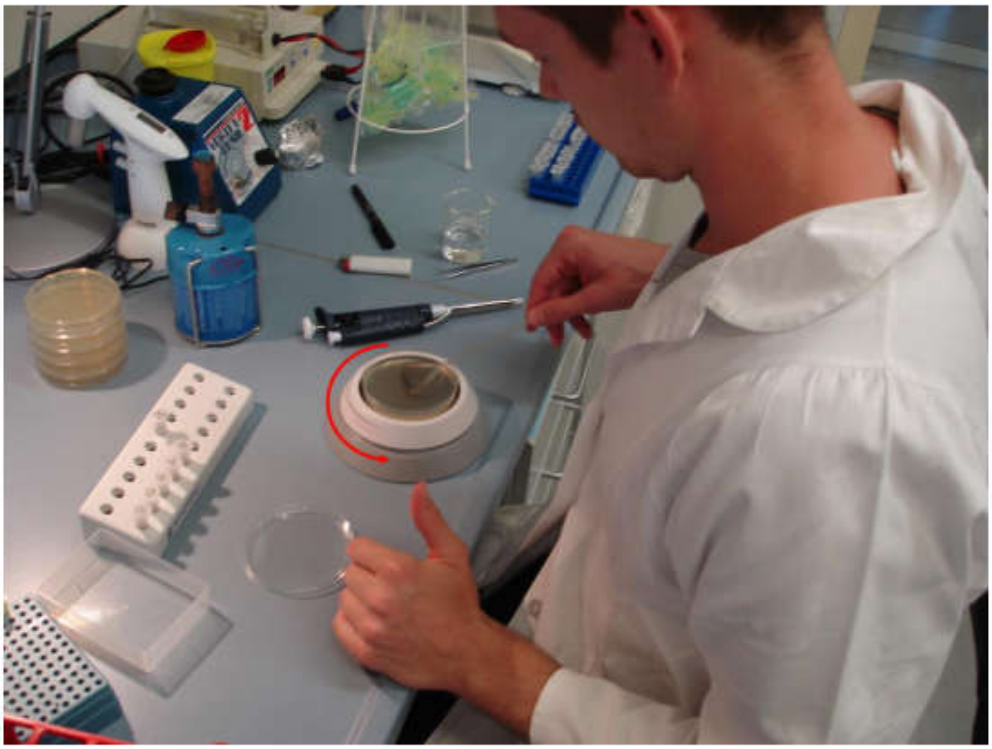

Figure 4-14: Dispensing

This results in an overall amount of 172 petri dishes, which are dispensed in scope of the EDEN Diosol Exposure Test:

- Dilution series: 120 petri dishes
- Falcon tubes: 20 petri dishes

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- Water flasks: 4 petri dishes
- Cultures: 28 petri dishes

Figure 4-14 demonstrates the dispensing workplace. Petri dishes and reaction tubes are placed ahead, pipette tips to the left, ethanol, the Bunsen burner, and vortex mixer to the right.

Dispensing is performed with the help of a turntable for petri dishes. After vortexing, 100µL of the bacterial suspension are removed with a pipette from the reaction tubes and given into the dedicated petri dishes. These are placed on the turntable for petri dishes. The bacterial suspension is dispensed with a Drigalski spatula. The Drigalski spatula is dipped into ethanol and breamed before and after every dispensing procedure. The bacterial suspension, which is pipetted onto the LBA nutrient medium into the petri dish is evenly distributed with the Drigalski spatula.

4.8 Incubation

After plating the falcon and reaction tubes, the petri dishes are incubated over night for a period of time of approximately 18h.

Figure 4-15: Incubating of petri dishes

Figure 4-15 shows the incubation chamber on the left and the petri dishes, which are located inside the incubation chamber on the right. The petri dishes are incubated upside down at 37°C (±1°C).

Placing petri dishes upside down into an incubator is a common method to incubate petri dishes, which are suspended with bacteria. Due to the heat, condensate begins to form on the lid of the petri dish. By incubating upside down, the dripping down of condensate into the culture is avoided, since the condensate potentially leads to a change of bacterial growth.

5 TEST SUMMARY AND CONCLUSION

The following section provides the results of the EDEN Diosol Exposure Test. Therefore, the results of the four test runs are plotted in the following figures 5-1, 5-3, 5-5, and 5-8.

Only the results of the samples, which were previously inoculated are respected in these figures. The samples, which are left empty before initiating the test procedure serve as control samples in order to prove sterility of the working procedure. For reasons of transparency, these curves are not plotted since they provide a zero-line in the diagram. The x-axis demonstrates the dilution of the dedicated control sample and the y-axis provides the CFU count. All the results are plotted on a logarithmic scale.

5.1 Results - Test Run 1

Table 5-1: Results – Test Run 1

Results: Test Run 1						
		PC.1	PC.2	NC.1	NC.2	NC.3
Dilution	E00	32	46	492	0	0
	E-01	2	0	348	0	0
	E-02	0	0	1	1	2
	E-03	0	0	9	0	0
	E-04	0	0	1	0	0
	E-05	0	0	0	0	0
	E-06	0	0	0	0	0

Figure 5-1 illustrates the results for the first test run, which is executed with the test agent Diosol 3.

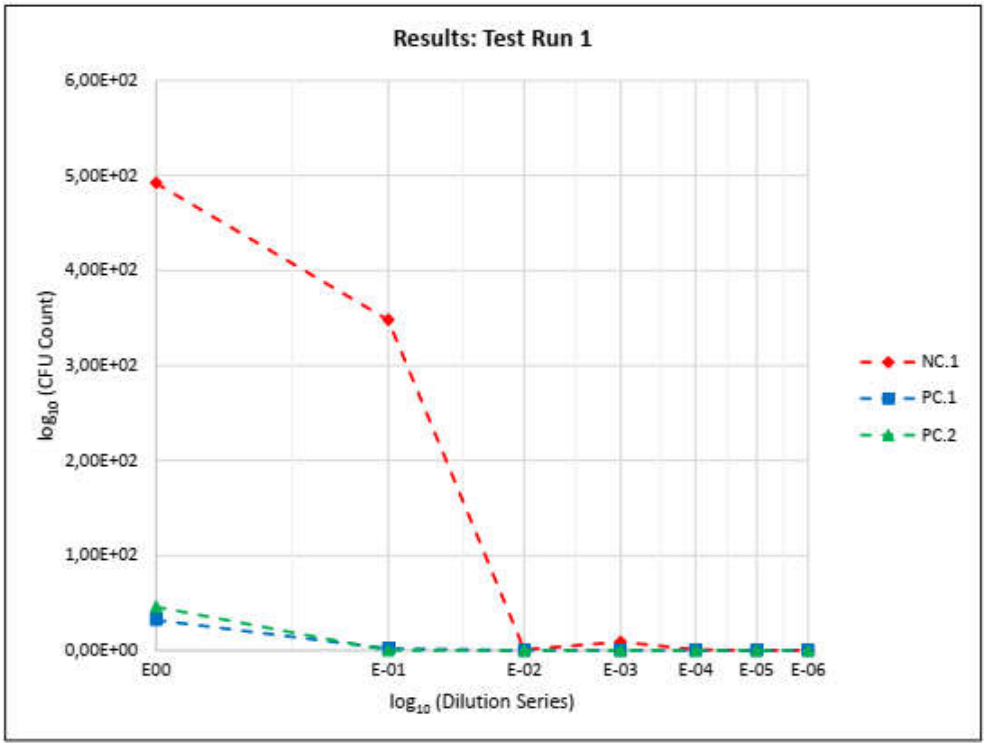

Figure 5-1: Results Test Run 1

The control sample placed next to the decontamination chamber (NC.1) is charted red, the positive control samples (PC.1 and PC.2), which are situated inside the decontamination chamber are charted blue and green.

Concerning the negative control sample (NC.1), the initial CFU count is 492. Proceeding the dilution series, the E-01 dilution provides a CFU count of 348, which is however expected to be approximately 49. Continuing, the CFU count does not exceed a threshold of nine, which is reached at the E-03 dilution.

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Concerning the positive control samples (PC.1 and PC.2), a remarkable reduction of the CFU count occurred. The undiluted bacterial solutions of the falcon tubes provide a CFU count of 32 and 46. Again, the E-01 dilution already results in a CFU count of zero. Proceeding the dilution series, the CFU count, which is observed in the graph above almost remains zero.

Figure 5-2: Dilution series – Bacterial solution (U2)

Figure 5-2 demonstrates the dilution series of the bacterial solution (OD_{600} = 1.442). Petri dishes U2 (E00) - U2.3 (E-03) are not countable due to the amount and expansion of the colonies into each other. Petri dishes U2.4 (E-04) and U2.5 (E-05) are uncountable, because of inaccurate dispensing technique. However, petri dish U2.6 (E-06) is countable. The counted CFUs on petri dish U2.6 are highlighted with red circles in figure 5-2 above.

A crucial issue is the evaluation of the procedure, in which *E. coli* is inoculated on the glass carriers and dried. The drying procedure is assumed to be torture to *E. coli*, by which the bulk of the colonies die. The number of bacteria, which dies during this procedure is calculated in the following section:

Primarily, 100µL of the untreated bacterial solution are dispensed in the petri dish U2 and diluted to E-06 (U2.6). Three colonies are counted in petri dish U2.6, which is the E-06 dilution. Therefore, the counted colonies of U2.6 are multiplied with 10^6 to receive the value of the undiluted solution (E00), which is inoculated on the slides:

$$CFU_{U2} = 3 * 10^6\ CFU \quad (5.1)$$

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Since only 50µL are inoculated on the glass slides, this value is divided by two to obtain the initial cell count:

$$CFU_{Glass\ slide} = 1.5 * 10^6\ CFU \quad (5.2)$$

Next, the decontamination procedure is executed and afterwards, the remaining colonies are washed off the glass slide by adding 15mL PBS to the falcon tube and vortexing. Therefore, 1µL of the solution in the falcon tube contains:

$$CFU_{Falcon\ tube} = \frac{1.5 * 10^6\ CFU}{15.000\mu L} = 100\ CFU/\mu L \quad (5.3)$$

Furthermore, 100µL of this initial amount are dispensed into the petri dishes. Therefore, the amount undried amount of CFU is:

$$CFU_{undried} = 100\frac{CFU}{\mu L} * 100\mu L = 10.000\ CFU \quad (5.4)$$

The value of (5.5) is extracted from the table 5-1 presenting the results for the negative control sample (NC.1):

$$CFU_{dried} = 492\ CFU \quad (5.5)$$

In order to calculate the amount of CFU, which died by the drying procedure ($CFU_\dagger$), the initial amount of CFU (5.4) and the amount of CFU after drying (5.5) are set in relation:

$$CFU_* = \frac{492\ CFU}{10.000\ CFU} * 100\% = 4.92\% \quad (5.6)$$

$$CFU_\dagger = 100\% - 4.92\% = 95.08\% \quad (5.7)$$

Finally, by setting (5.5) and (56) in relation, the amount of CFU, which survived the drying procedure is determined (5.7). Since only 4.92% survive the drying procedure, a total amount of 95.08% died during this process.

5.2 Results – Test Run 2

Table 5-2: Results – Test Run 2

Results: Test Run 2						
		PC.3	PC.4	NC.4	NC.5	NC.6
Dilution	E00	17	0	189	0	0
	E-01	0	0	1	0	0
	E-02	0	0	0	0	0
	E-03	0	0	0	0	1
	E-04	0	0	0	0	0
	E-05	0	0	0	0	0
	E-06	0	0	0	0	0

Figure 5-3 presents the results of test run two. Diosol 3 is used in scope of the second test run.

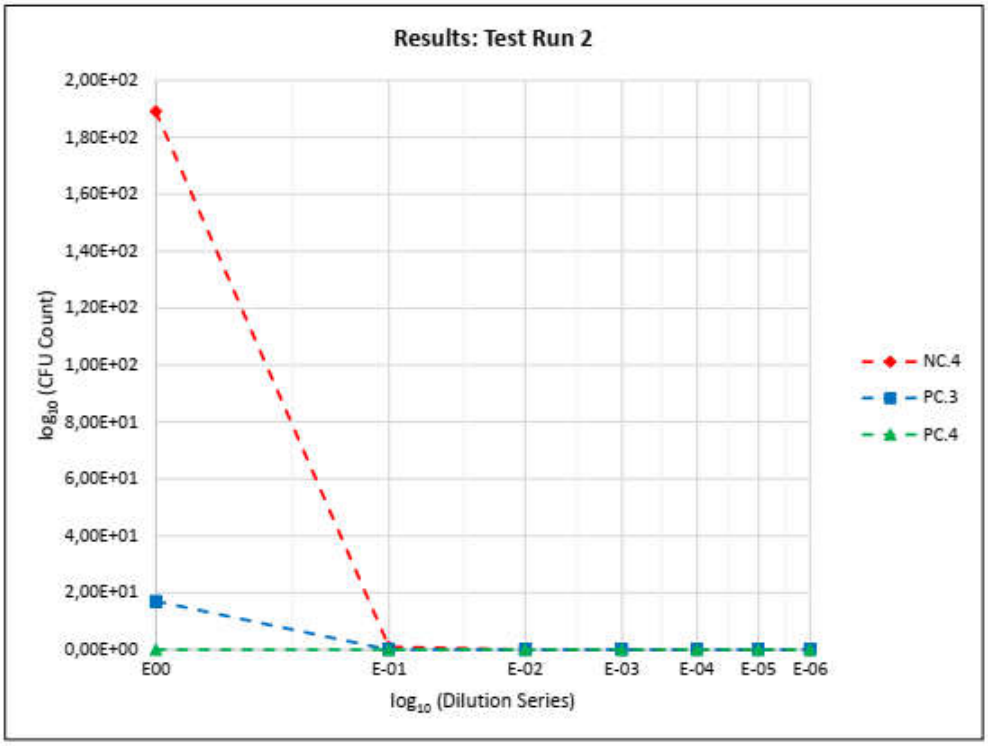

Figure 5-3: Results Test Run 2

The inoculated negative control sample (NC.4) remaining outside the decontamination chamber shows a CFU count of 189 for the undiluted sample E00 and is marked red in the figure above. Already for the E-01 dilution, the CFU count is reduced to one and remains zero for the following dilutions.

Positive control samples, which are situated inside the decontamination chamber are charted blue (PC.3) and green (PC.4) again. Counting the CFU, a remarkable reduction is observed:

PC.3 shows a maximum CFU count of 17 after the treatment with H_2O_2 in the undiluted sample. Continuing the dilution series, the amount of CFU accounts zero for the following dilutions. Concerning positive control PC.4, no CFU forms in the petri dishes at all.

Figure 5-4: Dilution series – Bacterial solution (U3)

Figure 5-4 demonstrates the dilution series of the bacterial solution (U3), which is used for the second test run. Petri dishes E00 - E-02 are not countable because of the CFU growth into each other. The innumerableness of petri dishes E-03 and E-04 is due to inaccurate plating technique. As highlighted with red circles in figure 5-4, ten CFU are counted on the petri dish with the dilution E-05.

Again, the influence of the drying procedure on *E. coli* is investigated. First of all, the CFU count of U3 is determined. As ten CFU are counted on petri dish E-05, the number of the CFU in 100µL undiluted bacterial solution is:

$$CFU_{U3} = 1 * 10^6\ CFU \quad (5.8)$$

Consequently, 50µL, which are initially inoculated in the glass slides contain:

$$CFU_{Glass\ slide} = 5 * 10^5\ CFU \quad (5.9)$$

As next step, the decontamination procedure is initiated and afterwards, the bacteria are washed off the glass slide by adding 15mL PBS and vortexing. The amount of 1µL in the solution is calculated as follows:

$$CFU_{Falcon\ tube} = \frac{5 * 10^5\ CFU}{15.000\mu L} = 33.33\ CFU/\mu L \quad (5.10)$$

Therefore, the calculated undried amount of CFU is:

$$CFU_{undried} = 33.33\frac{CFU}{\mu L} * 100\mu L = 3.333\ CFU \quad (5.11)$$

The value of (5.12) is extracted from the table 5-2 presenting the results of the negative control sample (NC.4):

$$CFU_{dried} = 189\ CFU \quad (5.12)$$

In order to calculate the amount of CFU, which died by the drying procedure ($CFU_\downarrow$), the initial amount of CFU (5.11) and the amount of CFU after drying (5.12) are set in relation:

$$CFU_\downarrow = \frac{189\ CFU}{3.333\ CFU} * 100\% = 5.67\% \quad (5.13)$$

$$CFU_\uparrow = 100\% - 5.67\% = 94.33\% \quad (5.14)$$

Finally, by setting (5.11) and (5.12) in relation, the amount of CFU, which survived the drying procedure is determined (5.13). Since only 5.67% survive the drying procedure, a total amount of 94.33% died during this process.

5.3 Results – Test Run 3

Table 5-3: Results – Test Run 3

Results: Test Run 3						
		PC.5	PC.6	NC.7	NC.8	NC.9
Dilution	E00	0	0	1522	0	0
	E-01	0	0	2	0	0
	E-02	0	0	0	0	0
	E-03	0	0	0	0	0
	E-04	0	0	0	0	0
	E-05	0	1	0	0	0
	E-06	0	0	0	0	0

The following third test run is executed with Diosol 12. Figure 5-5 presents the dedicated results of test run three.

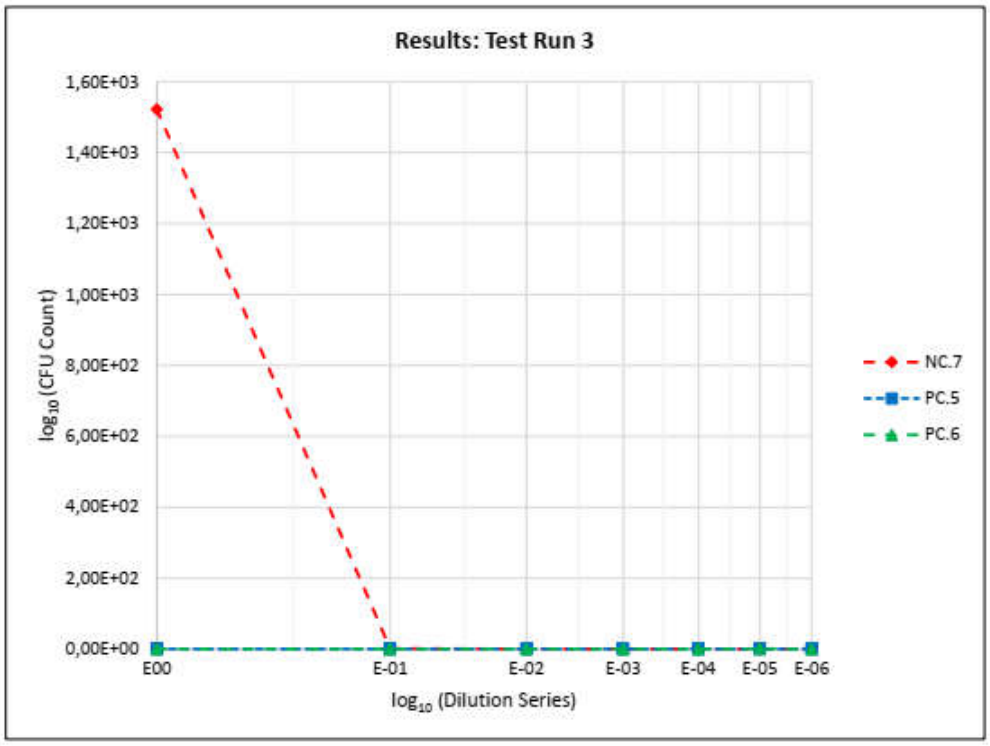

Figure 5-5: Results Test Run 3

The inoculated negative control sample NC.7 remains outside the decontamination chamber, whereas positive controls PC.5 and PC.6 are situated inside the chamber.

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Concerning NC.7, 1522 CFU are counted for the undiluted solution E00. The values for NC.7 are charted red in the following figure. Regarding positive control samples PC.5 and PC.6, no CFU are counted after the decontamination procedure. The values for PC.5 and PC.6 are charted blue and green in the following figure.

Figure 36 highlights the dilution series of the bacterial solution (U4), which is used for the third test run.

Figure 5-6: Dilution series – Bacterial solution (U4)

In the next step, the amount of *E. coli*, which are capable of forming CFU after the drying procedure is determined again. As figure 36 shows, dilutions E00 – E-04 are not countable. However, dilution E-05 is countable and the amount of CFU counted in the petri dish is 186. Furthermore, for the dilution E-06, the counted amount of CFU is 47.

Although dilution E-05 is countable, the following calculations are based on the counted results of dilution E-06, as the previous calculation originate from dilution E-06, too. Since 47 CFU are counted on petri dish E-06, the number of the CFU in 100µL undiluted bacterial solution is:

$$CFU_{U4} = 4.7 * 10^7\ CFU \quad (5.15)$$

Consequently, 50µL, which are initially inoculated in the glass slides contain:

$$CFU_{Glass\ slide} = 2.35 * 10^7\ CFU \quad (5.16)$$

Next, the decontamination procedure is executed and the bacteria are washed off the glass slide with 15mL PBS. Equation (5.17) provides information about the amount of bacteria contained in 1µL of the prepared solution:

$$CFU_{Falcon\ tube} = \frac{2.35 * 10^7\ CFU}{15.000\mu L} = 1566.67\ CFU/\mu L \quad (5.17)$$

Since the CFU count of 1µL is known, the total amount of 100µL dispensed bacterial solution is:

$$CFU_{undried} = 1566.67 \frac{CFU}{\mu L} * 100\mu L = 156.667\ CFU \quad (5.18)$$

The amount of CFU, which survived the drying procedure is extracted from the numerical results of negative control sample NC.7, which is listed in appendix D.3.

$$CFU_{dried} = 1522\ CFU \quad (5.19)$$

The amount of *E. coli*, which survived the drying procedure are set in relation with the CFU count of the undried dilution to calculate the surviving and dead CFU:

$$CFU_{\bullet} = \frac{1522\ CFU}{156.667\ CFU} * 100\% = 0.97\% \quad (5.20)$$

$$CFU_{\dagger} = 100\% - 0.97\% = 99.03\% \quad (5.21)$$

Equations (5.20) and (5.21) demonstrate that only 0.97% of the CFU survived the drying procedure during test run three. However, by calculating the surviving CFU with the value of dilution E-05 (186), approximately 2.5% of the CFU survive the drying procedure.

Furthermore, by deploying Diosol 12, a visible reaction occurred. The colony, which was previously inoculated on the glass slide and in particular the boundaries turned white after the decontamination procedure, which is shown in figure 37.

