REBUS: *in-situ* REsources Bio-Utilisation for Life support systems in Space

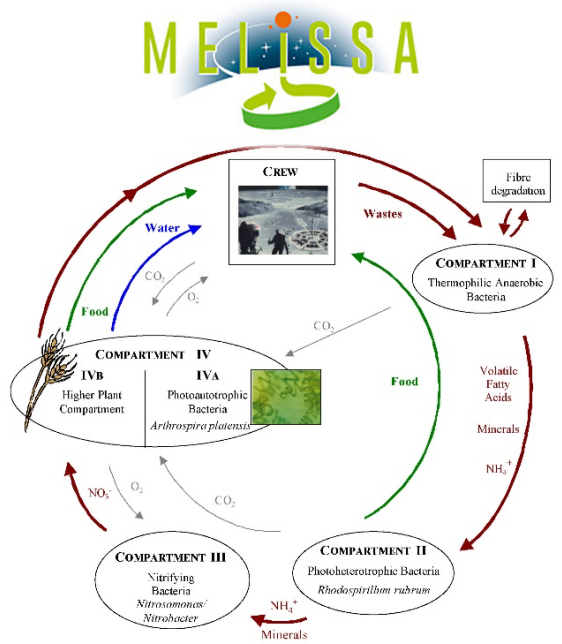
Stefania De Pascale *et al.*
Department of Agricultural Sciences
University of Naples Federico II, Portici (Naples), Italy

As mission targets move away from Low Earth Orbit, BLSSs become essential to regenerate the necessary resources for the crew and minimizing the need for supply from Earth

In-Situ Resource Utilization (ISRU) is the practice of collection, processing, storing and use of materials found or manufactured on other astronomical objects (the Moon, Mars, asteroids, etc.) to replace materials that would otherwise be brought from Earth.

ReBUS project Goals

- ✓ Complementarity and synergy with the MELiSSA program
- ✓ To develop a research line for the realization of a BLSS in Space based on ISRU
- ✓ To study the integration of different organisms (plants, cyanobacteria and different decomposing organisms), to minimize the use of exogenous resources and at the same time to maximize the use of resources available *in-situ (i.e.* lunar and martian soils, water, gases present in the atmosphere) and the recycling of organic matter produced in the system itself *(e.g.* crop residues, physiological waste from the crew)



Agenzia Spaziale Italiana

The project

BANDO DI RICERCA PER MISSIONI FUTURE DI ESPLORAZIONE UMANA DELLO SPAZIO

Area tematica Sistemi Biorigenerativi

agenzia spaziale italiana

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GIOVANNI VALENTINI

SILVIA MARI, MARTA DEL BIANCO, GABRIELE MASCETTI

STEFANIA DE PASCALE

Agenzia Spaziale Italiana

In Situ Resources BioUtilization per il Supporto alla Vita

ReBUS

CNR IRET

Istituto di Ricerca sugli Ecosistemi Terrestri

ENEA

1.700.645 €

OCT. 10, 2019

36 MONTHS

ISTITUTO SUPERIORE DI SANITÀ

Università di Roma Tor Vergata

KAYSER ITALIA

TELESPAZIO a LEONARDO and THALES company

ThalesAlenia Space a Thales / Leonardo company

In-situ REsources Bio-Utilisation for Life support systems in Space

WBS

Agenzia Spaziale Italiana

WP_1000 Prime UNINA

- WP_1100 Microgree…
- WP_1300 Effects of radiations on plants
- WP_1400 Effects of µg on plants

WP_2000 UR1 ENEA

- WP_2100 Anaerobic digestion
- WP_2200 Composting from bacteria and insects

WP_3000 UR2 UNITOV

- WP_3100 Cyanobacteria fo… ISRU

WP_4000 UR3 UNIPV

- …100 …ganic matter …gradation by microfungi

WP_5000 UR4 CNR

- WP_5100 Food quality and safety and waste characterization
- WP_5200 Prebiotics in… and from… ma…

WP_6000 UR5 ISS

- WP… P…

WP_7000 UR6 TAS-I

- WP_7100 Space context definition and analysis
- WP_7200 Space environment analogue research platforms
- WP… Spa… research… platforms on-board the ISS

WP_8000 UR7 KAYSER

- WP_8100 Modelling … human-sy… interac…
- …8200 …ort for the …finition of …xperimental facilities on-board the ISS
- WP_8300 Support for the definition of scientific experiments in µg

WP_9000 UR8 TPZ

- …VP_9100 …ssemination and Spin-off

Agrospace

Organic waste recovery

Food quality and safety

Technological support

WP 1100 – Microgreens

Youssef Rouphael

Stefania De Pascale

Antonio Pannico

Christophe El-Nakhel

WP 1100 – Objectives

- ✓ To evaluate the genotypic effects on microgreens production cycle, yield and quality characteristics
- ✓ To examine how light quality can be modulated for improving sensorial and phytochemical components of microgreens
- ✓ To examine the modulatory effects of natural fiber substrates, synthetic alternatives (capillary mat and cellulose sponge) and a novel substrate developed in the frame of this project on the nutritive and phytochemical composition of select microgreens species
- ✓ To assess the selenium and iodine biofortification impacts on the nutritive value, polyphenolic content, and bioactive constitution of different microgreens
- ✓ To examine how the modulation of the fertilization program and growth stage at harvest affects sensory quality and bioactive content of microgreens

Trends in Food Science & Technology 57 (2016) 103–115

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: http://www.journals.elsevier.com/trends-in-food-science-and-technology

ELSEVIER

Review

Micro-scale vegetable production and the rise of microgreens

Marios C. Kyriacou [a], Youssef Rouphael [b, *], Francesco Di Gioia [c], Angelos Kyratzis [a], Francesco Serio [d], Massimiliano Renna [e], Stefania De Pascale [b], Pietro Santamaria [e]

[a] Department of Vegetable Crops, Agricultural Research Institute, Nicosia, Cyprus
[b] Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy
[c] Institute of Food and Agricultural Sciences, South West Florida Research and Education Center, University of Florida, Immokalee, FL, United States
[d] Institute of Sciences of Food Production, National Research Council of Italy, Bari, Italy
[e] Department of Agricultural and Environmental Science, University of Bari Aldo Moro, Bari, Italy

frontiers in Plant Science

OPINION
published: 12 September 2017
doi: 10.3389/fpls.2017.01587

Microgreens as a Component of Space Life Support Systems: A Cornucopia of Functional Food

Marios C. Kyriacou[1], Stefania De Pascale[2], Angelos Kyratzis[1] and Youssef Rouphael[2]*

[1] Department of Vegetable Crops, Agricultural Research Institute, Nicosia, Cyprus, [2] Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy

WP 1200 – *In-situ* soils utilization

Paola Adamo

Antonio G. Caporale