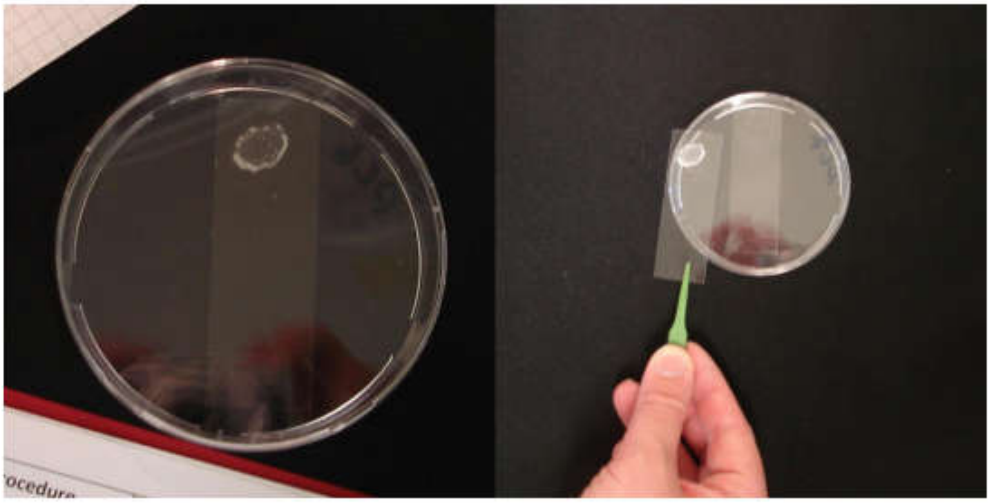

Figure 5-7: Positive Catalase Test

The white coloring arises from the fact that the bacteria exhibit a positive catalase reaction after the exposure to a 12%-solution of H_2O_2. As the term catalase already suggests, this reaction is the result of an enzymatic reaction.

Catalase is a natural enzyme, which is broadly spread among living organisms, which are exposed to oxygen. High levels of H_2O_2 trigger the release of catalase decomposing cytotoxic H_2O_2 into the harmless compounds O_2 and H_2O. As a consequence, instant sputtering and the formation of bubbles occurs while undergoing this reaction. The white residues of this reaction are demonstrated in figure 37. [69]

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5.4 Results – Test Run 4

Table 5-4: Results – Test Run 4

Results: Test Run 4						
		PC.7	PC.8	NC.10	NC.11	NC.12
Dilution	E00	0	0	11	0	0
	E-01	0	0	0	0	0
	E-02	0	0	0	0	0
	E-03	0	0	0	0	0
	E-04	0	0	1	0	0
	E-05	0	0	0	0	0
	E-06	0	0	0	0	0

Figure 5-8 charts the results of the fourth test run. Test run 4 is executed with Diosol 12.

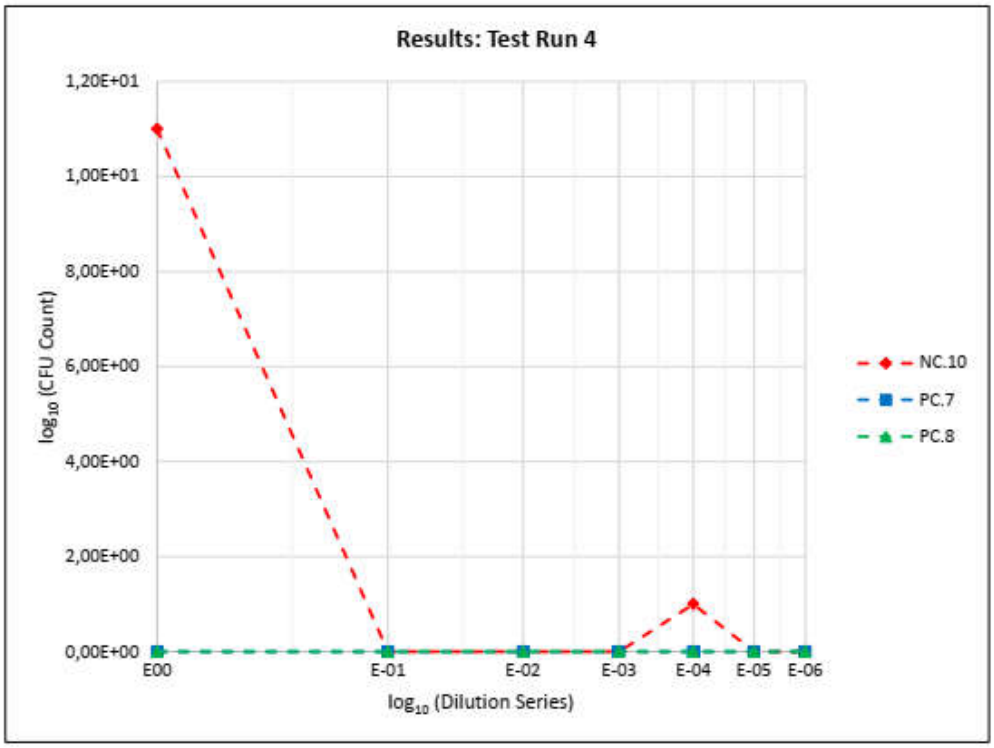

Figure 5-8: Results Test Run 4

The negative control sample NC.10 is placed outside the decontamination chamber and the positive control samples PC.7 and PC.8 are placed inside the decontamination chamber.

Concerning sample NC.10, the counted amount of CFU after finishing the decontamination procedure is eleven. On the contrary, the counted amount of CFU for the samples PC.7 and PC.8 is zero.

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The chart shows a second maximum for the E-04 dilution. This maximum is highlighted in this chart due to the overall low CFU count and is considered to be an airborne germ.

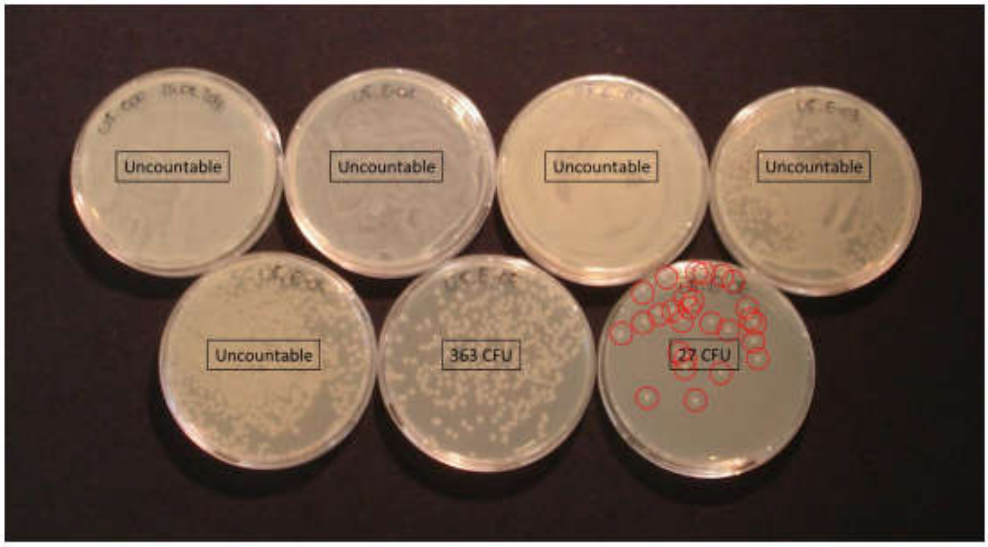

Figure 5-9: Dilution series – Bacterial solution (U5)

Figure 5-9 demonstrates the dilution series of the bacterial solution (U5), which is used for the fourth test run.

While the samples E00 – E-04 are not countable, the CFU on the petri dishes E-05 and E-06 are countable. As a result, for the E-05 dilution, 363 CFU are counted and for the E-06 dilution, 27 CFU are counted.

Again, the amount of CFU, which survived the drying procedure, is calculated with the value of dilution E-06. Therefore, 100µL of the previously dispensed solution contain:

$$CFU_{U5} = 2.7 * 10^7\ CFU \quad (5.22)$$

Consequently, 50µL inoculated on the glass slides contain:

$$CFU_{Glass\ slide} = 1.35 * 10^7\ CFU \quad (5.23)$$

After finishing the decontamination procedure, the remaining CFU are washed off the glass slide with 15mL PBS:

$$CFU_{Falcon\ tube} = \frac{1.35 * 10^7\ CFU}{15.000\mu L} = 900\ CFU/\mu L \quad (5.24)$$

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As 1µL of the diluted solution contains 900 CFU, equation (3.39) provides information about the amount of CFU in 100µL:

$$CFU_{undried} = 900\frac{CFU}{\mu L} * 100\mu L = 90.000\ CFU \quad (5.25)$$

From the numerical results, which are listed in appendix D.4, the amount of CFU on the dried sample (NC.10) is known:

$$CFU_{dried} = 11\ CFU \quad (5.26)$$

As next step, the calculated amount of CFU before and after the drying procedure are set in relation to determine the amount of the CFU, which survived and died:

$$CFU_{\downarrow} = \frac{11\ CFU}{90.000\ CFU} * 100\% = 0.01\% \quad (5.27)$$

$$CFU_{\uparrow} = 100\% - 0.01\% = 99.99\% \quad (5.28)$$

Equations (5.27) and (5.28) demonstrate that only 0.01% of the CFU survived the drying procedure during test run four. Therefore, 99.99% of the CFU died during this drying procedure.

5.5 Results – Summary

The following section provides a summary of the results, which are presented in chapter 5.1 – 5.4. Subsequently, important values are calculated and listed in equations (5.29) – (5.39):

- (5.29) – (5.34): Relative decontamination efficiency (X_{rel}) of each positive control sample
- (5.35) – (5.36): Average reduction rates of Diosol 3 ($E\emptyset_{Diosol\ 3}$) and Diosol 12 ($E\emptyset_{Diosol\ 12}$)
- (5.37) – (5.38): Absolute logarithmic decontamination efficiency of Diosol 3 ($\downarrow Log_{Diosol\ 3}$) and Diosol 12 ($\downarrow Log_{Diosol\ 12}$)
- (5.39): Average amount of CFU dying during the drying procedure ($CFU_{\emptyset r}$)

For the following summary, the results of the undiluted samples (E00) are compared among each other, since they provide countable results for all samples. The surviving CFU ($X_{\bullet}$) are extracted from the tables, which provide the numerical results. Therefore, the relative decontamination efficiency (X_{rel}) of each sample is calculated as follows:

$$PC.1_{\bullet} = \frac{32\ CFU}{492\ CFU} * 100\% = 6.50\% \quad (5.29)$$

$$PC.1_{rel} = 100\% - 6.50\% = 93.50\% \quad (5.30)$$

For the positive control sample PC.1, a relative decontamination efficiency of *E. coli* of 93.50% is achieved as only 6.50% survive the decontamination procedure.

$$PC.2_{\bullet} = \frac{46\ CFU}{492\ CFU} * 100\% = 9.35\% \quad (5.31)$$

$$PC.2_{rel} = 100\% - 6.50\% = 90.65\% \quad (5.32)$$

For the positive control sample PC.2, a relative decontamination efficiency of E. coli of 90.65% is realized.

$$PC.3_{\bullet} = \frac{17\ CFU}{189\ CFU} * 100\% = 8.99\% \quad (5.33)$$

$$PC.3_{rel} = 100\% - 8.99\% = 91.01\% \quad (5.34)$$

For the positive control sample PC.3, a relative decontamination efficiency of 91.01% is accomplished.

Continuing, zero CFU are counted for the positive control samples PC.4 – PC.8, which means a relative decontamination efficiency of 100% is achieved. Processing these values, an average decontamination efficiency for Diosol 3 ($E\emptyset_{Diosol\ 3}$) and Diosol 12 ($E\emptyset_{Diosol\ 12}$) is calculated in equations (5.35) and (5.36):

$$E\emptyset_{Diosol\ 3} = \frac{93.50\% + 90.65\% + 91.01\% + 100.00\%}{4} = 93.79\% \quad (5.35)$$

$$E\emptyset_{Diosol\ 12} = 4 * 100.00\% = 100.00\% \quad (5.36)$$

Evaluating the results of the average decontamination efficiency for Diosol 3 and Diosol 12, a significant overall reduction of *E. coli* is observed.

The absolute logarithmic are calculated in the following section. As an example, the logarithmic reduction of the first test run for PC.1 is calculated as follows:

$$\downarrow Log_{Diosol\ 3} = 4.92\ E02 - 3.2\ E01 = 4.6\ E02 \quad (5.37)$$

By vaporizing Diosol 3, a logarithmic reduction for of at least two grades is achieved, which is proved in equation (3.51).

Subsequently, the absolute logarithmic reduction rates of the third test run for PC.5 is calculated:

$$\downarrow Log_{Diosol\ 12} = 1.522\ E03 - 00\ E00 = 1.522\ E03 \quad (5.38)$$

Regarding Diosol 12, a reduction of not less than three logarithmic grades (E-03 → E00) is realized, which is proved in equation (3.38).

The average amount of *E. coli* dying during the drying procedure ($CFU_{\emptyset\uparrow}$) is determined as follows:

$$CFU_{\emptyset\uparrow} = \frac{95.08\% + 94.33\% + 99.03\% + 99.99\%}{4} = 97.11\% \quad (5.39)$$

The average amount of CFU, which does not survive the drying procedure before initiating the test runs is 97.11%. This is an important fact, which has to be respected in further testing in scope of the EDEN H_2O_2 Decontamination Test.

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6 DISCUSSION

All the graphical results of the previous chapters are incorporated in figure 6-1 to allow a reasonable discussion.

In the top row, the graphs of test run one and two present the decontamination efficiency of Diosol 3. In the bottom row, the graphs three and four present the results of the decontamination efficiency of Diosol 12. All four graphs provide evidence of a significant reduction of *E. coli* after the treatment with Diosol (comparing the red with the blue and green curves).

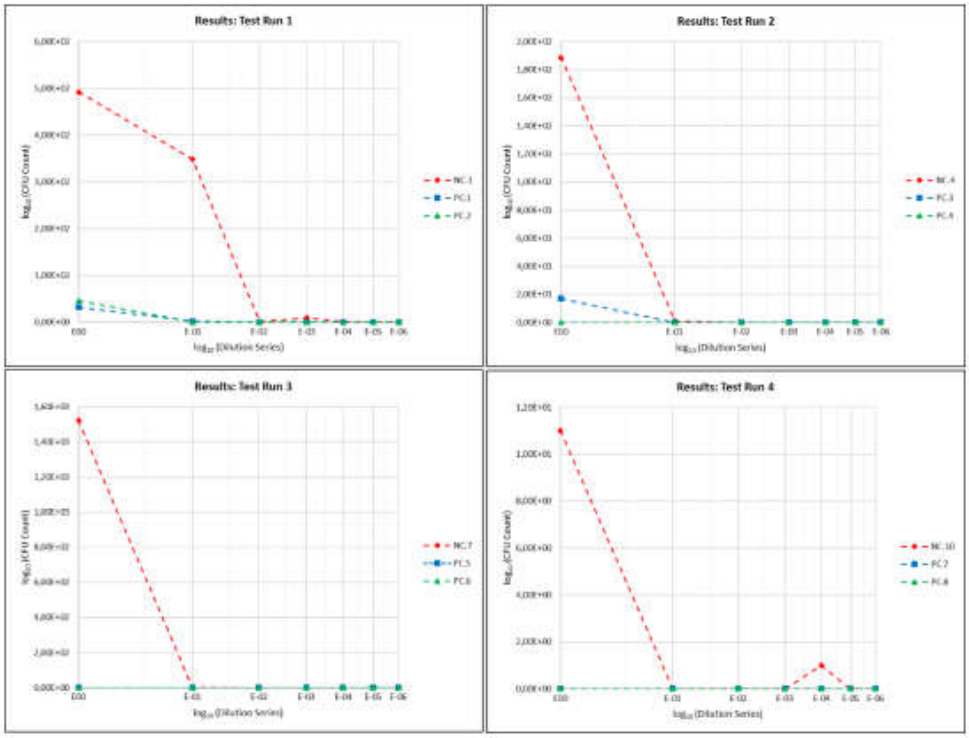

Figure 6-1: Discussion of the Results

By comparing the previously calculated results, the better reduction of *E. coli* is achieved by using Diosol 12, as zero CFU survive the decontamination procedure. Nevertheless, a decent decontamination efficiency is achieved by using Diosol 3, as approximately 93% of the CFU are killed though the decontamination procedure.

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Although the EDEN Diosol Exposure Test provides conclusive results, some issues have to be discussed critically:

1. The results are in most of the cases only countable in dilution series E00 and E-01.
2. The average amount of CFU, which die during the drying procedure.
3. The low amount of CFU counted for the reference negative control sample NC.10 during test run four.

Firstly, examining the red curves in figure 6-1, it is clearly shown that the curves of the dilution series develop as expected. However, in most of the cases, CFUs are only counted for the undiluted solutions (E00) and in some cases for the 10-fold dilutions (E-01). For the proceeding dilutions (E-02 - E-06), no viable results are identified. The reason for this effect arises from the fact that the bulk of *E. coli* does not survive the drying process and the decontamination procedure is initiated with a maximum CFU count of E03 (Test Run 3) on the glass slides. Therefore, a result is only expected to be seen up to the E-03 dilution.

Two exceptions of the previously described behavior of the dilution series are identified in the results and reflected in the following section.

Exceptions are the dilution series of NC.1 and representatively the sample NC.3 (E-02) as well as the sample NC.10 (E-04) for low CFU counts ≤ 2.

Dilution series NC.1 is emphasized, since it provides the only E-03 dilution, which obtains a viable result, since still 9 CFUs are counted on the dedicated petri dish. This is a unique feature among all dilution series, which is the reason for highlighting the dilution series of sample NC.1 in this section.

Concerning the low CFU counts (≤ 2), these occur on some of the petri dishes although they are not expected to emerge there. These low CFU counts potentially originate from airborne germs referring to the representative samples NC.3 (E-02) and NC.10 (E-04). For this reason, these low CFU counts are henceforth negligible.

Secondly, as calculated in the results for each test run, the fact that the bulk of *E. coli* dies during the drying procedure (97.11%) is a crucial issue that has to be considered in further testing. This value will be discussed with the microbiologists from DLR who perform the microbiological evaluation in scope of the EDEN H_2O_2 Decontamination Test.

Third, the amount of CFU counted for the negative control sample NC.10 during the 4th test run is eleven. Comparing this value to the CFU count of NC.1, NC.4, and NC.7 a remarkable disparity is recognizable. The greatest discrepancy (CFU_D) in the CFU count is calculated between samples NC.10 and NC.7:

$$CFU_D = \frac{11\,CFU}{1522\,CFU} * 100\% = 0.72\% \quad (6.1)$$

Equation (6.1) proves that the initial amount of CFU, which is inoculated on glass slide NC.10 amounts only 0.72% in comparison to glass slide NC.7.

There are two possible influencing factors, which explain the occurrence of this incidence:

The first possible influencing factor is the drying time itself. Comparing NC.1 to NC.4 as well as NC.7 to NC.10, the initial amount of CFU decreases the longer the samples dry. On Tuesday, samples NC.1 and NC.4 are inoculated at the same time. On Wednesday, samples NC.7 and NC.10 are inoculated at the same time. Both days, the amount of CFU counted after the test runs executed at a later time, is significantly lower than the amount of CFU counted after the test runs executed at an earlier time.

The second possible influencing factor is the incomplete decomposition of H_2O_2. According to the manual, the manufacturer DIOP declares that an exposure time of 90 minutes after initiating the decontamination process is appropriate for Diosol 3 and Diosol 12. Apart from this, harmless concentrations of H_2O_2 are reached after this time has passed. Therefore, 90 minutes after initiating the decontamination procedure, all samples are removed from the decontamination chamber after each test run. Afterwards, the doors of the decontamination chamber are left opened to guarantee an adequate air circulation under the cleanbench and inside the decontamination chamber. Thereby, a complete removal of H_2O_2 is supposed to be assured. After at least 120 minutes have passed, the samples for the following test runs are placed at their respective positions. Still, it is possible that H_2O_2 particles of the previous test run did not completely decompose. In particular regarding Diosol 12, the following occasion indicates that H_2O_2 is not completely decomposed within 90 minutes.

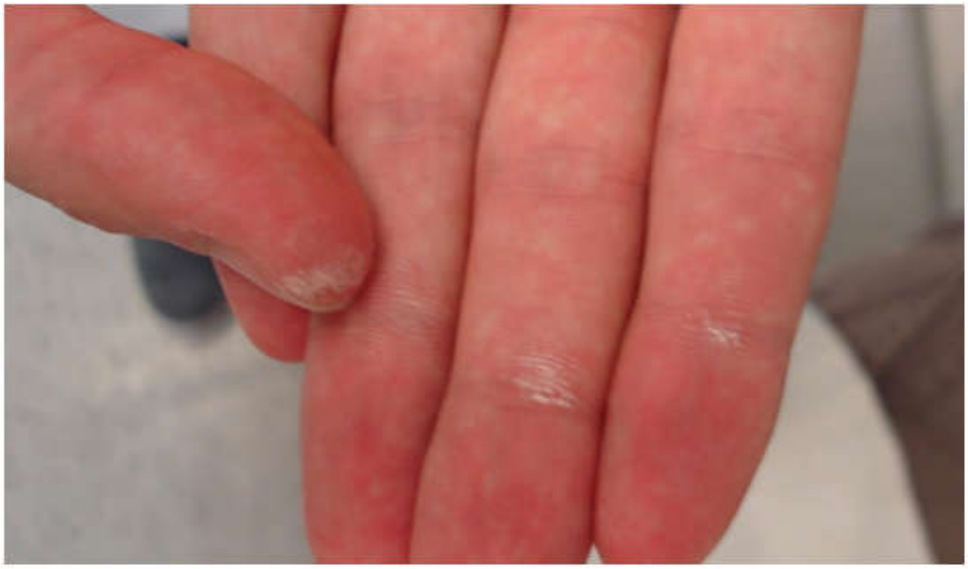

Figure 6-2: Caustic and bleaching effect on skin

Figure 6-2 demonstrates the impact of H_2O_2 on skin 90 minutes after the decontamination procedure was initiated. By touching the slightly wet petri dishes, a caustic effect on the skin occurred. The white patches, which appear after touching the petri dishes (and therefore liquid H_2O_2) are a result of the emerging oxygen, which forms by the decomposition process in the skin.

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The figure above proves that H_2O_2 is not completely decomposed after 90 minutes. Therefore, it is not excludable that Diosol and in particular Diosol 12 has a sanitizing impact on the glass slides, which are situated under the cleanbech for the subsequent test runs, 120 minutes after the previous decontamination test run was initiated. This impact might lead to the lower amount of CFU counted on the petri dishes in test run #2 and especially test run #4.

Summarizing the discussion, an effective decontamination is achieved with both of the decontamination agents: Diosol 3 (approx. 93%) and Diosol 12 (100%). Although the EDEN Diosol Exposure Test provides conclusive results, some factors are identified, which have a potential impact on the results.

The impact of the listed issues one (countable results in dilution series E00 and E-01) and two (average amount of CFU dying during the drying procedure) is clarified, whereas the impact of issue three (the potentially incomplete decomposition of H_2O_2) on the results is difficult to assess by the given information.

7 CONCLUSION

The conclusion of the EDEN Diosol Exposure Test provides inputs for the large-scale experiment, since the determined H_2O_2 solution is used for further testing in scope of the EDEN H_2O_2 Decontamination Test.

Evaluating the acquired results, Diosol 12 is not considered for further testing. In particular figure 6-2 demonstrates the impact of 12% H2O2 solutions on cells and living organisms. Certainly, Diosol 12 provides the more effective reduction of bacteria compared to Diosol 3. An absolute reduction efficiency of at least three log-stages and a relative reduction efficiency of 100% is achieved. However, it is very likely to kill the test plants in the large-scale experiment. This potential negative impact on the test plants would violate the second crucial success criterion and therefore lead to a failure of the experiment.

Concerning Diosol 3, no negative impact on skin is observed after removing the slightly wet petri dishes from the decontamination chamber. This occasion provides information about the lower impact of 3% H_2O_2 solutions on cells and living organisms. Nevertheless, an absolute bacterial reduction of approximately two log-stages and a relative reduction efficiency of ~93% is achieved. However, the lower reduction efficiency of Diosol 3 could lead to an insufficient reduction of microorganisms, especially since less susceptible microorganisms besides *E. coli* are used for further testing. This would violate the first success criterion and therefore result in a failure of the experiment.

Assessing the reduction efficiencies and their potential impact on plants, the establishment of a compromise between 12% and 3% H_2O_2 solutions seems beneficial. Therefore, a solution of 6% H_2O_2 is considered to be the best compromise in order to realize the issued success criteria. Thereby, enhanced reduction efficiency on microorganisms (than 3% solutions of H_2O_2) and a less negative impact on plants (than 12% solutions of H_2O_2) is supposed to be achieved. The impact of 6% H_2O_2 solutions on these issues is investigated in scope of the EDEN H_2O_2 Decontamination Test.

To give a short outlook, there are several possibilities, which provide an advancement of the EDEN Diosol Exposure Test. Ideally, the EDEN Diosol Exposure Test is executed under the same conditions with the 6% H_2O_2 solution. However, due to a lack of time and money, a resumption of this test is not possible.

In order to improve the results of the EDEN Diosol Exposure Test, an extension of the air circulation period after each test run seems to be helpful, as the impact of the H_2O_2 solutions of the previous test runs is difficult to assess.

Besides, another modification of this test, which proves the actual decontamination efficiency, is to investigate the impact of H_2O_2 solutions on undried colonies, as the bulk of the CFU does not survive the drying procedure.

On top of that, the execution of more test runs would lead to more stable results, as the statistical significance is increased.

Another enhancement of the test could be realized by using a variety of robust microorganisms as it is done in the large-scale experiment. In scope of the EDEN Diosol Exposure Test, only the gram-negative bacterium *E. coli* is used. In order to achieve stable and versatile results, gram-positive and spore-forming microorganisms could be used additionally.

A particularly interesting issue is to investigate the development of resistances. This could be examined by repeating this experiment several times. The CFU, which survive the decontamination procedure are cultivated and decontaminated again and again while the results are observed.

As shown in the section above, the EDEN Diosol Exposure Test is arbitrarily expandable in any direction. However, the relevant inputs are identified and transferred to the large-scale experiment, the EDEN H_2O_2 Decontamination Test, which is presented in the following chapter.

8 ABBREVIATIONS

AN	A. niger
AS	Hersteller der PEN3/MLA-VI
AST	Astrium Space Transportation
ATP	Authorization to proceed
B/B	Breadboard
BS	B. subtilis
BG	Breath Gas
CE	Communauté Européenne (Europäische Gemeinschaft)
CFU	Colony forming units
CM	Configuration Management
COM	Serielle Schnittstelle
COTS	Commercial off-the-shelf
DC	Direct Current
DLR	Deutsches Zentrum für Luft- und Raumfahrt
DMS	Data Management System
DRD	Document Requirements Description
DRL	Document Requirements List
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EC	E. coli
EDU	Enrichment and desorption unit
EM	Engineering Model
EMC	ElectroMagnetic Compatibility
E-Nose	Electronic nose
E-Nose 2	Upgrade of the Electronic nose
EP	Experiment Procedure
ERD	Experiment Requirements Document
FM	Flight model
FM	Flight Model
GSE	Ground Support Equipment
H/W	Hardware
HK	Housekeeping
I/F	Interface
I/O	Input/Output
IBMP	institute biomedical problems
IMBP	Institute of Medical and Biological Problems
IMR	Ionen-Molekularreaktions-Massenspektrometrie
ISS	International Space Station
LCD	Liquid Cristal Display
LDA	Linear Discriminant Analysis
LLI	Life Limited Item
LOD	Limit of detection
LSE	Laboratory Support Equipment
LST	Lance Sampler Tip

LTU	Laptop Unit
MAIT	Manufacturing, Intergration and Test
MLA	Material für Langzeitanwendungen im Weltraum
MOS	Metal Oxide Semiconductor
MS	Massenspektrometrie
MVOC	Microbial Volatile Organic Compounds
n/a	Not available
NMML	Non Metallic Material List
P/L	PayLoad
PA	Product Assurance
PI	Principle Investigator
PA&S	Product Assurance & Safety
PC	Personal Computer
PCA	Principal Component Analysis
PCB	Printed Circuit Board
PCU	Power Control Unit
PDR	Preliminary Design Review
PDRD	Performance and Design Requirements Description
PE	P. expansum
PEN3	Portable Electronic Nose 3
PLS	Partial Least Squares
PPM	Parts per million
PTFE	Polytetrafluorethylen
PVC	Polyvinylchlorid
QA	Quality Assurance
QM	Qualification model
Qty.	Quantity
Req	Requirement
Resp	Institution responsible for the corresponding requirement
RFQ	Request for Quotation
ROM	Rough Order of Magnitude
S/W	Software
SRR	System Requirement Review
SFPT	Short functional performance test
TBC	To be confirmed
TBC	To Be Confirmed
TBD	To Be Defined
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
UFT	Zentrum für Umweltforschung und nachhaltige Technologien der Universität Bremen
USB	Universal Serial Bus
VOC	Volatile organic compound
w.r.t.	With reference to

9 ANNEX AND ATTACHMENTS

AIRBUS DEFENCE & SPACE

EDEN ISS

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Ausgabe/Issue:	01	Datum/Date:	19.12.2016	
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Titel:
Title:

EDEN H_2O_2 Decontamination Test

Dokumenten Typ: Document Type:	Test Report	Konfigurations-Nr.: Configuration Item No.:	
Referenz- Nr.: Reference No.:		Klassifikations-Nr.: Classification No.:	
Lieferbedingungs-Nr.: DRL/DRD No.:		Freigabe Nr.: Release No.:	
Gruppierung (Dok.): Group (Doc.-related):		Gruppierung (Version): Group (Version-related):	
Thema: Subject:	EDEN – Investigation of 6 % H_2O_2 Treatment on Microorganisms and Plants		

Kurzbeschreibung:
Abstract:

The report investigates and evaluates the influences of countermeasures on microbiological contaminations for bio-regenerative closed-loop life support systems. The test exhibits a significant decrease in plant growth and development of biomass as well as an inadequate reduction of microorganisms treated with 6 % H_2O_2.

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AIRBUS DEFENCE & SPACE

EDEN ISS

Dok.Nr./Doc.No.:	EN-TR-AST-0069				
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TABLE OF CONTENTS

1 INTRODUCTION

A crucial objective of the EDEN ISS project is to study the microbiological behavior and dedicated countermeasures in plant-based closed-loop ecosystems. High humidity and temperatures provide not only favorable growth conditions for plants, but also for microorganisms like bacteria and fungi. Since these microorganisms can spoil plants, the development of a microbiological decontamination system is necessary to preserve the plants from spoiling. Thus, this report analyses the decontamination system and agent (6 % H_2O_2) under examination.

Success criteria of the experiment are to verify the system and agent on:

1. No impairments of the plants to the test agent (H_2O_2)
2. Efficiency to reduce microorganisms

This report provides information about:

- Test Material and Methods
- Results of the microbiological evaluation and plant health monitoring
- Discussion
- Conclusion

2 APPLICABLE AND REFERENCE DOCUMENTS

2.1 Applicable Documents

The following table shows the top level applicable documents.

AD	Doc. Number	Issue	Date	Title	Applicability
AD0	-	1	2016-10-20	EDEN ISS-ABDS WP2.3-D2.5-Design Report-v1.0	all
ADD1	-	1	2016-02-08	EDEN ISS-ADS-WP3.5-CDR E-Nose and Decontamination System-v0.2	all
ADD2	EN-TR-AST-0068	1	2016-22-08	EN-TR-AST-0068 EDEN Diosol Exposure Test Report on Escherichia coli	all

2.2 Reference Documents

The following table shows the reference documents.

RD	Doc. Number	Issue	Date	Title	Applicability
RDD1	-	1	2016-07-28	Gefährdungsbeurteilung ArbSchG und BetrSichV_V1.0	all

3 TEST ARTICLE

The following articles were used:

No.	Article	Part Number	Model	S/N	Number of Items	CIDL/ABCL or Drawing:
1.	Diosol 6% without Ag-complex					
2.	*E. coli* *Staphylococcus cohnii* *Bacillus atrophaeus spores*					
3.	Decontamination System					
4.	Test plants (cucumber, lettuce)					
5.	Greenhouse compartments					
6.						
7.						
8.						
9.						
10.						
11.						

A detailed description of test articles can be found in chapters 'Material' and 'Methodology' as well as in the Test Report of the EDEN Diosol Exposure Test.

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4 TEST DESCRIPTION

4.1 Stakeholders and Competences

This chapter is inserted to give an overview over the different stakeholders of the EDEN ISS project involved in the EDEN H_2O_2 Decontamination Test. Figure 4-1 illustrates the correlations between the stakeholders and outlines the mutual inputs provided for each other. Project management is performed by Airbus DS cooperating with German Aerospace Centre Institute of Aerospace Medicine (DLR-ME) and Wageningen University Greenhouse Horticulture (WUR). Test dates are fixed in consultation with the mentioned project partners. The test procedures are authored by Airbus DS while DLR-ME and WUR deliver the necessary inputs in their fields of expertise. Additionally, Airbus DS carries out the plant analysis instructed by the professionals from WUR. The final test report is published by Airbus DS as well. The crop science professionals incorporated in the project collaborate closely with the local greenhouse experts from Bleiswijk (NL) where the test is executed. WUR acts as contact ensuring appropriate plant cultivation and treatment during the period of four weeks while the test runs. Additionally, WUR shares competence with Airbus DS by providing instructions in plant analysis.

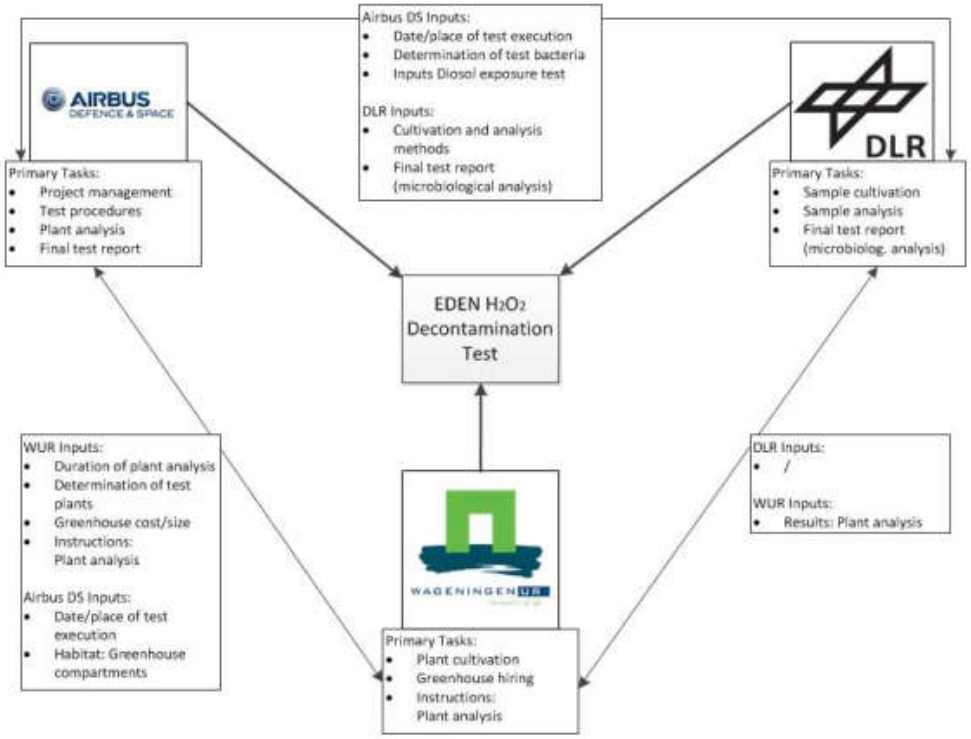

Figure 4-1: Stakeholders and Competences

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Biologists from DLR-ME perform the microbiology in scope of this test. This includes the cultivation, transport, and analysis of the microorganisms brought to the greenhouse compartments in Bleiswijk. DLR-ME delivers sample cultivation and analysis methods to Airbus DS as well as the necessary inputs for the final test report.

4.2 Material

Since the EDEN Diosol Exposure Test is closely linked to the EDEN H_2O_2 Decontamination Test, the materials are already introduced in chapter 3.1. These materials are not repetitively listed in this chapter. Only further essential materials used in the EDEN H_2O_2 Decontamination Test are described subsequently in the following sections.

4.2.1 Greenhouse Compartments

Two greenhouse compartments are rent in Bleiswijk (NL) for a period of four weeks. One compartment serves as test compartment (C1) containing the test samples and the other as reference compartment (C2), in which the control samples are situated. Each compartment has a size of 24 m² and a height of 5.5 m resulting in a total volume of 132 m³. Three tables are placed in each compartment having a length of 3.9 m, width 1.3 m, and a height of 0.8 m. Plant samples are placed on these tables as well as the tablets with microorganisms.

4.2.2 Microorganisms

Figure 4-2: Arrangement of Glass Slides on Tablets

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Three different microorganisms are used in scope of this test. The selection of microorganisms covers a broad range since besides the gram-negative *Escherichia coli* bacterium (DSM No. 613), a gram-positive *Staphylococcus cohnii* (DSM No. 20260), and *Bacillus atrophaeus spores* (DSM No. 675) are utilized. The microorganisms are arranged in triplicates on glass slides as shown in Figure 4-2. Overall, 18 of these tablets are prepared for the test:

- 14 test tablets placed in C1 (treated with H_2O_2)
- 2 test tablets placed in C2 (untreated controls)
- 1 test tablet remaining inside the car (transport control - TC)
- 1 test tablet remaining inside the laboratory (laboratory control - LC)

4.2.3 Decontamination System

The decontamination system deployed in this test is already described in detail in the EDEN Diosol Exposure Test. However, as concluded from the Diosol Exposure Test, a solution of 6 % H_2O_2 is further utilized to run the system.

4.2.4 Test Plants

The following table 5 demonstrates an extract of a report investigating the practicability of different crops on their suitability for cultivation in space.

Table 4-1: Suitable Crops for Cultivation in Space

Crop	Score	Crop	Score
Lettuce	48.8	Spinach	14.6
Cucumber	37.9	Swiss chard	11.9
Dwarf tomato	26.9	Bell pepper	10.8
Chives	26.3	Red mustard	8.0
Tomato	26.3	Coriander	7.9
Strawberry	17.3	Water cress	6.7
Radish	16.9	Basil	2.9
Parsley	14.7		

The literature 'Choosing crops for cultivation in space' is authored by the project partners from Wageningen University (WUR) Greenhouse Horticulture (Tom Dueck et al., 2016). These listed crops are investigated on their suitability for cultivation in ISPR racks and the FEG. These crops are particularly examined on three previously determined selection criteria:

- Human quality aspects
  e.g.: edibility, appearance, pungency
- Cultivation aspects
  e.g.: plant height, harvest time, disease resistance
- Yield aspects
  e.g.: production efficiency, light and energy use, harvest index

Cucumber and lettuce achieved the highest overall score in the respective crop ranking shown inTable 4-1: Suitable Crops for Cultivation in Space. Thus, these plants are further used as test plants in the EDEN H_2O_2 Decontamination Test. Choosing cucumber and lettuce, a foliage plant, and a particularly sensitive plant (the most sensitive among fruits and vegetables) are selected. This choice is considered to provide transferable results about possible impacts of H_2O_2 on potential other plants

4.3 Methodology

4.3.1 Schedule

The following chapter provides the test schedule for the EDEN H_2O_2 Decontamination Test. Figure 4-3 illustrates the related test schedule as Gantt chart.

	Vorgangsname	Dauer	Anfang	Fertig stellen
1	EDEN H2O2 Decontamination Test	27 Tage?	So 06.11.16	Do 08.12.16
2	Arrival in Bleiswijk	1 Tag?	So 06.11.16	So 06.11.16
3	Test installation & Instruction	1 Tag?	Mo 07.11.16	Mo 07.11.16
4	Test Execution 1 & Return to Cologne	1 Tag?	Di 08.11.16	Di 08.11.16
5	Evaluation of Microorganisms @ DLR Cologne	3 Tage?	Mi 09.11.16	Fr 11.11.16
6	Plant short-term Monitoring of batch & Return to FN	1 Tag?	Do 10.11.16	Do 10.11.16
7	Plant mid-term Monitoring	1 Tag?	Di 22.11.16	Di 22.11.16
8	Arrival in Bleiswijk	1 Tag?	So 04.12.16	So 04.12.16
9	Plant long-term Monitoring	2 Tage?	Mo 05.12.16	Di 06.12.16
10	Test Execution 2 & Return to Cologne	1 Tag?	Di 06.12.16	Di 06.12.16
11	Evaluation of Microorganisms @ DLR Cologne and Return to FN	2 Tage?	Mi 07.12.16	Do 08.12.16

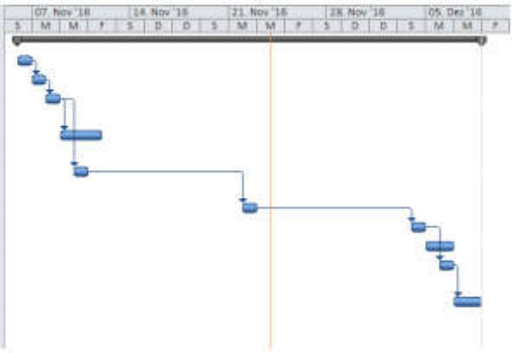

Figure 4-3: Test Schedule

Travel from Friedrichshafen to Bleiswijk is done on 6 November. Test installation and required preparations are performed on 7 November. Moreover, the greenhouse personnel and the local experts give instructions concerning the plant health monitoring. The treatment with H_2O_2 is executed on 8 November. After finishing the HPV procedure, the microorganisms are brought to DLR-ME in Cologne to commence the microbiological analysis straight away. Plant health monitoring is performed in four steps including immediate, short-term, mid-term, and long-term monitoring over a period of four weeks. Destructive measurements are only carried out at the end of the experiment on 5 and 6 December to avoid impairments in plant growth biasing the results. After removing the plants from the compartments, a repetition of the experiment is performed since no plants in a compartment is assumed to have the same impact on the ventilation as having small plants inside the compartment.

Table 4-2 provides the schedule for the plant health monitoring. Immediate plant health monitoring is performed immediately after the HPV procedure is finished. A visual inspection (VI) is performed as a non-destructive measurement. Short-term plant health monitoring is performed two days after executing the experiment. Again, only a VI is carried out as a non-destructive measurement to avoid impairments in plant

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growth. Mid-term plant health monitoring is undertaken fourteen days after executing the treatment with H_2O_2. A further VI is performed as well as the counting of flowers and buds, if the plant already developed these. Long-term plant health monitoring is realized four weeks after executing the experiment. As shown in the table below, non-destructive measurements (VI, NF) are performed as well as final destructive measurements of the leaf area index (LAI), fresh weight (FW), and dry weight (DW).

Table 4-2: Plant Health Monitoring Schedule

Step	Date	Monitoring	Remarks
Immediate Monitoring	8 November	VI	Non-Destructive Measurements
Short-term Monitoring	10 November	VI	Non-Destructive Measurements
Mid-term Monitoring	22 - 23 November	VI	Non-Destructive Measurements
Long-term Monitoring	6 - 7 December	VI, LAI, NF, FW, DW	Non-Destructive Measurements & Final Destructive Measurements

4.3.2 Preparation and Recovery of Microorganisms

Treatment of microorganisms is performed to the specifications given by DLR-ME for the EDEN H_2O_2 Decontamination test. The methods are performed equally in scope of this experiment.

First, the preculture is prepared by transferring one single colony from the stock culture plate to the preculture plate. The preculture plate is incubated upside down at 37 °C for 24 hours. Besides, glass slides inoculated with microorganisms in a further step, are fixed on tablets. The glass slides and tablets are washed in the laboratory dishwasher first. Then, the glass slides are fixed on the clean tablets with adhesive tape. Subsequently, the tablets and glass slides are autoclaved at 121 °C for at least 20 minutes to guarantee sterility. In the next step, the test inoculum is prepared. Therefore, one single colony is transferred from the preculture plate to an Erlenmeyer flask (size: 100 mL) filled with 200 mL LBB medium (receipt is previously described in scope of the Diosol Exposure Test in chapter 3.2.2 'Preparation of Culture Media Buffer and Liquids'). The Erlenmeyer flasks are kept in motion with 150 rpm in an incubation chamber at 37 °C for 24 hours. After incubating, the LBB medium is centrifuged at 3000 rpm for 15 minutes to guarantee the removal of high concentrations of microorganisms from the medium. The supernatant is discarded and the pellet is suspended with PBS (receipt is previously described in scope of the Diosol Exposure Test in chapter 3.2.2 'Preparation of Culture Media Buffer and Liquids'). The PBS containing the pellet is centrifuged at 3000 rpm for 15 minutes as well and the supernatant is discarded again. The pellet is suspended with PBS. The viable cell count is checked in a counting chamber (e.g. Neubauer improved, factor $4x10^6$). The suspension is diluted with PBS to the target concentration of $4x10^6$ CFU/mL is achieved. The previously sterilized tablets with the glass slides are unpacked under a laminar cleanbench. 50 mL of the prepared suspension are inoculated on the glass slides arranged as determined in figure 41. The inoculum is dried under a laminar cleanbench overnight. Finally, the tablets with the dried (inactivated) microorganism are ready for transport and exposure to H_2O_2.

After exposure to H_2O_2, the tablets with the glass slide samples are unpacked under a laminar cleanbench. Each glass slide is removed and transferred to a falcon tube (size: 50 mL) filled with 15 mL PBS. The falcon tubes are shaken on a vortex shaker to remove the microorganisms from the glass slides. Then, appropriate dilutions are produced with the recovered bacterial suspension. The bacterial suspension is casted on previously prepared petri dishes with solid LBA medium. The petri dishes are incubated upside down at 37 °C for at least 24 hours. After the incubation, the amount of CFUs is counted and the results are calculated.

4.3.3 Treatment and Plant Health Monitoring

The following chapter describes the treatment of the test plants and the plant health monitoring. Lettuce is sown in soil tablets and cucumber is sown in rock wool slabs as shown in Figure 4-4 and Figure 4-5.

Figure 4-4: Cucumber Seedlings

Figure 4-5: Lettuce Seedlings

Lettuce is irrigated by hand shower and cucumber is irrigated by drippers. While lettuce and cucumber have different preferable climatic conditions, an average is chosen as basic setting:

- Meaning day temperature (T_D): 20 °C
- Meaning night temperature (T_N): 18 °C
- Relative Humidity (rH): 80 % (70 % for the first three days)

The crop maintenance is minimal. While lettuce receives nothing else than irrigation, cucumber is winded on a stick if necessary. Concerning cucumber, one fruit per node is maintained and the side shots are taken off.

Several parameters are investigated in scope of the plant health monitoring to guarantee significant results. The parameters, which are under investigation, are already named distinguishing between destructive and non-destructive measurements. The following section describes these evaluation methods in detail.

To begin with the non-destructive measurements, two parameters are under investigation: VI, NF.

Visual Inspection (VI): The issue of performing the VI is identifying visible changes of the test plants. Since the Diosol Exposure Test revealed information about the catalase effect, colorimetric investigations are emphasized to identify any negative effects of H_2O_2 on the test plants. Therefore, a VI is performed four times as immediate, short-term, mid-term, and long-term monitoring. This includes the recording of photos and documentation of any occurring conspicuous features.

Number of Flowers (NF): The number of flowers provided by a plant is counted in scope of the plant health monitoring.

Figure 4-6: Flowering of Cucumber

The flowering is only counted for cucumber (since lettuce is not supposed to develop flowers). Besides the fully developed flowers, the evolving buds and upcoming fruits are counted as well.

Three parameters are investigated as destructive measurements after executing the non-destructive measurements as described in the schedule: LAI, FW, and DW.

Leaf Area Index (LAI): The leaf area index is measured for both, cucumber and lettuce. Measuring the leaf area provides information about the plant growth and possible negative impacts. The leaf size is calculated with the open source software EasyLeafArea. Figure 4-7 demonstrates the graphical interface of the mentioned freeware. Any image in the formats tagged image file format (.TTIF) or Joint Photographic Experts Group (.JPEG) is importable. The software analyses each pixel of the image differentiating the RGB values to identify red, green, and blue areas. The recognition sensitivity is adjustable in the right column of the interface shown in Figure 4-7. Detected green pixels are highlighted in lime green and the area covered by green pixels is output in cm². The red square illustrated in Figure 4-7 serves as reference, which must be included in every image evaluated with the software. Entering the size of the red square in the interface, the area covered by the green pixels is calculated automatically.

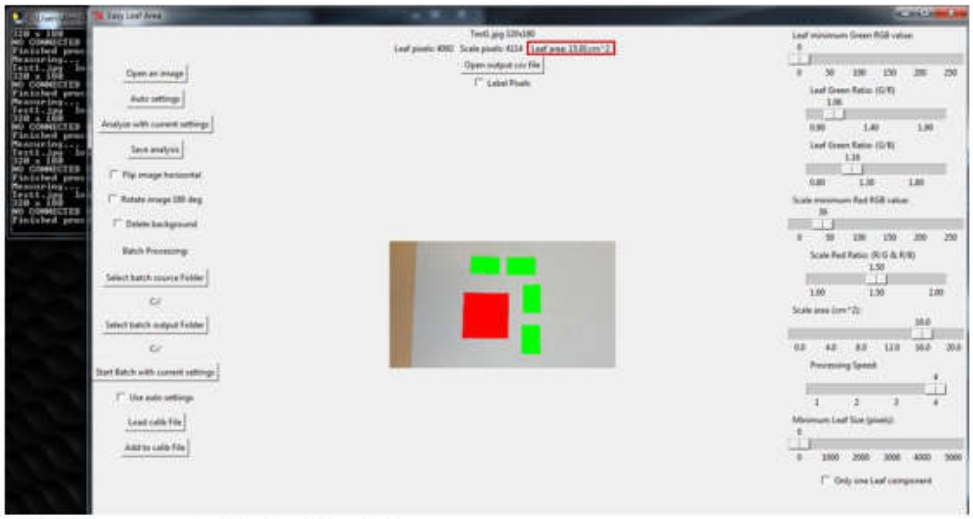

Figure 4-7: Measurement of the Leaf Area Index

Figure 4-7 shows an example with one red square and four green rectangles. The surface of the red square covers 16 cm² while each green rectangle covers an area of 4 cm² (L = 2.5 cm, W = 1.6 cm) summing 16 cm². The result calculated by the software is marked with a red rectangle in figure 46. The calculated result (15.91 cm²) only shows a minor deviation as 16 cm² (4 cm * 4 cm) are actually covered by green pixels.

Figure 4-8 demonstrates a more complex example with the picture containing additional elements. Besides the red square and the green elements proposed to be recognized by the software, additional elements are added in Figure 4-8. This example proves the software's ability to distinguish different colors and shades.

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Figure 4-8: Measurement of the Leaf Area Index (complex example)

Again, a red square with a size of 16 cm² is implicated in the image as reference. Next to the square, a ferruginous coin is placed as well as another fawn square. Additionally, blue rectangles are added next to the green rectangles having the same size (2.5 cm²). Thus, the flagged pixels emphasized in this image cover a surface of exactly 16.20 cm². The deviation is slightly higher in comparison to the example previously discussed in Figure 4-7. Nevertheless, the results demonstrate the applicability of the software, since the measurement of length and width of a leaf provides more inaccurate information about any changes of the leaf growth.

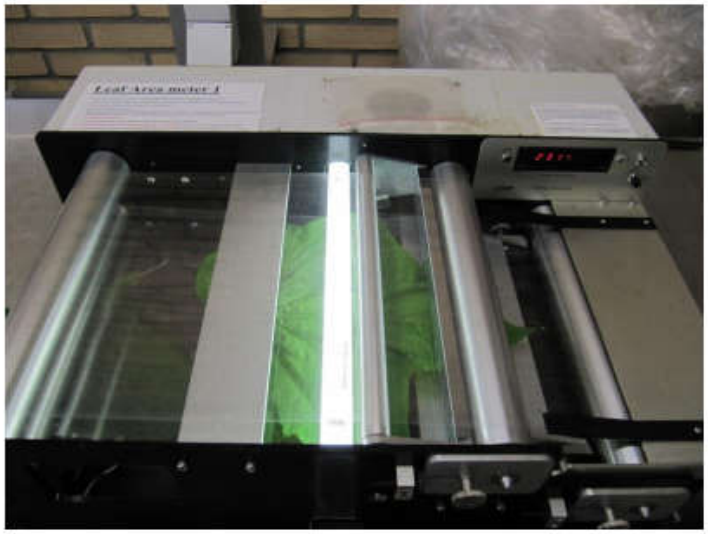

Figure 4-9: Leaf Area Meter

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Additionally, the LAI is measured with a leaf area meter depicted in Figure 4-9 on the following page. Since every single leaf has a different shape, an instrument with the ability to smoothen the leaves is deployed. After placing the leaves on the conveyor, every leaf passes through a press smoothing the leaf to its two-dimensional shape, because non-smoothened leaves and the measurement of their three-dimensional shape leads to underrated results for the LAI. This procedure provides an accurate method to measure the area of every leaf to a hundredth of a square centimeter. The LAI is measured as first destructive measurement followed by FW and subsequent DW measurements.

Fresh Weight (FW): Measuring the FW implies the measurement of the plant weight with the water content. While measuring the FW without harming the plant is basically possible, the act of removing the plant from its soil can impair the ongoing growth and therefore bias the results. Thus, FW measurements are executed as a final examination at the end of the experiment since FW measurements are considered as destructive measurements. To measure FW, the plants are carefully removed from the soil. In the next step, the loose soil is washed off. Then, the wet plants are dried by wiping them with a cloth. It is important to weight the plants immediately after washing and drying due to the rapid loss of water.

Dry Weight (DW): Measuring the DW implies the measurement of the plant weight without the water content. Measuring the DW provides more reliable results comparing the FW measurements since the water content varies among species in dependency of environmental conditions. DW measurements are destructive measurements, which are not survivable for a plant. As the plants have previously been removed from the soil, washed, dried, and weight, the next step is eliminating the water content. Therefore, the plants are exposed to low heat of 80 °C in an oven for at least 48 hours. Thereby, the plants lose their entire water content. After drying, the plant remains are finally weight with a fine scale.

4.3.4 Test Installation and Execution

The following section provides an overview of the test installation and execution. The figure demonstrates a schematic drawing of the samples and their respective arrangement in the test compartment C1. The decontamination system and the nozzle are installed at the bottom right corner of the compartment C1. Boxes are placed under the nozzle to ensure the same height of test plants and decontamination system. The nozzle is aligned to the center of the compartment C1 and fixed with tape to prevent tilting. In both compartments, lettuce is transplanted to soil boxes. Three soil boxes are arranged on each table with the central box containing ten lettuce plants while the top and bottom box of each table contains eleven lettuce plants. 14 cucumber plants are arranged around the soil boxes on each table as depicted. Overall, 48 cucumber plants and 96 lettuce plants are grown in each compartment resulting in an overall sum of 288 test plants deployed in the EDEN H_2O_2 Decontamination Test. Likewise, the 14 test tablets are placed in the compartment C1). Tablets 1-5 (highlighted with red arrows) are arranged on the side walls of the compartment C1 and fixed with tape. Tablets 6-8 (highlighted with a light blue arrow) are aligned in a diagonal line from the nozzle below the tables. Tablets 9-14 (highlighted with yellow arrows) are centrally placed on the tables in between of the soil boxes and the cucumber plants.

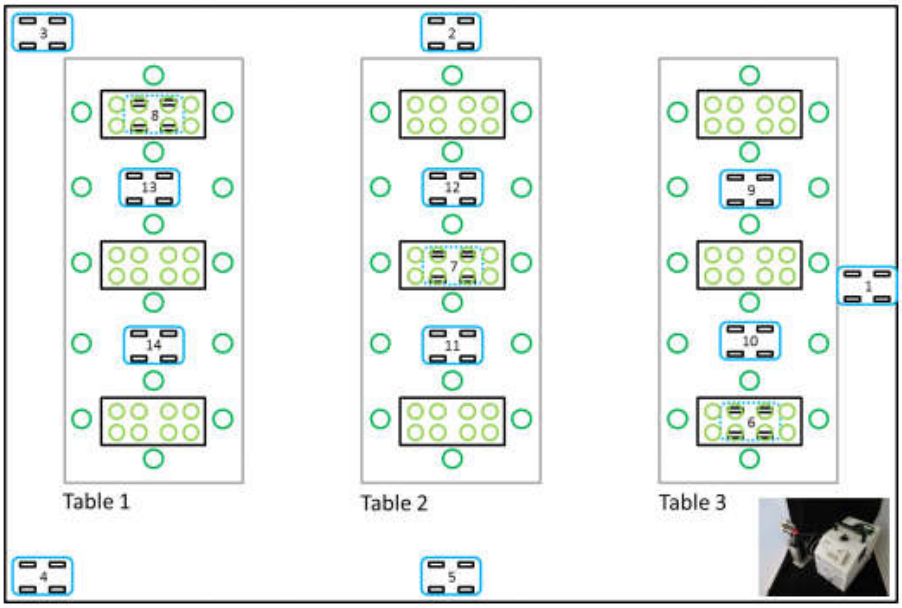

Figure 4-10: Schematic Sample Arrangement in Compartment C1

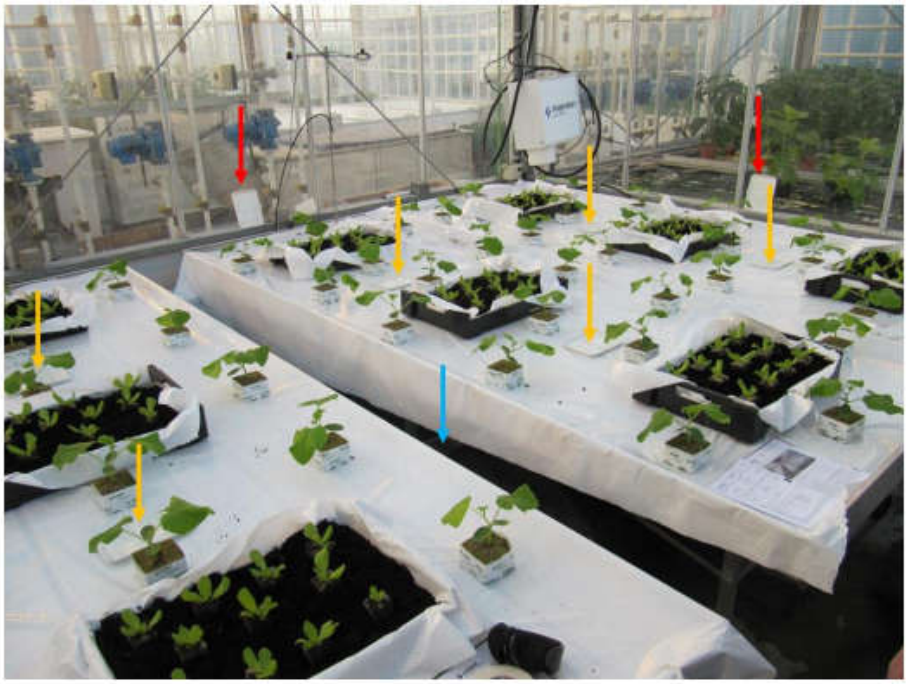

Figure 4-11: Arrangement of Tablets in Compartment C1

The movable tables are placed in a position guaranteeing the same distance of approximately 30 cm to each other. The tables adjacent to the bordering side walls are vertically positioned.

Figure 4-12 illustrates the arrangement of the plants and tablets in the control compartment C2 schematically. Lettuce and cucumber plants are arranged just as in the test compartment C1. Control tablets 15 and 16 are placed on the tables as shown in the figure.

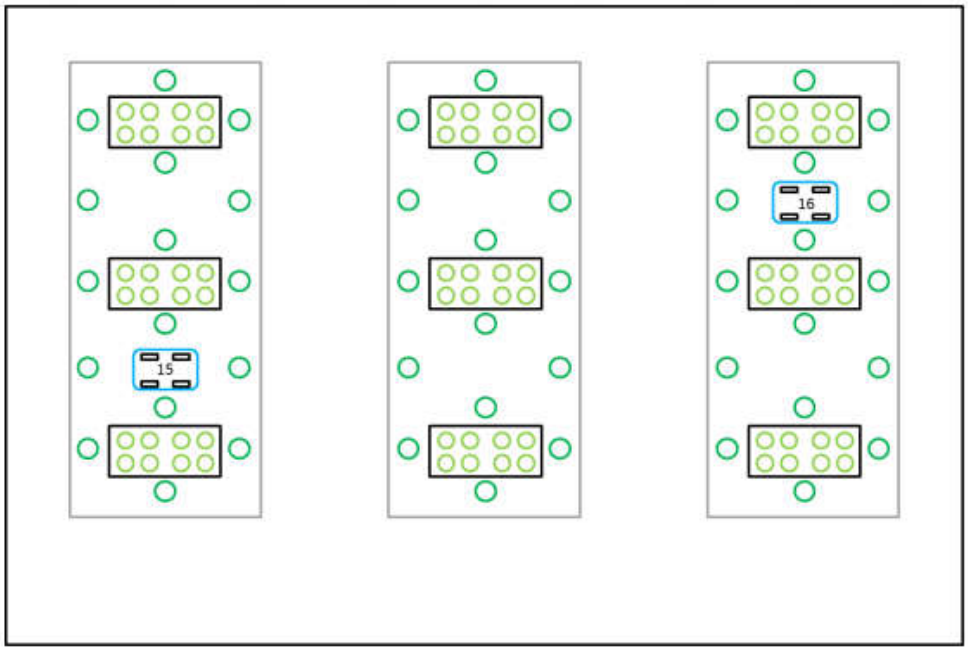

Figure 4-12: Schematic Sample Arrangement in Compartment C2

After arranging the test plants, the decontamination system is installed. As mentioned, the nozzle is placed on a couple of boxes providing the same level for test plants and microorganisms.

Figure 4-13: Installation and Alignment of the Decontamination System

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The generator is adjusted to a volume of approximately 140 m³. Since 2-3 mL are vaporized per m³, a total amount of approximately 300-400 mL H_2O_2 is vaporized during the decontamination. The duration of vaporization takes not more than 10 minutes, however due to safety and efficiency reasons, the compartment is closed for the following 90 minutes after initiating the procedure. When the 90 minutes have passed, both compartments are ventilated for a period of 30 minutes (according to safety regulations) to ensure that no H_2O_2 remains inside the compartment before entering.

4.3.5 Climatic Conditions

The following chapter provides information about the climate data prevailing inside the two compartments. Since the climate of both compartments is fully controlled, the entire data is recorded with an appropriate sensor system every five minutes. The data identified as the most striking is demonstrated subsequently. Each diagram depicts the recorded data from the day of the test execution and the stay in Bleiswijk (November 8, 2016 from 8.00 a.m. to 2.00 p.m.).

The data of the test compartment C1 is illustrated red, the data of the control compartment C2 is highlighted green, and the basic settings are presented blue.

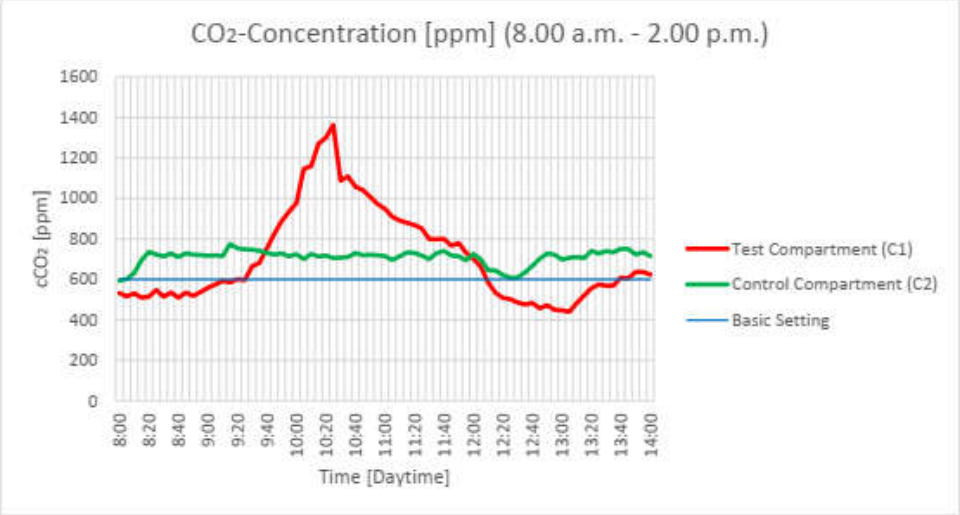

Figure 4-14: Concentration of CO_2 (cCO_2) in [ppm]

Figure 4-12 depicts the concentration of carbon dioxide (cCO_2) of the period during test execution. The basic setting is set to 600 points per million (ppm). By day, the average concentration of CO_2 is higher than during night due to plant metabolism. Starting from 9.00 a.m. to 10.30 a.m., a remarkable increase of cCO_2 peaking at 1363 ppm can be observed. The reason for this rapid increase is due to the breathing of involved persons who prepared the test. After initiating the test at 10.30 a.m., a decrease is shown in the figure above. At noon, a further decrease below the basic settings is shown caused by the ventilation lasting for 30 minutes. Starting at 1.00 p.m., the cCO_2 curves of both compartments converge to the line of the basic settings to 600

ppm. The reason for this approximation is explainable by the termination of sample packing and taking photos for the immediate plant health monitoring. The residual climate data does not exhibit any conspicuities for the cCO_2. Therefore, cCO_2 is not considered to have negative impacts on the growth of the test plants.

A further relevant parameter closely examined is the change in temperature during the period of test execution on November 8, 2016.

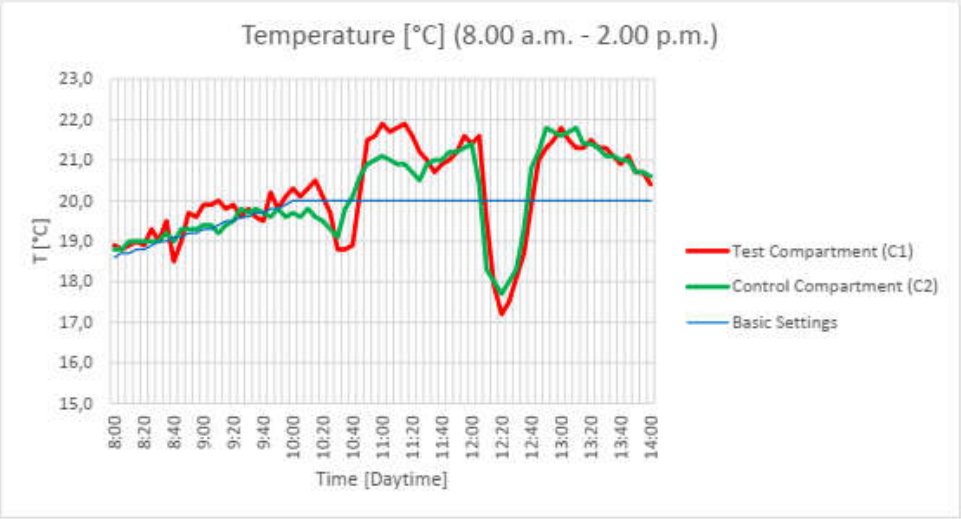

Figure 4-15: Temperature (T) in [°C]

The red curve represents the temperature in the test compartment C1, the green curve demonstrates temperature in the control compartment, and the blue graph the basic temperature settings. The basic temperature settings vary between 18 °C during night and 20 °C during daytime.

Figure 4-12 shows an almost linear increase of the basic temperature settings until the line stays constant at 20 °C. At 10.30 a.m., a first minimum can be observed. The reason for this minimum is due to preparing the experiment since the doors of the compartment are opened several times. During the period of test execution, the temperature increases to a maximum of 21.9 °C. This maximum is a result of the increased radiation measured in W/m². After finishing the decontamination procedure, a period of subsequent ventilation starting at noon is performed for 30 minutes. Ventilation is carried out by tipping the windows by 15°. The data exhibits a rapid temperature decrease in both compartments to a minimum of 17.2 °C followed by an equally rapid increase. Figure 4-11 demonstrates no significant differences in temperature for both compartments as well as the residual of the recorded climate data. Therefore, temperature is also not considered to have a negative impact on growth of the test plants.

Figure 4-12 illustrates the relative humidity (rH) as third relevant parameter processed from the climate data.

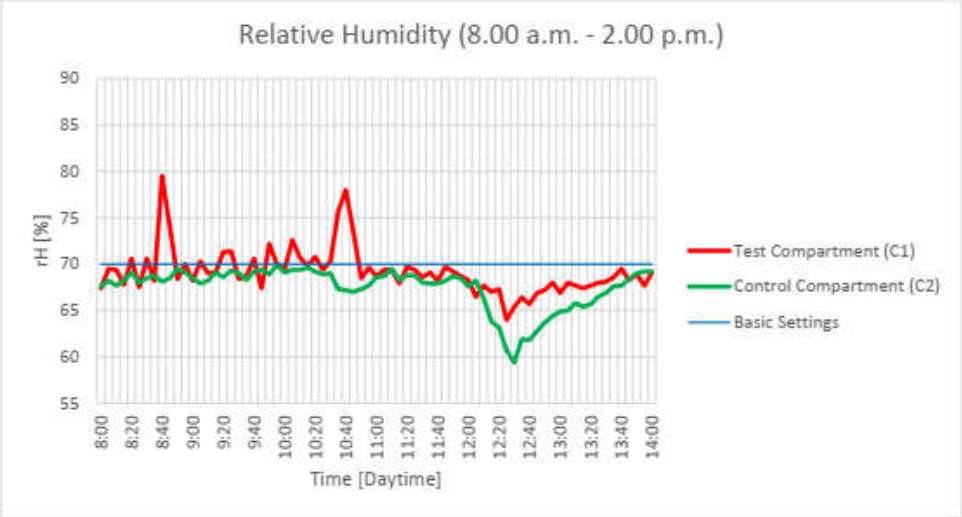

Figure 4-16: Relative Humidity (rH) in [%]

The red curve demonstrates the rH inside test compartment C1, the green curve represents the rH in the control compartment C2, and the blue line the basic settings for the rH set to a value of 70 % for the first three days. After three days, the rH is increased to 80 % providing optimal growth conditions for the test plants. The red and the green curve exhibit a similar trend except two maxima.

The first maximum can be observed at 8.45 a.m. in C1 and is an exception since such a sudden increase is never recorded in the greenhouse data again. Comparing the rH to the temperature data at 8.45 a.m., a temperature drop of 1°C can be observed just as an increase in rH of 10 %. The reason for this increase is explainable by opening the compartment door for irrigation purposes by the greenhouse personnel. The second maximum is detected at 10.30 a.m. after initiating the decontamination system. By vaporizing approximately 400 mL H_2O_2, the rH increases from 70 % to 78 %. At noon, both curves decrease reaching a minimum of 60 % (C2) and 65 % (C1) due to the ventilation after 90 minutes have passed. Until 2.00 p.m., the curves converge to the basic settings for rH of 70 %.

Since these are only minor deviations (and an increase in rH is assumed to provide even better growth conditions), rH, cCO_2, and temperature are not considered to have negative impacts on the growth of the plants. Similar results are recorded during the second test run and are therefore not further delineated in this chapter.

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5 RESULTS

5.1 Immediate Plant Health Monitoring

A first VI, the immediate plant health monitoring, is performed directly after the decontamination procedure and subsequent ventilation has finished. The plants are particularly investigated for white spots on their leaves. However, no changes in color or firmness are observed in scope of the immediate plant health monitoring.

5.2 Short-Term Plant Health Monitoring

Another VI is carried out, the short-term plant health monitoring, on 10 November (exactly 48 hours after executing the decontamination procedure). Again, no white spots or changes in firmness can be observed. Therefore, the HPV treatment with 6 % H_2O_2 does not exhibit any visible negative impacts over a period of two days.

5.3 Mid-Term Plant Health Monitoring

The next VI is performed in scope of the mid-term plant health monitoring, two weeks after treating the plants with 6 % H_2O_2. Still, there are no white spots or changes in firmness on the leaf surfaces visible.

Figure 5-1 demonstrates an example of a cucumber plant of test compartment C1 on the left and a plant of the control compartment C2 on the right. The C1 sample (on the left) is 33 cm high, whereas the C2 sample (right) is approximately 60 cm high. Figure 56 depicts only one plant of each compartment. Nevertheless, differences in growth are already visible comparing the whole batches of plants. The untreated control plants are on average 10 cm higher than the plants treated with 6 % H_2O_2.

Figure 5-1: Mid-term Plant Health Monitoring – Cucumber (left: C1, right: C2)

A similar inhibition of growth can be observed for lettuce as well. Figure 5-2demonstrates lettuce plants after a period of two weeks.

Figure 5-2: Mid-term Plant Health Monitoring – Lettuce (left: C1, right: C2)

The plants treated with 6 % H_2O_2 (lettuce and cucumber) from test compartment C1 appear to be smaller in comparison to the untreated control plants from control compartment C2.

However, mid-term plant health monitoring is performed to receive an impression about the growth of the plants. The long-term plant health monitoring provides detailed viable results examining and comparing the biomass of test and control plants.

5.4 Long-term Plant Health Monitoring

Besides a further VI, four measurements are carried out in scope of the long-term plant health monitoring: NF, LAI, FW, and DW.

In all subsequent bar charts, the test plants are illustrated by a red bar while the bars of the controls are colored green. The analysis is performed per tables calculating a mean value of 14 cucumber and 32 lettuce plants per table numbered from left to right.

Visual Inspection (VI): Visual inspection is performed to identify visible changes.

After four weeks, the plants and particularly the leaves treated with 6% H_2O_2 exhibit visible changes. The following Figure 5-3 demonstrates a comparison of the first pair of leaves developed by the plants.

The first striking feature of the leaves in the top row are emerging white spots on the leaf surface. The second striking feature of the leaves in the top row is augmented surface dried out at the edges of the leaves. Additionally, the color of the leaves in the bottom row is dark-green while the color of the treated leaves is slightly brighter.

Figure 5-3: Impact of H_2O_2 on Cucumber Leaves (Top row – treated; Bottom row – untreated)

Besides these features, the test plants seem to be smaller in average height than the control plants. Therefore, an unscheduled additional height measurement of each cucumber plant is performed. The mean height of cucumber plants per tables is shown inFigure 5-4.

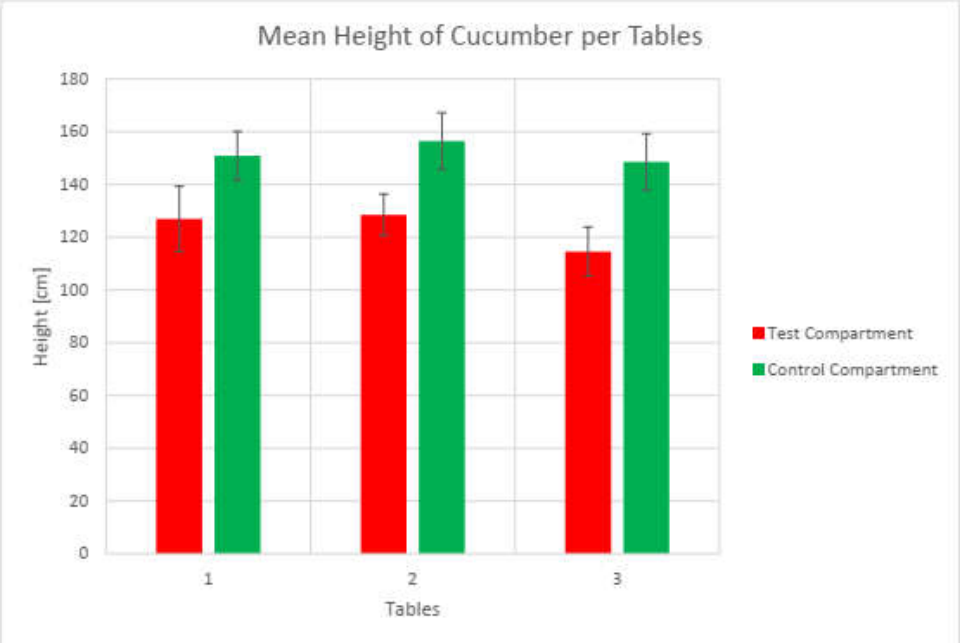

Figure 5-4: Mean Height of Cucumber

The measurements are performed per tables including 14 cucumber plants per bar. Since the reduction is observed on every table, a significant growth reduction of the test plants is stated with a mean difference in growth of minimally 24 cm and maximally 34 cm The relative growth reduction is approximately 18.8 % (calculated by the means of all three tables).

Number of Flowers (NF): The number of evolving buds and flowers is counted.

The mean values of counted NF is depicted in Figure 5-5 on the following page. For the number of flowers (as well as evolving buds and upcoming fruits), the plots show a similar result as observed during the height measurements of cucumber plants since all test plants treated with 6 % H_2O_2 demonstrate a remarkable reduction in their potential to evolve fruits. While the mean value of controls remains stable at approximately 19 NF per plant, the treated test plants are not able to develop more than 13 NF in average per table. On table 1, the strongest reduction occurred since not even 12 NF evolved in average. The relative reduction for the NF is approximately 35.7 %. Thus, counting the NF shows a significant reduction in the plant's potential to evolve fruits since the green bars are remarkably higher than the red bars.

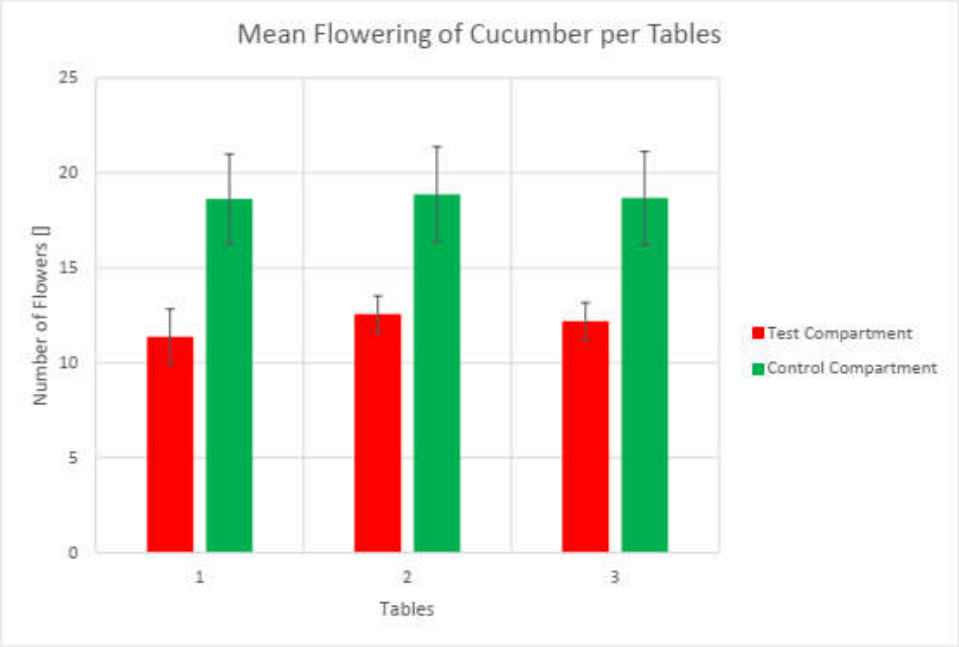

Figure 5-5: Mean NF of Cucumber

Leaf Area Index (LAI): Surface area of the leaves is measured.

The LAI is measured for both, cucumber and lettuce. The results of the LAI of cucumber are charted in

Figure 5-6 and lettuce in Figure 5-7 on the following page. The mean LAI of cucumber remains constant for all three tables at approximately 6000 cm² while a difference compared to the treated samples is

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recognizable. The test plants treated with H_2O_2 show a clear reduction in the LAI. The lowest value (~4700 cm²) is measured on table three. The relative reduction of the LAI is approximately 16.1 %.

The mean LAI for lettuce is calculated from 32 lettuce plants per table. The relative LAI of test plants is 12.7 % lower compared to the test plants treated with 6 % H_2O_2. However, the standard deviation (SD) for the LAI of lettuce is particularly high, which should be considered in further evaluations.

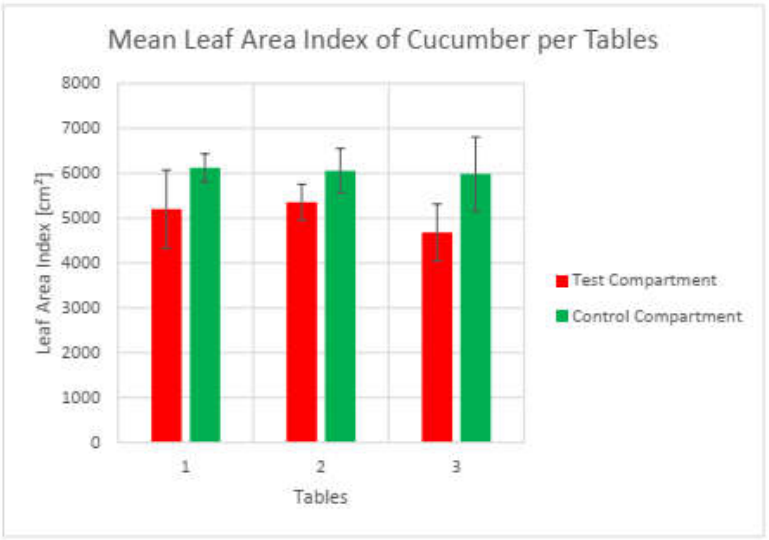

Figure 5-6: Mean LAI of Cucumber

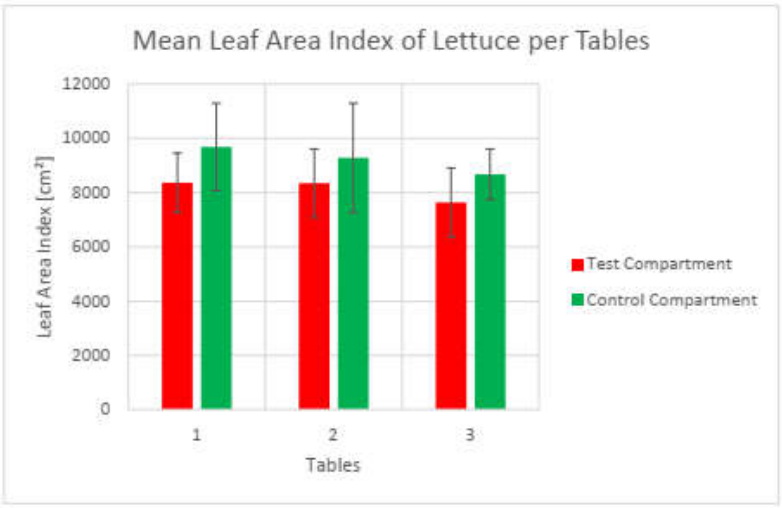

Figure 5-7: Mean LAI of Lettuce

Fresh Weight (FW): Measurement of plant weight including water content is done.

The following figures provide an overview of the FW measurements for cucumber and lettuce. The FW of cucumber stems and leaves is measured separately. Figure 5-8 shows the FW measurements of the cucumber stems.

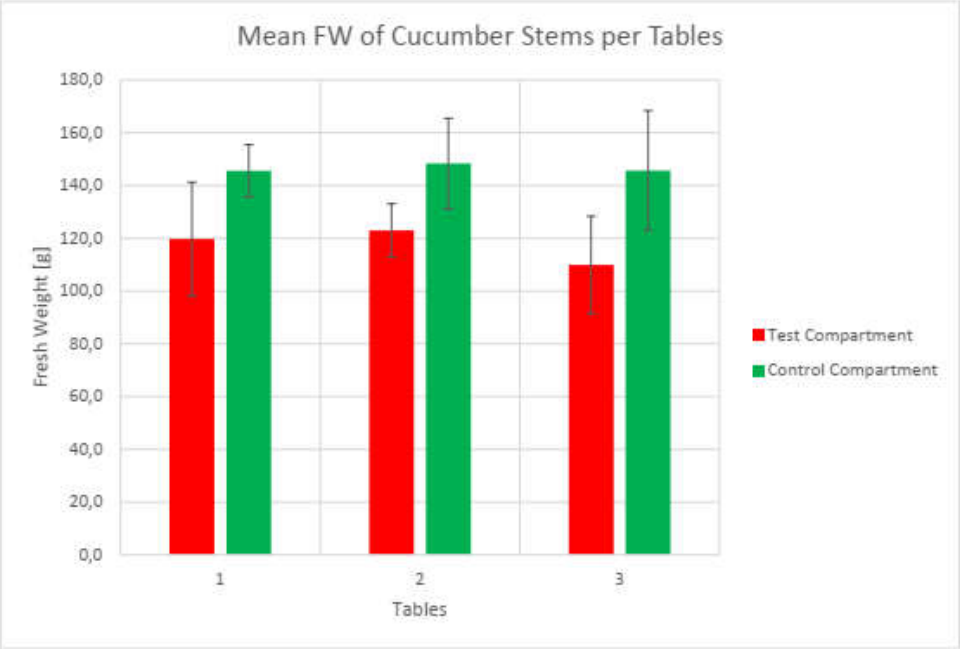

Figure 5-8: Mean FW of Cucumber Stems

Again, the chart demonstrates stable results on all three tables for the FW of cucumber stems. Comparing the control to the test samples, a distinct decrease in FW for cucumber and particularly for the stems is observed. The difference accounts minimally 25 g (table 2) and maximally 36 g (table 3). Comparing the FW average of controls and test samples, the biomass of plants treated with 6 % H_2O_2 is relatively reduced approximately 19.6 %.

Figure 5-9 identifies the FW measurements for the cucumber leaves. Again, a reduction of the biomass is observed since the bars of the three controls are higher than the bars of the treated plants. Also, the results of the controls are stable at 95 g while the treated plants show increased differences among all three tables. Calculating the differences between the mean FW of control and test samples, a relative discrepancy of approximately 15.2 % occurred.

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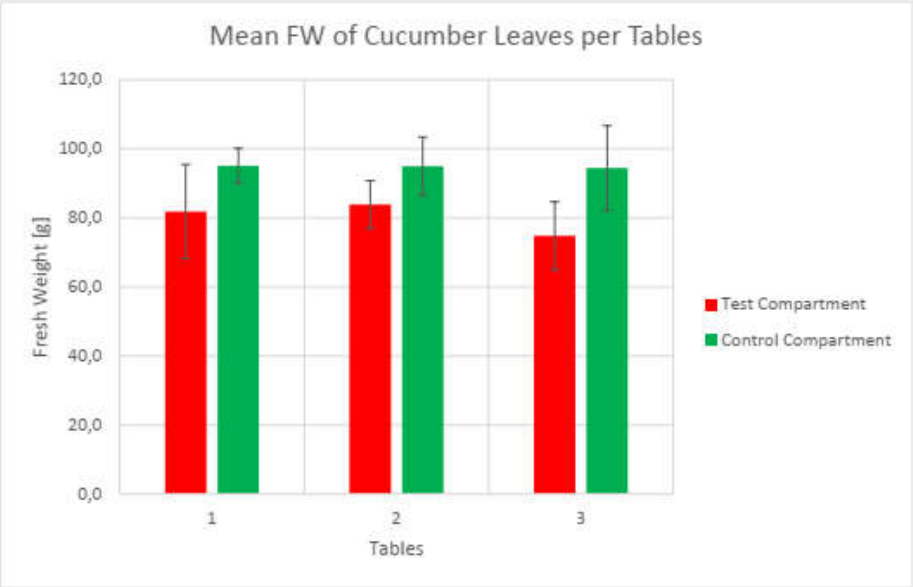

Figure 5-9: Mean FW of Cucumber Leaves

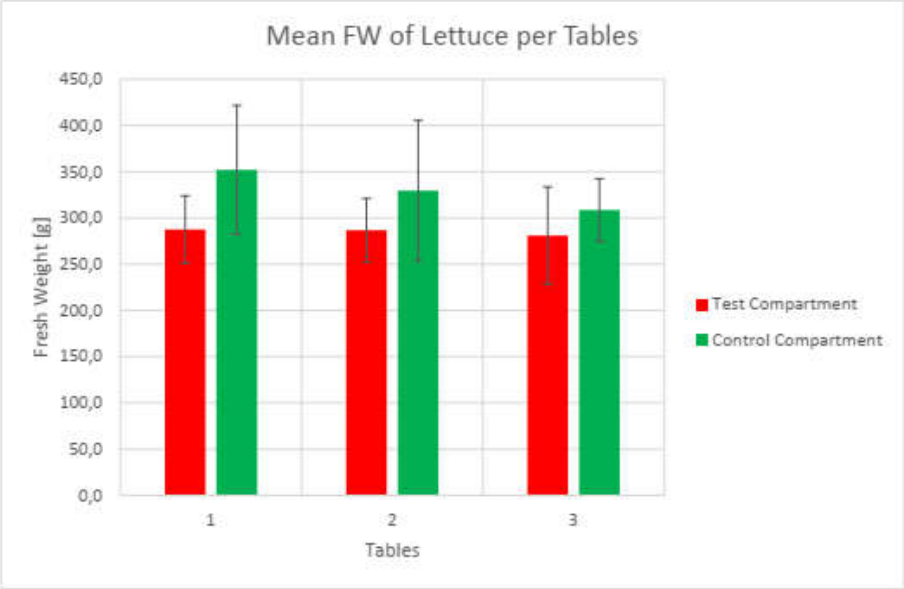

Figure 5-10: Mean FW of Lettuce

Figure 5-10 highlights the mean FW of lettuce per tables. The diagram shows a higher FW amount for the controls compared to the test samples. In this figure, the value of the test samples remains stable at approximately 285 g while a discrepancy within the control samples occurred. Nevertheless, a relative reduction of 15.5 % comparing the means of test and controls is observed.

Dry Weight (DW): Measurement of the plant weight excluding water content.

The following figures demonstrate the results of the DW measurements. Cucumber stems and leaves are measured separately (in the same way as for the FW). Figure 5-11 depicts the results of the DW measurements of cucumber stems.

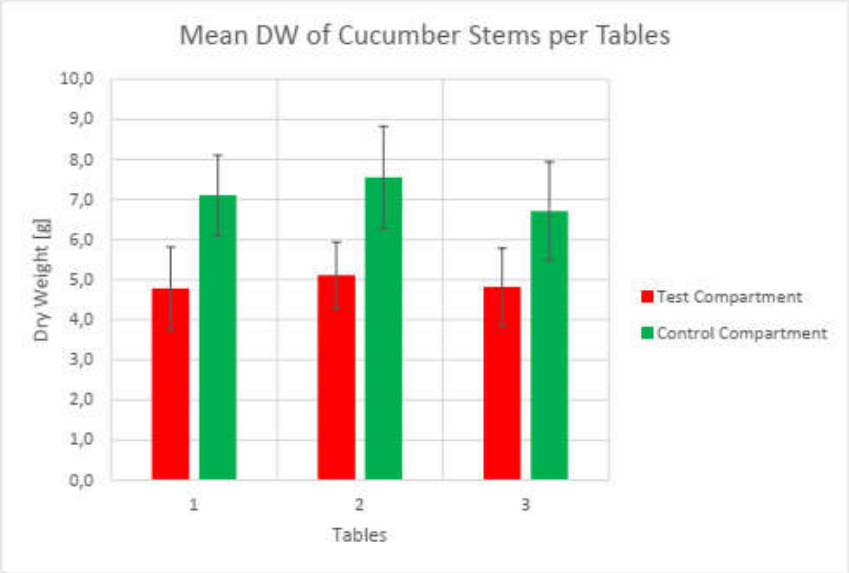

Figure 5-11: Mean DW of Cucumber Stems

The chart clearly indicates that the relative amount of biomass is even higher for the DW measurements compared to the FW measurements. Calculating the means of all three tables, the relative dry biomass (without water content) of stems treated with 6 % H_2O_2 is 31.2 % lower compared to the untreated controls.

Figure 5-12on the following page presents the results of the DW of cucumber leaves. The relative reduction for treated plants accounts 19.2 %.

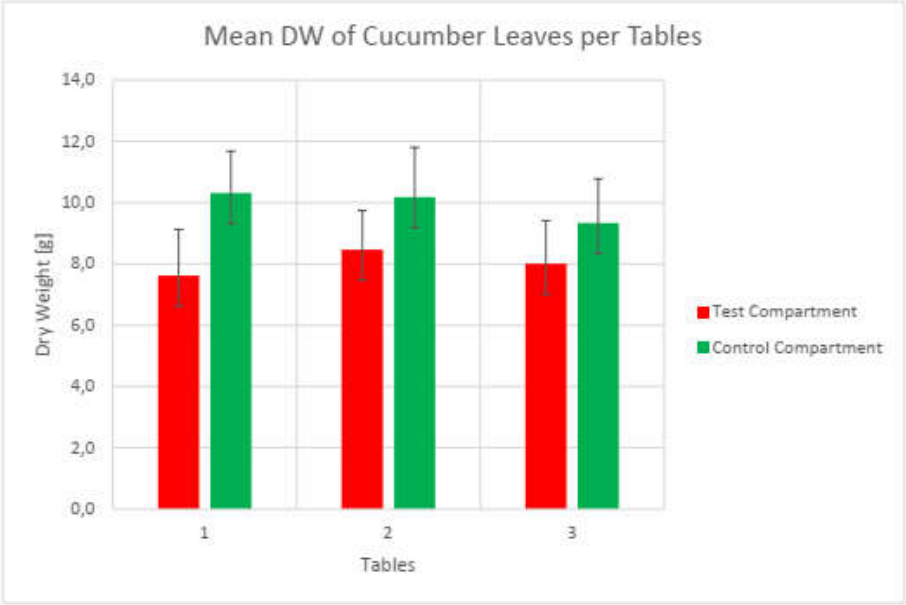

Figure 5-12: Mean DW of Cucumber Leaves

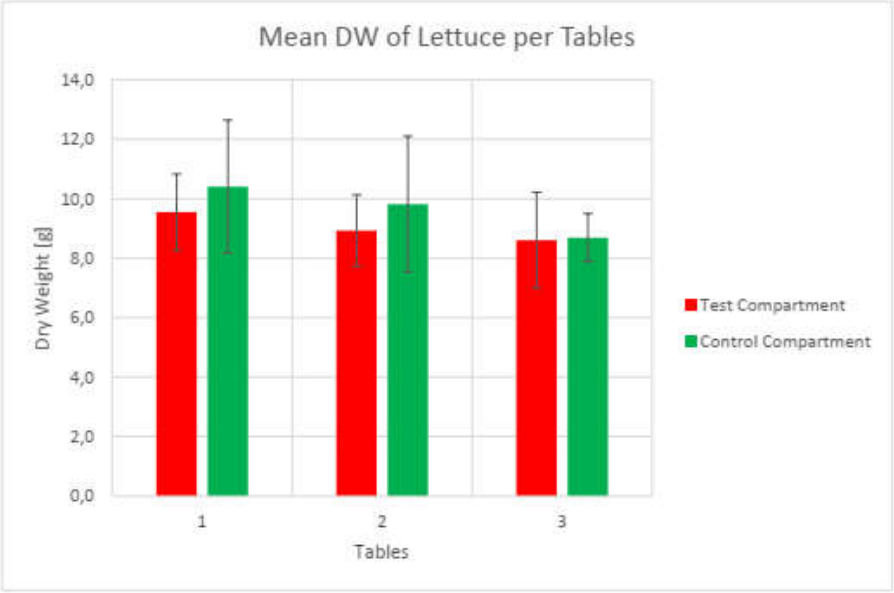

Figure 5-13: Mean DW of Lettuce

Figure 5-13 depicts the results of the DW measurements of lettuce per tables. On tables 1 and 2, a decrease in DW can be observed while the DW on table 3 is only slightly higher for the untreated plants

compared to the plants, which experienced the H_2O_2 HPV treatment. Likewise, the calculated reduction of all means accounts 8.4 %, which is the lowest relative discrepancy of all measurements performed in scope of the long-term plant health monitoring.

Table 5-1: Relative Growth/Biomass Discrepancies provides a summarizing overview of the absolute and relative discrepancies in growth/biomass calculated by the means of all test samples compared to their respective controls.

Table 5-1: Relative Growth/Biomass Discrepancies

Relative Discrepancies						
Parameter	Cucumber			Lettuce		
	C1	C2	Relative	C1	C2	Relative
Height	123,33 cm	151,96 cm	18.8 %	n/a	n/a	n/a
NF	12,04	18,73	35.7 %	n/a	n/a	n/a
LAI	5077,4 cm^2	6053,78 cm^2	16.1 %	7083,96 cm^2	8113,50 cm^2	12.7 %
FW S	113,78 g	141,44 g	19.6 %	n/a	n/a	n/a
FW L	77,59 g	92,46 g	15.2 %	246,96 g	282,18 g	15.5 %
DW S	4,75 g	6,90 g	31.2 %	n/a	n/a	n/a
DW L	7,78 g	9,63 g	19.2 %	7,86 g	8,58 g	8.4 %

The height of cucumber treated with 6 % H_2O_2 is reduced 18.8 %. The NF value representing the amount of provided fruits, inferior evolvement of 35.7 % occurred. Concerning the LAI of cucumber, the measured leaf area of test samples is 16.1 % smaller compared to the controls. Likewise, a reduction for the LAI of lettuce occurred accounting 12.7 %. The FW of cucumber stems treated with H_2O_2 is 19.6 % smaller compared to the controls. Next, the FW of cucumber leaves is 15.2 % lower while the FW of lettuce is 15.5 % reduced. The DW of cucumber stems is reduced approximately one third to 31.2 %. Finally, the DW of cucumber leaves is 19.2 % lower compared to the controls, while a relative reduction of 8.4 % occurred for lettuce.

5.5 Microbiological Analysis

The following subchapter provides the results of the microbiological evaluation performed by DLR. The HPV treatment on microorganisms is performed twice. The first test run is executed on 8 November and the second test run is carried out on 6 December. The reason for this interjacent period of four weeks is due to several factors. First, during the period of test execution, there is only a limited availability of greenhouse compartments. Second, the experiment cannot be repeated until the test compartment is empty since the plants are intended to be treated only once with hydrogen peroxide. Third, the repetition of the experiment is only executed with microorganisms and without a new batch of test plants considering that no plants inside a greenhouse compartment have the same impact on the circulation of the H_2O_2 aerosol as having small plants.

Test Run #1:

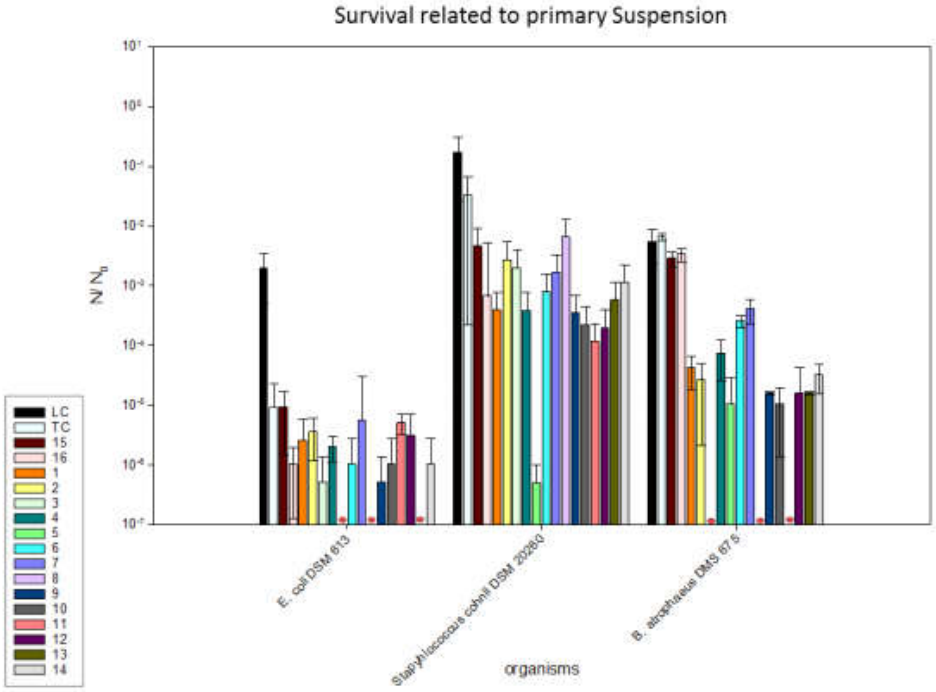

Figure 5-14: Survival of Microorganisms related to Suspension - Test Run #1

Figure 5-14: Survival of Microorganisms related to Suspension - Test Run #1 depicts the overall results of the microbiological analysis after the exposure to 6 % H_2O_2 related to the primary suspension for the first test run. The bars in the figure below are ordered by microorganisms from left to right: *Escherichia coli* DSM 613, *Staphylococcus cohnii* DSM 20260, and *Bacillus atrophaeus spores* DSM 675. The values are plotted by dividing the test sample (N) by the related control (N_0).

E. coli exhibits the highest reduction rates among the three microorganisms under investigation. On tablets 5, 8, and 13, no surviving colonies are counted after the exposure to H_2O_2. Comparing the laboratory control (LC) to the primary suspension, a reduction of almost three log levels occurred by drying the microorganisms under the laminar cleanbench. A further reduction of more than two log levels is observed by comparing LC to the transport control (TC). Thus, the bulk (approximately five log levels) of *E. coli* is already lost during preparation and transport. Control samples 15 and 16 show a high variance among the CFU count with a mean differentiating approximately one log level. The following figure 70 demonstrates the 14 test samples related to the mean of control samples 15 and 16 equally exposed to 70 % relative humidity.

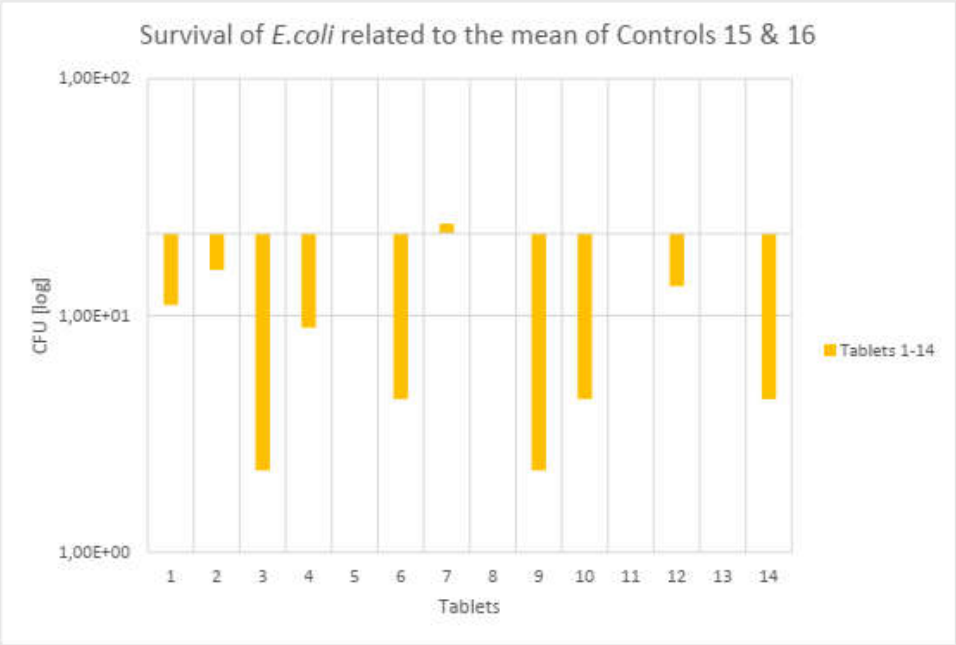

Figure 5-15: Survival of *E. coli* related to Controls 15 and 16

Related to the mean of control samples 15 and 16, a relative reduction of microorganisms is observed on twelve test samples. The CFU count on sample 7 is higher than the mean while the count on tablet 11 matches the calculated mean at 2.22 E01. However, since a standard deviation of approximately one log level is observed on the control samples, the reduction of *E. coli* is not considered significant. Furthermore, the bulk of approximately five log levels of *E. coli* is lost during drying and transport. Due to these reasons and the findings from the Diosol Exposure Test, *E. coli* drops out as test strain using this preparation method for further testing.

Staphylococcus cohnii exhibits the lowest reduction rates among the three organisms under investigation. Comparing the CFU counts for LC and TC to the rates of *E. coli*, Staphylococcus cohnii turns out to be more robust pertaining to drying and transport since not even two log levels are lost. However, one sample of the triplicates showed higher reduction rates on the TC leading to an increased negative value for the standard deviation of almost three log levels. On sample 5, a disproportional high reduction occurred compared to the other test samples.

Figure 5-16: Survival of *Staphylococcus cohnii* related to Controls 15 and 16 depicts the survival of *Staphylococcus cohnii* related to the mean of controls 15 and 16 calculated to 1.15 E04. Samples two and eight exhibit higher CFU counts compared to the related controls. The remaining samples show a reduction of *Staphylococcus cohnii* of approximately one log level, which is not considered significant regarding the standard deviation within the control samples

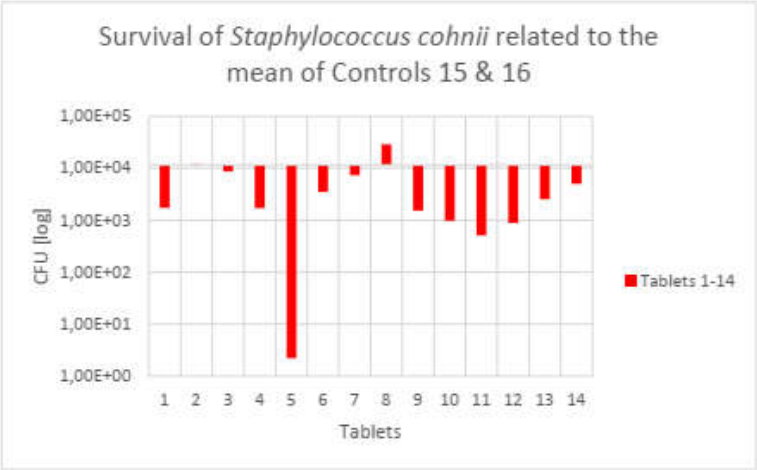

Figure 5-16: Survival of *Staphylococcus cohnii* related to Controls 15 and 16

Bacillus atrophaeus spores turn out to lose about two log levels by drying. Comparing LC and TC to the respective controls of *E. coli* and *Staphylococcus cohnii*, no further CFUs are lost during the transport.

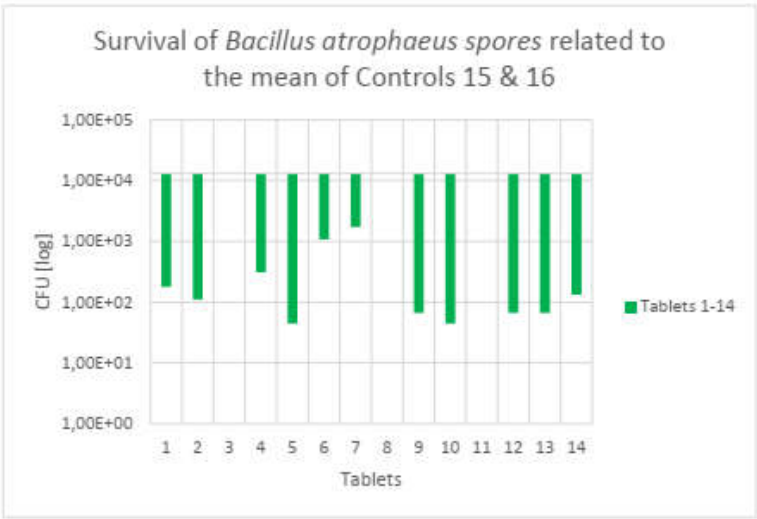

Figure 5-17: Survival of *Bacillus atrophaeus spores* related to Controls 15 and 16

Moreover, Bacillus atrophaeus spores present itself more robust to humidity comparing the mean reduction of controls 15 and 16 to the transport control. Nevertheless, a reduction of approximately one log level is achieved. No CFUs are counted on the samples 3, 8, and 11. The highest reduction rates can be observed on samples 5 and 10. Figure 5-17demonstrates the survival of Bacillus atrophaeus spores related to the mean of controls 15 and 16. Compared to E. coli and Staphylococcus cohnii, there is no sample possessing a value above the mean of the controls calculated to 1.31 E04. In addition, all samples present a consistent reduction

for Bacillus atrophaeus spores of approximately two log levels except samples 6 and 7. Considering that these samples are placed below the tables, a reduction of still one log level is achieved. Regarding the standard deviation of the control samples (there is almost no deviation), the reduction achieved by the treatment with the HPV procedure is considered significant.

Test Run #2:

Figure 5-18 presents the survival of *Staphylococcus cohnii* and *Bacillus atrophaeus spores* related to a different primary suspension used for the second test run ordered by organisms. Since the bulk of E. coli died during drying and transport of the first test run (due to the method), this strain is not incorporated in further experiments and evaluations.

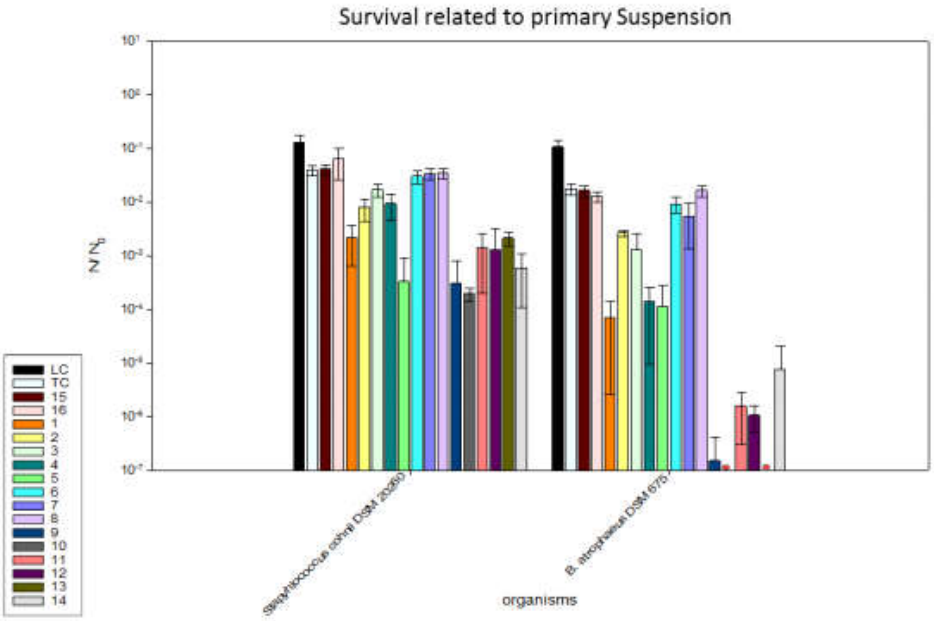

Figure 5-18: Survival of Microorganisms related to Suspension - Test Run #2

Staphylococcus cohnii provides stable results in scope of the second test run. Examining LC, only one log level is lost during drying the microorganisms. A difference compared to the first test run is observed since no further reduction occurred during transport (TC) and exposure to a high relative humidity in the control compartment (15 and 16). The samples placed below the tables (6, 7, and 8) exhibit the lowest reduction rates while the highest reduction rates can be stated for samples 5, 9, and 10. Figure 5-19 provides an overview of the survival of *Staphylococcus cohnii* related to the mean of controls 15 and 16. According to the data, a relative reduction compared to the controls occurred on every sample. However, the reduction on the samples placed under the tables (6, 7, and 8) is neglectable due to the small rates. Except samples 2, 3, and 4, a reduction of at least one log level is observed.

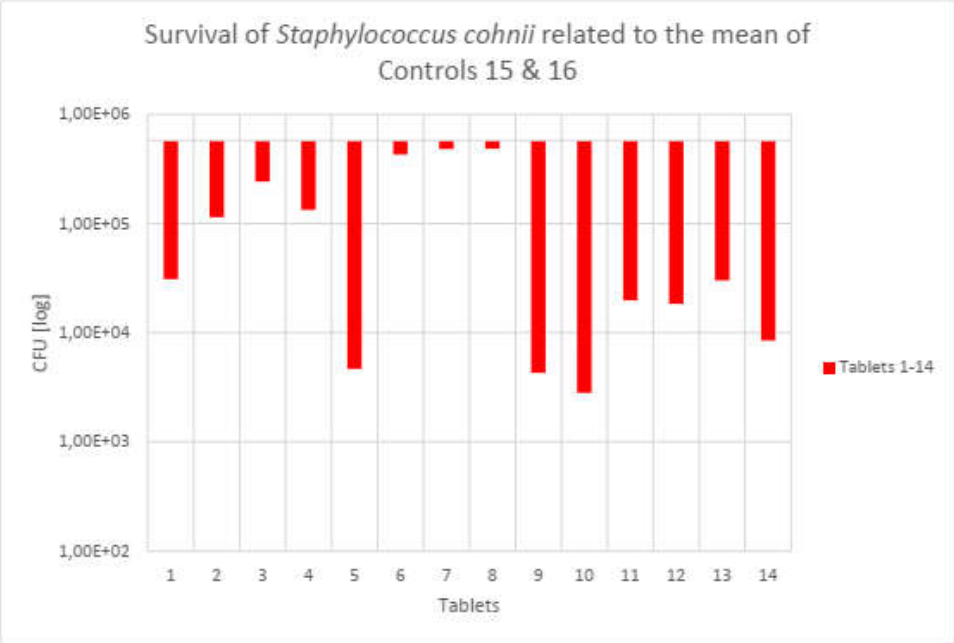

Figure 5-19: Survival of *Staphylococcus cohnii* related to Controls 15 and 16

Regarding the standard deviation of the control samples, there is almost no deviation within the samples at all. Thus, a minor significant reduction of Staphylococcus cohnii of at least on log level is stated for the samples positioned on the tables (9-14).

Bacillus atrophaeus spores provide stable results for the second test run. Figure 5-18 shows only a small range of standard deviation for the controls LC, TC, 15, and 16. During the drying procedure, approximately one log level CFUs is lost (see LC). Another log level is lost during transport (TC), while no further impairments are observable for the controls exposed to a relative humidity of 70 % (comparing TC to 15 and 16). No CFUs are countable on the glass slides of samples 8, 10 and 13. The highest reduction rates are documented on samples 9, 10, 11 and 14. The lowest reduction rates are again observed for the samples 6 and 7 positioned under the tables inside the test compartment. Figure 5-20 exhibits no values above the mean of the control 15 and 16 resulting in a stateable reduction for all samples, on which CFUs are countable after the HPV treatment. The vertically positioned samples 1-5 show a significant relative reduction rate of one to two log levels, while a significant relative reduction of at least three log levels is observed for the samples 9, 11, 12, and 14 placed on top of the tables. A minor neglectable reduction of *Bacillus atrophaeus spores* is observed on the samples 6 and 7 positioned below the tables inside the test compartment. This minor reduction is considered insignificant.

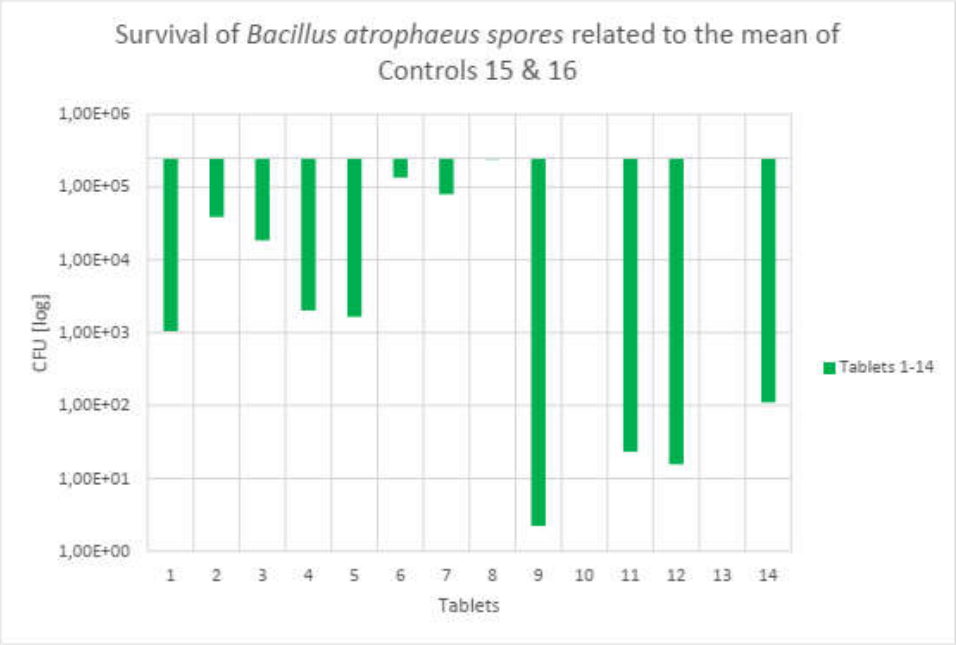

Figure 5-20: Survival of *Staphylococcus cohnii* related to Controls 15 and 16

To sum up, the method (drying and transport) turns out to be inadequate for *E. coli*. Moreover, the controls exhibit a high standard deviation leading to insignificant results of the test samples. The observed results of the first test run are equally to the results gained from the Diosol Exposure Test emphasizing that the bulk of E. coli dies during the drying procedure (and transport). Therefore, the *E. coli* strain is not considered in further experimental test runs and evaluations. *Staphylococcus cohnii* provides stable results in both test runs. However, a minor significant reduction can only be stated for the test samples (in test run two) positioned on top of the tables. *Bacillus atrophaeus spores* exhibit significant results especially for the samples positioned on top of the tables for both test runs. The vertically positioned samples show only minor significance. A neglectable insignificant reduction is observed on the samples placed below the tables.

6 DISCUSSION

The following chapter provides the discussion of the results gained from the EDEN H_2O_2 Decontamination Test.

The plant health monitoring is performed in four steps. No visible changes or differences in growth were observed in scope of the immediate and short-term plant health monitoring. The first differences in growth were recognized in scope of the mid-term plant health monitoring. Nevertheless, the visible impacts (see figure 58) were not seen until the long-term plant health monitoring was performed, although it is assumed that these changes already showed up at this particular time (mid-term monitoring).

The analysis of the long-term plant health monitoring reveals that a reduction in growth and biomass occurred for both plants treated with 6 % H_2O_2. This effect is stateable without exception for every parameter (Height, Flowering, Leaf Area Index, Fresh Weight, and Dry Weight) investigated in scope of the plant health monitoring. Comparing the results of both test plants, the impairments are even more obvious for cucumber (since it is a fast-growing plant). Moreover, the HPV treatment executed with the decontamination system indicates no directional dependencies for plants since the reduction rates of growth/biomass are evenly distributed on all three tables.

A minor issue arising during the long-term measurements depicted in figure 76 is the infestation by thrips (small insects) on a few control plants on table 1.

Figure 6-1: Infestation by Thrips

The infestation by thrips occurred recently before performing the final measurements (if not, thrips had spread all over the compartment). Besides, this recent infestation of thrips is assumed to have no negative impacts on the results (since only a few controls on table 1 were infested). This assumption corresponds with the data gained from the measurements since no negative impacts or conspicuities can be stated for the data of table 1 compared to tables 2 and 3.

The climate data is checked carefully to exclude biased results due to different growth conditions. Minor favorable growth conditions are observed for the plants treated with 6 % H_2O_2. Nevertheless, since the test plants exhibit a significantly worse outcome, these minor different conditions are neglectable (and even considering these significant, they provide favorable growth conditions for the plants treated with H_2O_2).

However, a crucial issue not clarified by the measurements performed in scope of the plant health monitoring is, whether the damage induced by the HPV treatment is temporary or permanent. Moreover, these measurements reveal no information about the processes that triggered the damage. Consulting the experts from WUR to discuss this issue, it is assumed to be most likely that a damage in the photosynthetic machinery eventuated.

To prove this assumption, it is necessary to perform further investigations on plant metabolism processes. In order to receive viable data on these metabolic processes, measurements (e. g. chlorophyll fluorescence) should have been performed before, subsequently, and further after executing the HPV treatment on 8 November. Since changes or differences in growth and biomass were considered but expected to be very unlikely as described in literature (investigating the impacts of HPV treatment with even 10 % H_2O_2 on lettuce without any remarkable changes), no further measurements on plant metabolism were executed.

The results presented in chapter four reveal a distinct negative impact on growth and biomass of cucumber and lettuce (since influences by climate data and thrips are excludable) after a HPV treatment with 6 % H_2O_2. However, a distinct reason explaining the processes that triggered the damage cannot be determined by the results of the measurements performed in scope of the plant health monitoring.

The microbiological evaluation presents varying results among the three organisms under investigation.

E. coli exhibits the highest reduction rates compared to *Staphylococcus cohnii* and *Bacillus atrophaeus spores*. However, the laboratory control (LC) and transport control (TC) identify that the treatment with 6 % H_2O_2 does not induce these high reduction rates. As already recognized in scope of the EDEN Diosol Exposure Test, in which 97.11 % died (and now confirmed by the first test run of the EDEN H_2O_2 Decontamination Test), the applied method of drying and transport is unsuitable to carry out experiments with *E. coli*. Comparing the primary suspension to LC, almost three log levels of CFU are lost while further two log levels are lost during transport (TC). High variances and standard deviations among the controls 15 and 16 lead to insignificance regarding the results for *E. coli*.

Staphylococcus cohnii turns out to be robust to drying and transport comparing the primary suspension to LC and TC. One log level is lost during drying while another log level is lost during transport. *Staphylococcus cohnii* presents itself more sensitive to humidity in the first test run comparing TC to controls 15 and 16. Particularly, a high reduction is observed on tablet 5 for both test runs. Variance and deviation of the controls 15 and 16 lead to insignificant results for the vertically positioned samples (1-5). A significant reduction can be stated for the tablets positioned on top of the tables in test run two (9-14) while the least reduction is achieved on the tablets placed under the tables (6-8).

Bacillus atrophaeus spores are just as *Staphylococcus cohnii* robust to the applied method of drying and transport (comparing LC and TC to the primary suspension). Furthermore, there is no reduction observed while exposing controls 15 and 16 to 70 % relative humidity inside the control compartment for 120 minutes. *Bacillus atrophaeus spores* show significance (due to low variance and SD) in both test runs varying dependent on their position inside the test compartment:

- Tablets 9-14 (on the tables): 3-4 log levels reduction
- Tablets 1-5 (vertically positioned): 1-2 log levels reduction
- Tablets 6-8 (under the tables): no reduction

Therefore, *Bacillus atrophaeus spores* turn out to be the most sensitive organism to the HPV treatment with 6 % H_2O_2 among the organisms under investigation. Considering the results of the microbiological evaluation, it can definitely be stated that the 6 % H_2O_2 vaporized by the decontamination system is not distributed homogenously all over the compartment (as assumed from the plant health monitoring). The highest reduction rates are achieved on the tablets positioned on top of the tables struck by the spray jet of the nozzle. A medium reduction is achieved for the tablets positioned vertically on the walls of the compartment while almost no reduction can be observed for the tablets positioned under the tables.

Still, two further issues arose during the experiment and the respective evaluation. These issues are discussed in the subsequent section:

1. Particularly high reduction rates on tablet 5
2. Discrepancies among controls 15 and 16 during the first test run

Particularly, high reduction rates are observed for the samples on tablet 5 in both test runs. The following figure depicts tablet 5 and reveals information about a potential impact of the accumulation of water condensate on this tablet. The formation of water condensate is highlighted with red circles in Figure 6-2 and can also be observed on the window around the tablet.

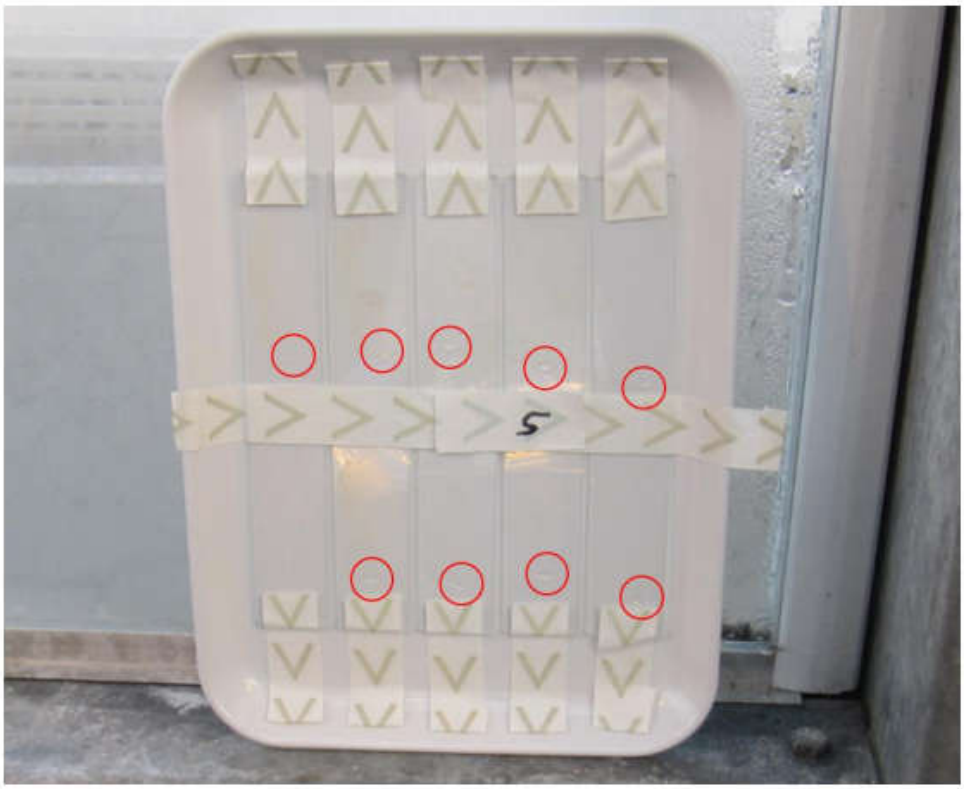

Figure 6-2: Accumulation of Water Condensate on Tablet 5

Tablet 5 is the only tablet positioned at this side wall. Conspicuously, this side wall is the only one bordering outdoors – the other side walls are bordering other greenhouse compartments. Since the test is executed in November and December, low temperatures of approximately 5 °C prevail during this time of the year, while the temperature inside the compartment is controlled at 18-19 °C. Due to the ability of warm air to store more humidity than cooler air (equal pressure is required), a boundary layer forms at the side wall accumulating water condensate at the surfaces of the tablet and side walls. Additionally, the suspension inoculated on the glass slides is laced with PBS (a salty buffer) being even more susceptible to water.

For these reasons, the glass slides on tablet 5 are exposed to water condensate probably washing the suspension off as the tablet is positioned vertically. Since this issue cannot be clarified afterwards, tablet 5 is not respected in test run one. In scope of the second test run, it is ensured that no suspension poured off the glass slides. Nevertheless, the results of tablet 5 are still considered critically due to the accumulation of water condensate.

The second issue identified is the deviation among controls 15 and 16 during the first test run. An inferior CFU count occurred on the glass slides of tablet 16 positioned on table 3 inside the compartment.

One reason for this deviation is explainable by the positioning of the humidifiers controlling the relative humidity inside the compartment. The nozzles of the humidifiers are mounted closely to the position of tablet 16, next to table 3, in both compartments. Therefore, tablet 16 has potentially experienced more humidity than tablet 15. However, the activity of the nozzles cannot be reproduced since they are activated automatically if necessary to control the humidity at the predetermined value. Thus, investigating the impact of condensate and rH on samples prepared with the method of drying and transport provides tasks for future work.

Closing the discussion, some aspects like the accumulation of water condensate could not be excluded from the experiment (but still they are taken into account). Furthermore, an aspect that always has to be considered in microbiological experiments is the fact that living organisms sometimes do not behave as expected. Nevertheless, a distinct impairment on the test plants as well as reduction of *Bacillus atrophaeus spores* can be stated by the HPV treatment with 6% H_2O_2.

7 CONCLUSION

The overall goal of this test is to investigate and evaluate the influences of the decontamination system and agent applied with the HPV procedure in a bio-regenerative closed-loop life support system like the FEG, which will be sent to Antarctica in October 2017. Previously, within the scope of work, two critical success criteria are defined examining the applied system and method on its suitability for decontamination purposes inside a BLSS, further transient facilities for a space flight to Mars, or a permanent habitable lunar station (see chapter 1.1):

1. Impairments of the plants to the test agent (H_2O_2)
2. Efficiency to reduce microorganisms

Two experiments are performed in this thesis investigating the system on these success criteria (EDEN Diosol Exposure Test and EDEN H_2O_2 Decontamination Test).

Rating the results and considering that a significant decrease in growth and development of biomass occurred for lettuce and particularly cucumber, the plants assessed the most suitable for space applications according to literature, the method is evaluated inadequate for disinfection purposes in bio-regenerative closed-loop life support systems.

The HPV procedure using a solution of 6 % H_2O_2 does not provide the desired results of reducing microorganisms. The decontamination system exhibits a highly inhomogeneous distribution of H_2O_2 inside the compartment since the samples located in the jet of the nozzle demonstrate the higher reduction rates compared to the samples positioned under the tables. Furthermore, the application of H_2O_2 as broadband disinfectant turns out to be inappropriate since inadequate reduction of *Staphylococcus cohnii* is achieved. Additionally, further findings proved (in both experiments) that the applied method is unsuitable to perform experiments with *E. coli* since the bulk of CFUs dies due to the procedure.

Concluding, the violation of both success criteria leads to the result that the decontamination system and agent used in scope of the project EDEN ISS are inadequate for disinfection purposes in bio-regenerative closed-loop life support systems.

7.1 Limitations

The overall goal of scientific research is to exclude all potential limitations and criteria influencing the results of an experiment in a negative way. However, in some cases, these limitations arise due to a lack of time and budget or are not considered until results are available.

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First, beginning with the Diosol Exposure Test, critical limitations occurred due to a lack of time and budget. Providing scientific valuable results, at least three test runs should have been executed with an arrangement of triplicates regarding the microbiological samples. Besides, the impacts of remaining H_2O_2 inside the cleanbench could not be determined for further test runs. Additionally, the bulk of *E. coli* died due to the applied method of drying, which was not discovered until the CFUs were counted after executing the test. Since only two test runs for each agent were executed and the impacts discussed in chapter 3.4 were not clearly assignable, *E. coli* was still included in the EDEN H_2O_2 Decontamination Test, although being assumed to be susceptible to the method. This strain could have been replaced by another one investigating the effects of the applied system and method.

In the same way, only two test runs with microbiological samples are executed in scope of the EDEN H_2O_2 Decontamination Test. Receiving more stable results, at least a third test run should have been executed. Furthermore, as it is demonstrated within the discussion, the impact of relative humidity and accumulating water condensate is not distinctly clarified considering the results of tablet 5 and controls 15/16 from the first test run. It is assumed to have a negative impact on growth and surviving of CFU, however additional research is required to solve this issue.

Concerning the plant health analysis, only one batch containing 96 lettuce and 48 cucumber plants treated with 6 % H_2O_2 was evaluated (and the same amount of controls). Nevertheless, treating and examining two more batches of plants with this method would provide even better and more significant results. Besides, some limitations having a possible negative influence (climate data, thryps) were taken into account but not considered to bias the results. A further issue identified and already mentioned within the discussion is the measurement of plant metabolism processes in order to determine if the damage induced by the HPV procedure is temporary or permanent.

7.2 Future Work

The determination and exclusion of such limitations provides the opportunity for new approaches generating enhanced results in scope of future work.

Since the plant health monitoring performed in this thesis gives information about the inhibition of growth and the reduction of biomass, further investigations on plant metabolism have to be carried out examining, if the growth inhibition and damage is temporary or permanent. There are several approaches accomplishing these further investigations. The most suitable method is a continuous measurement of chlorophyll fluorescence via Pulse-Amplitude-Modulation (PAM). Furthermore, since discrepancies concerning the impact of H_2O_2 on the test plants occurred, additional experiments investigating the influences of the applied method on other plants should be executed.

The microbiological analysis provides even more space for future work. A crucial issue is to examine the impacts of relative humidity and water condensate on samples prepared by the method described in this thesis.

Besides, further strains should be investigated and the fact why *Bacillus atrophaeus spores* act more sensitive to H_2O_2 than *Staphylococcus cohnii* (since spores were expected to be more robust to disinfectants than Staphylococcus).

Avoiding directional dependent disinfections (as it occurred in this experiment), the development of a sprinkling system integrated with several nozzles in a bio-regenerative closed-loop life support system appears to be a beneficial solution.

On top of that, as H_2O_2 induced a severe damage to the test plants, the choice of an alternative disinfectant should be considered. Literature proposes natural disinfectants like citral, cinnamaldehyde, or tea polyphenols recoverable from herbs and naturals exhibiting significant reduction among *Staphylococcus aureus* and *Salmonella Enteridis*.

Finally, although the decontamination system applying the HPV procedure could not be stated suitable for disinfection purposes in the EDEN ISS project, the experiments performed in scope of this thesis uncovered essential findings. Moreover, continuing research in this field of work is of importance since the results provide not only advancements for the space sector. The findings achieved in scope of this work can also be transferred to terrestrial applications like the agriculture sector (vertical farms) or the medical sector (broadband disinfection of microorganisms).

8 ABBREVIATIONS

AN	A. niger
AS	Hersteller der PEN3/MLA-VI
AST	Astrium Space Transportation
ATP	Authorization to proceed
B/B	Breadboard
BS	B. subtilis
BG	Breath Gas
CE	Communauté Européenne (Europäische Gemeinschaft)
CFU	Colony forming units
CM	Configuration Management
COM	Serielle Schnittstelle
COTS	Commercial off-the-shelf
DC	Direct Current
DLR	Deutsches Zentrum für Luft- und Raumfahrt
DMS	Data Management System
DRD	Document Requirements Description
DRL	Document Requirements List
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EC	E. coli
EDU	Enrichment and desorption unit
EM	Engineering Model
EMC	ElectroMagnetic Compatibility
E-Nose	Electronic nose
E-Nose 2	Upgrade of the Electronic nose
EP	Experiment Procedure
ERD	Experiment Requirements Document
FM	Flight model
FM	Flight Model
GSE	Ground Support Equipment
H/W	Hardware
HK	Housekeeping
I/F	Interface
I/O	Input/Output
IBMP	institute biomedical problems
IMBP	Institute of Medical and Biological Problems
IMR	Ionen-Molekularreaktions-Massenspektrometrie
ISS	International Space Station
LCD	Liquid Cristal Display
LDA	Linear Discriminant Analysis
LLI	Life Limited Item
LOD	Limit of detection
LSE	Laboratory Support Equipment
LST	Lance Sampler Tip

LTU	Laptop Unit
MAIT	Manufacturing, Intergration and Test
MLA	Material für Langzeitanwendungen im Weltraum
MOS	Metal Oxide Semiconductor
MS	Massenspektrometrie
MVOC	Microbial Volatile Organic Compounds
n/a	Not available
NMML	Non Metallic Material List
P/L	PayLoad
PA	Product Assurance
PI	Principle Investigator
PA&S	Product Assurance & Safety
PC	Personal Computer
PCA	Principal Component Analysis
PCB	Printed Circuit Board
PCU	Power Control Unit
PDR	Preliminary Design Review
PDRD	Performance and Design Requirements Description
PE	P. expansum
PEN3	Portable Electronic Nose 3
PLS	Partial Least Squares
PPM	Parts per million
PTFE	Polytetrafluorethylen
PVC	Polyvinylchlorid
QA	Quality Assurance
QM	Qualification model
Qty.	Quantity
Req	Requirement
Resp	Institution responsible for the corresponding requirement
RFQ	Request for Quotation
ROM	Rough Order of Magnitude
S/W	Software
SRR	System Requirement Review
SFPT	Short functional performance test
TBC	To be confirmed
TBC	To Be Confirmed
TBD	To Be Defined
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
UFT	Zentrum für Umweltforschung und nachhaltige Technologien der Universität Bremen
USB	Universal Serial Bus
VOC	Volatile organic compound
w.r.t.	With reference to

9 ANNEX AND ATTACHMENTS

Climate Data

Table 9-1: Climate Data cCO_2 in ppm

CO_2-Concentration (cCO_2) in [ppm]			
Time	C1	C2	BS
8:00	532	594	600
8:05	516	601	600
8:10	531	632	600
8:15	510	695	600
8:20	515	736	600
8:25	548	723	600
8:30	515	714	600
8:35	535	728	600
8:40	510	710	600
8:45	535	729	600
8:50	519	722	600
8:55	537	721	600
9:00	559	717	600
9:05	575	719	600
9:10	593	715	600
9:15	585	775	600
9:20	601	755	600
9:25	597	748	600
9:30	664	747	600
9:35	682	742	600
9:40	756	731	600
9:45	826	723	600
9:50	888	728	600
9:55	935	714	600
10:00	978	724	600
10:05	1146	701	600
10:10	1159	725	600

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10:15	1271	713	600
10:20	1300	718	600
10:25	1363	706	600
10:30	1088	708	600
10:35	1109	710	600
10:40	1058	731	600
10:45	1041	719	600
10:50	1008	722	600
10:55	973	719	600
11:00	948	715	600
11:05	910	695	600
11:10	891	714	600
11:15	880	733	600
11:20	869	731	600
11:25	852	718	600
11:30	800	700	600
11:35	798	729	600
11:40	801	741	600
11:45	767	718	600
11:50	780	714	600
11:55	732	694	600
12:00	702	725	600
12:05	663	699	600
12:10	585	646	600
12:15	534	644	600
12:20	509	621	600
12:25	503	608	600
12:30	486	609	600
12:35	476	634	600
12:40	484	667	600
12:45	457	702	600
12:50	473	728	600
12:55	450	722	600

13:00	447	698	600
13:05	440	707	600
13:10	482	711	600
13:15	520	705	600
13:20	557	740	600
13:25	575	728	600
13:30	569	739	600
13:35	569	734	600
13:40	609	751	600
13:45	605	750	600
13:50	636	723	600
13:55	637	734	600
14:00	624	715	600

Table 9-2: Climate Data T in °C

Temperature (T) in [°C]			
Time	C1	C2	BS
8:00	18,9	18,8	18,6
8:05	18,8	18,8	18,7
8:10	18,9	19,0	18,7
8:15	19,0	19,0	18,8
8:20	18,9	19,0	18,8
8:25	19,3	19,0	18,9
8:30	19,0	19,0	19,0
8:35	19,5	19,2	19,0
8:40	18,5	19,0	19,1
8:45	19,0	19,3	19,1
8:50	19,7	19,3	19,2
8:55	19,6	19,3	19,2
9:00	19,9	19,4	19,3
9:05	19,9	19,4	19,3
9:10	20,0	19,2	19,4
9:15	19,8	19,4	19,5
9:20	19,9	19,5	19,5
9:25	19,6	19,8	19,6
9:30	19,8	19,7	19,6
9:35	19,6	19,8	19,7
9:40	19,5	19,7	19,7
9:45	20,2	19,6	19,8
9:50	19,8	19,8	19,8
9:55	20,1	19,6	19,9
10:00	20,3	19,7	20,0
10:05	20,1	19,6	20,0
10:10	20,3	19,8	20,0
10:15	20,5	19,6	20,0
10:20	20,1	19,5	20,0
10:25	19,7	19,3	20,0

10:30	18,8	19,1	20,0
10:35	18,8	19,8	20,0
10:40	18,9	20,1	20,0
10:45	20,2	20,6	20,0
10:50	21,5	20,9	20,0
10:55	21,6	21,0	20,0
11:00	21,9	21,1	20,0
11:05	21,7	21,0	20,0
11:10	21,8	20,9	20,0
11:15	21,9	20,9	20,0
11:20	21,6	20,7	20,0
11:25	21,2	20,5	20,0
11:30	21,0	20,9	20,0
11:35	20,7	21,0	20,0
11:40	20,9	21,0	20,0
11:45	21,0	21,2	20,0
11:50	21,2	21,2	20,0
11:55	21,6	21,3	20,0
12:00	21,4	21,4	20,0
12:05	21,6	20,4	20,0
12:10	19,5	18,3	20,0
12:15	17,9	18,0	20,0
12:20	17,2	17,7	20,0
12:25	17,5	18,0	20,0
12:30	18,1	18,3	20,0
12:35	18,7	19,3	20,0
12:40	19,9	20,8	20,0
12:45	21,0	21,2	20,0
12:50	21,3	21,8	20,0
12:55	21,5	21,7	20,0
13:00	21,8	21,6	20,0
13:05	21,5	21,7	20,0
13:10	21,3	21,8	20,0

13:15	21,3	21,4	20,0
13:20	21,5	21,4	20,0
13:25	21,3	21,3	20,0
13:30	21,3	21,1	20,0
13:35	21,1	21,1	20,0
13:40	20,9	21,0	20,0
13:45	21,1	21,0	20,0
13:50	20,7	20,7	20,0
13:55	20,7	20,7	20,0
14:00	20,4	20,6	20,0

Table 9-3: Climate Data rH in %

Relative Humidity (rH) in [%]			
Time	C1	C2	BS
8:00	67	68	70
8:05	70	68	70
8:10	69	68	70
8:15	68	68	70
8:20	71	69	70
8:25	68	68	70
8:30	71	69	70
8:35	68	69	70
8:40	80	68	70
8:45	74	69	70
8:50	68	70	70
8:55	70	69	70
9:00	68	69	70
9:05	70	68	70
9:10	69	68	70
9:15	69	69	70
9:20	71	69	70
9:25	71	69	70
9:30	68	69	70
9:35	69	68	70
9:40	71	69	70
9:45	67	69	70
9:50	72	69	70
9:55	70	70	70
10:00	69	69	70
10:05	73	69	70
10:10	71	69	70
10:15	70	70	70
10:20	71	69	70
10:25	69	69	70

10:30	70	69	70
10:35	76	67	70
10:40	78	67	70
10:45	74	67	70
10:50	69	67	70
10:55	70	68	70
11:00	69	69	70
11:05	69	69	70
11:10	69	70	70
11:15	68	68	70
11:20	70	69	70
11:25	69	69	70
11:30	69	68	70
11:35	69	68	70
11:40	68	68	70
11:45	70	68	70
11:50	69	69	70
11:55	69	69	70
12:00	68	68	70
12:05	67	68	70
12:10	68	66	70
12:15	67	64	70
12:20	67	63	70
12:25	64	61	70
12:30	65	59	70
12:35	66	62	70
12:40	66	62	70
12:45	67	63	70
12:50	67	64	70
12:55	68	64	70
13:00	67	65	70
13:05	68	65	70
13:10	68	66	70

13:15	67	65	70
13:20	68	66	70
13:25	68	67	70
13:30	68	67	70
13:35	69	68	70
13:40	70	68	70
13:45	68	68	70
13:50	69	69	70
13:55	68	69	70
14:00	69	69	70

Results: Plant Health Monitoring

Table 1: Mean Height of Cucumber in cm

Cucumber			
Number	**Test Compartment**	**Number**	**Control Compartment**
1111	105	2111	130
1112	117	2112	153
1113	130	2113	152
1114	134	2114	148
1115	148	2115	167
1121	135	2121	157
1122	139	2122	143
1123	106	2123	158
1124	129	2124	149
1125	129	2125	151
1126	136	2126	157
1131	109	2131	142
1132	122	2132	138
1133	136	2133	155
1134	123	2134	153
1135	133	2135	161
MEAN	**126,94**		**150,88**
STABW	**12,39**		**9,20**
1211	128	2211	146
1212	127	2212	140
1213	133	2213	165
1214	122	2214	153
1215	137	2215	149
1221	122	2221	148
1222	112	2222	164
1223	119	2223	162
1224	136	2224	160
1225	133	2225	164
1226	138	2226	160

1231	124	2231	147
1232	125	2232	137
1233	125	2233	167
1234	136	2234	171
1235	139	2235	170
MEAN	**128,50**		**156,44**
STABW	**7,83**		**10,76**
1311	109	2311	147
1312	119	2312	169
1313	111	2313	158
1314	131	2314	153
1315	122	2315	145
1321	100	2321	144
1322	117	2322	141
1323	114	2323	149
1324	102	2324	141
1325	123	2325	150
1126	113	2326	164
1331	103	2331	136
1332	106	2332	162
1333	113	2333	128
1334	129	2334	145
1135	121	2335	145
MEAN	**114,56**		**148,56**
STABW	**9,30**		**10,66**

Table 9-5: Mean NF of Cucumber

Cucumber			
Number	**Test Compartment**	**Number**	**Control Compartment**
1111	9	2111	13
1112	11	2112	20
1113	12	2113	19
1114	13	2114	20
1115	13	2115	21
1121	12	2121	17
1122	13	2122	16
1123	9	2123	19
1124	11	2124	20
1125	10	2125	20
1126	13	2126	20
1131	9	2131	15
1132	11	2132	18
1133	12	2133	18
1134	12	2134	20
1135	12	2135	22
MEAN	**11,38**		**18,63**
STABW	**1,45**		**2,36**
1211	12	2211	16
1212	12	2212	14
1213	12	2213	22
1214	11	2214	18
1215	13	2215	20
1221	12	2221	18
1222	12	2222	19
1223	12	2223	19
1224	13	2224	19
1225	13	2225	19
1226	13	2226	22
1231	12	2231	17

1232	12	2232	16
1233	13	2233	18
1234	14	2234	22
1235	15	2235	23
MEAN	**12,56**		**18,88**
STABW	**0,96**		**2,50**
1311	12	2311	17
1312	13	2312	20
1313	13	2313	18
1314	13	2314	21
1315	14	2315	19
1321	10	2321	17
1322	12	2322	15
1323	12	2323	16
1324	11	2324	20
1325	13	2325	20
1126	12	2326	21
1331	11	2331	17
1332	12	2332	24
1333	12	2333	15
1334	13	2334	19
1135	12	2335	20
MEAN	**12,19**		**18,69**
STABW	**0,98**		**2,44**

Table 9-6: Mean LAI of Cucumber in cm²

Cucumber			
Number	**Test Compartment**	**Number**	**Control Compartment**
1111	3466,2	2111	5503,3
1112	4049,2	2112	6463,6
1113	5215,6	2113	5901,3
1114	5952,4	2114	6082
1115	5493,1	2115	5636,3
1121	5375,6	2121	6157,8
1122	5281,0	2122	6277,2
1123	3562,9	2123	6592,8
1124	5708,1	2124	5773,1
1125	5595,3	2125	6247,9
1126	5724,6	2126	5927,3
1131	4513,6	2131	6186,7
1132	5541,3	2132	6462,3
1133	6576,3	2133	6215,5
1134	5219,3	2134	6046,6
1135	5918,8	2135	6479,0
MEAN	**5199,58**		**6122,04**
STABW	**871,35**		**313,06**
1211	5148,5	2211	5636,8
1212	5707,2	2212	5831,9
1213	5507,3	2213	6551,6
1214	5230,3	2214	5990,6
1215	5119,7	2215	5293,7
1221	5218,3	2221	5461,0
1222	4719,8	2222	6644,1
1223	4731,4	2223	6370,0
1224	5852,4	2224	6513,4
1225	5509,4	2225	5420,0
1226	5167,9	2226	5949,8
1231	5464,9	2231	6292,6

1232	5069,4	2232	5377,4
1233	5154,9	2233	6331,2
1234	6161,8	2234	6725,8
1235	5867,1	2235	6470,3
MEAN	**5351,89**		**6053,76**
STABW	**402,06**		**496,17**
1311	4723,2	2311	5810,6
1312	5581,7	2312	6989,1
1313	4956,3	2313	6100,9
1314	5812,2	2314	7023,3
1315	5691,3	2315	6280,0
1321	3822,0	2321	5230,5
1322	4988,5	2322	5205,1
1323	4901,6	2323	5233,3
1324	4063,6	2324	6442,9
1325	4886,4	2325	6397,5
1126	4179,6	2326	6724,3
1331	3746,1	2331	4573,3
1332	4374,3	2332	7322,0
1333	4301,8	2333	4786,2
1334	4502,5	2334	5721,2
1135	4360,5	2335	5928,4
MEAN	**4680,73**		**5985,54**
STABW	**632,01**		**823,67**

Table 9-7: Mean LAI of Lettuce in cm²

Lettuce			
Number	**DM Leaves [g]**	**Number**	**DM Leaves [g]**
111	8277,2	211	9402,6
112	7315,1	212	8236,3
113	9494,0	213	11412,3
MEAN	**8362,10**		**9683,73**
STABW	**1091,93**		**1606,56**
121	9543,3	221	9968,2
122	7029,0	222	7002,6
123	8461,0	223	10856,3
MEAN	**8344,43**		**9275,70**
STABW	**1261,20**		**2018,02**
131	7039,6	231	9742,3
132	6777,1	232	8049,9
133	9095,5	233	8221,0
MEAN	**7637,40**		**8671,07**
STABW	**1269,55**		**931,65**

Table 9-8: Mean FW of Cucumber Stems and Leaves in g

Test Compartment C1			Control Compartment C2		
Cucumber			Cucumber		
Number	FW Stem [g]	FW Leaves [g]	Number	FW Stem [g]	FW Leaves [g]
1111	75,2	53,1	2111	120,4	85,2
1112	94,6	65,6	2112	151,4	100,0
1113	118,7	80,7	2113	142,7	92,0
1114	133,3	91,8	2114	140,0	92,7
1115	129,1	85,4	2115	145,3	87,8
1121	131,9	94,6	2121	143,9	96,8
1122	126,4	81,2	2122	138,6	95,6
1123	76,8	56,3	2123	158,7	99,7
1124	131,1	90,2	2124	136,7	88,6
1125	129,4	86,0	2125	145,1	95,6
1126	133,6	87,9	2126	155,2	95,7
1131	103,4	74,0	2131	142,2	99,7
1132	123,0	87,5	2132	150,4	97,3
1133	152,7	104,0	2133	147,0	95,4
1134	121,0	80,3	2134	147,6	94,9
1135	135,4	90,3	2135	163,9	105,1
MEAN	**119,73**	**81,81**		**145,57**	**95,13**
STABW	**21,52**	**13,66**		**9,93**	**5,06**
1211	123,5	79,9	2211	127,1	88,5
1212	133,4	91,5	2212	127,6	89,7
1213	124,1	84,1	2213	160,5	100,8
1214	116,0	79,8	2214	150,0	94,8
1215	119,0	78,6	2215	131,9	83,6
1221	115,7	83,1	2221	123,5	82,5
1222	113,3	78,6	2222	157,6	102,4
1223	106,2	70,9	2223	153,7	97,9
1224	132,0	90,5	2224	159,4	101,0
1225	123,8	82,6	2225	141,2	85,8
1226	122,1	81,1	2226	157,4	98,7

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1231	125,8	90,4	2231	145,5	101,1
1232	116,4	79,8	2232	127,1	81,0
1233	114,0	80,1	2233	158,4	100,5
1234	144,3	96,4	2234	175,0	106,1
1235	138,4	94,5	2235	177,6	104,8
MEAN	**123,00**	**83,87**		**148,34**	**94,95**
STABW	**10,03**	**6,89**		**17,16**	**8,45**
1311	107,8	77,6	2311	136,5	89,4
1312	131,4	89,4	2312	169,7	109,6
1313	114,5	76,7	2313	154,0	96,2
1314	143,5	92,0	2314	176,0	111,5
1315	142,6	92,6	2315	153,3	99,1
1321	81,6	61,3	2321	120,2	82,6
1322	112,4	78,3	2322	114,9	78,6
1323	117,7	81,0	2323	130,4	87,2
1324	94,6	62,5	2324	159,2	101,6
1325	120,2	75,7	2325	160,8	96,7
1126	94,6	68,2	2326	160,1	103,7
1331	83,2	61,2	2331	110,2	72,1
1332	98,7	70,0	2332	182,2	113,4
1333	98,8	67,1	2333	115,2	79,5
1334	111,0	72,3	2334	140,7	91,1
1135	106,3	71,6	2335	148,1	98,8
MEAN	**109,93**	**74,84**		**145,72**	**94,44**
STABW	**18,45**	**9,84**		**22,67**	**12,30**

Table 9-10: Mean FW of Lettuce in g

Lettuce			
Number	**DM Leaves [g]**	**Number**	**DM Leaves [g]**
111	284,6	211	342,4
112	252,3	212	287,8
113	325,4	213	426,1
MEAN	**287,43**	**MEAN**	**352,10**
STABW	**36,63**	**STABW**	**69,66**
121	315,5	221	343,5
122	248,5	222	247,8
123	296,0	223	397,7
MEAN	**286,67**	**MEAN**	**329,67**
STABW	**34,46**	**STABW**	**75,90**
131	253,7	231	340,6
132	247,7	232	311,9
133	341,6	233	273,2
MEAN	**281,00**	**MEAN**	**308,57**
STABW	**52,57**	**STABW**	**33,82**

Table 9-11: DW of Cucumber Stems and Leaves in g

Test Compartment C1			Control Compartment C2		
Cucumber			Cucumber		
Number	DW Stem [g]	DW Leaves [g]	Number	DW Stem [g]	DW Leaves [g]
1111	3,38	5,02	2111	5,26	8,51
1112	3,85	6,34	2112	6,72	10,08
1113	4,25	7,14	2113	7,43	9,99
1114	5,19	8,67	2114	6,95	10,55
1115	6,49	9,61	2115	8,14	11,19
1121	5,24	8,61	2121	6,87	10,28
1122	5,75	8,69	2122	6,32	9,21
1123	2,65	4,56	2123	7,38	9,58
1124	4,58	7,43	2124	6,78	9,35
1125	4,78	7,11	2125	7,23	10,43
1126	5,27	7,92	2126	8,76	12,78
1131	3,83	6,66	2131	6,81	10,61
1132	4,62	7,50	2132	5,76	8,68
1133	5,99	9,62	2133	6,88	9,65
1134	4,68	7,49	2134	7,19	10,45
1135	5,98	9,54	2135	9,26	13,72
MEAN	**4,78**	**7,62**	**MEAN**	**7,11**	**10,32**
STABW	**1,03**	**1,51**	**STABW**	**1,00**	**1,36**
1211	4,85	7,72	2211	6,49	9,85
1212	5,34	8,85	2212	5,91	9,08
1213	5,11	8,14	2213	7,94	10,81
1214	4,73	7,68	2214	7,96	11,30
1215	5,88	9,41	2215	7,33	10,98
1221	4,40	8,35	2221	5,95	8,35
1222	3,89	6,52	2222	7,11	9,47
1223	4,01	6,76	2223	6,75	8,69
1224	5,27	8,79	2224	7,78	9,61
1225	4,94	8,26	2225	7,87	9,75
1226	6,48	10,15	2226	9,48	13,86

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1231	5,12	8,91	2231	7,29	10,22
1232	4,68	7,72	2232	5,73	7,39
1233	4,34	7,09	2233	8,03	9,69
1234	6,12	9,89	2234	8,93	10,96
1235	6,64	11,25	2235	10,29	12,85
MEAN	**5,11**	**8,47**	**MEAN**	**7,55**	**10,18**
STABW	**0,82**	**1,28**	**STABW**	**1,28**	**1,62**
1311	3,98	7,28	2311	5,58	8,11
1312	4,68	8,22	2312	7,18	9,69
1313	4,01	6,58	2313	6,94	8,88
1314	5,65	8,79	2314	8,01	10,62
1315	6,45	11,06	2315	7,96	10,64
1321	2,91	5,75	2321	5,73	8,48
1322	4,32	7,28	2322	4,26	6,89
1323	4,62	7,88	2323	5,91	7,87
1324	3,97	6,18	2324	6,15	9,04
1325	5,58	8,84	2325	6,87	8,80
1126	5,78	9,19	2326	8,06	11,02
1331	4,29	7,08	2331	5,21	7,64
1332	4,83	7,72	2332	8,61	11,46
1333	4,27	7,17	2333	5,89	8,57
1334	5,69	8,81	2334	7,05	9,83
1135	6,10	10,35	2335	7,93	11,77
MEAN	**4,82**	**8,01**	**MEAN**	**6,71**	**9,33**
STABW	**0,96**	**1,40**	**STABW**	**1,23**	**1,45**

Table 9-12: DW of Lettuce

Lettuce			
Number	**DM Leaves [g]**	**Number**	**DM Leaves [g]**
111	10,23	211	9,89
112	8,07	212	8,48
113	10,35	213	12,86
MEAN	**9,55**	**MEAN**	**10,41**
STABW	**1,28**	**STABW**	**2,24**
121	9,69	221	10,15
122	7,54	222	7,39
123	9,56	223	11,92
MEAN	**8,93**	**MEAN**	**9,82**
STABW	**1,21**	**STABW**	**2,28**
131	8,08	231	9,55
132	7,32	232	8,59
133	10,42	233	7,94
MEAN	**8,61**	**MEAN**	**8,69**
STABW	**1,62**	**STABW**	**0,81**

Table 9-13: Survival of *E. coli* related to the mean of Controls 15 and 16

E. coli	
Tablet	CFU Count
Mean 15, 16	2,22E+01
1	1,11E+01
2	1,56E+01
3	2,22E+00
4	8,89E+00
5	0,00E+00
6	4,44E+00
7	2,44E+01
8	0,00E+00
9	2,22E+00
10	4,44E+00
11	2,22E+01
12	1,33E+01
13	0,00E+00
14	4,44E+00

Table 9-14: Survival of *Staphylococcus cohnii* related to the mean of Controls 15 and 16

Staphylococcus cohnii	
Tablet	CFU Count
Mean 15, 16	1,15E+04
1	1,73E+03
2	1,20E+04
3	8,69E+03
4	1,71E+03
5	2,22E+00
6	3,48E+03
7	7,30E+03
8	2,87E+04
9	1,53E+03
10	9,77E+02
11	5,12E+02
12	8,81E+02
13	2,54E+03
14	4,99E+03

Table 9-15: **Survival of *Bacillus atrophaeus spores* related to the mean of Controls 15 and 16**

Bacillus atrophaeus spores	
Tablet	CFU Count
Mean 15, 16	1,31E+04
1	1,78E+02
2	1,11E+02
3	0,00E+00
4	3,11E+02
5	4,44E+01
6	1,08E+03
7	1,73E+03
8	0,00E+00
9	6,67E+01
10	4,44E+01
11	0,00E+00
12	6,67E+01
13	6,67E+01
14	1,33E+02

Table 9-16: Survival of *Staphylococcus cohnii* related to the mean of Controls 15 and 16

Staphylococcus cohnii	
Tablet	CFU Count
Mean 15, 16	5,78E+05
1	3,10E+04
2	1,14E+05
3	2,42E+05
4	1,33E+05
5	4,67E+03
6	4,27E+05
7	4,80E+05
8	4,83E+05
9	4,28E+03
10	2,81E+03
11	1,98E+04
12	1,84E+04
13	3,01E+04
14	8,48E+03

Table 9-17: Survival of *Bacillus atrophaeus spores* related to the mean of Controls 15 and 16

Bacillus atrophaeus spores	
Tablet	CFU Count
Mean 15, 16	2,50E+05
1	1,05E+03
2	3,90E+04
3	1,85E+04
4	2,03E+03
5	1,65E+03
6	1,35E+05
7	7,97E+04
8	2,41E+05
9	2,22E+00
10	0,00E+00
11	2,33E+01
12	1,56E+01
13	0,00E+00
14	1,10E+02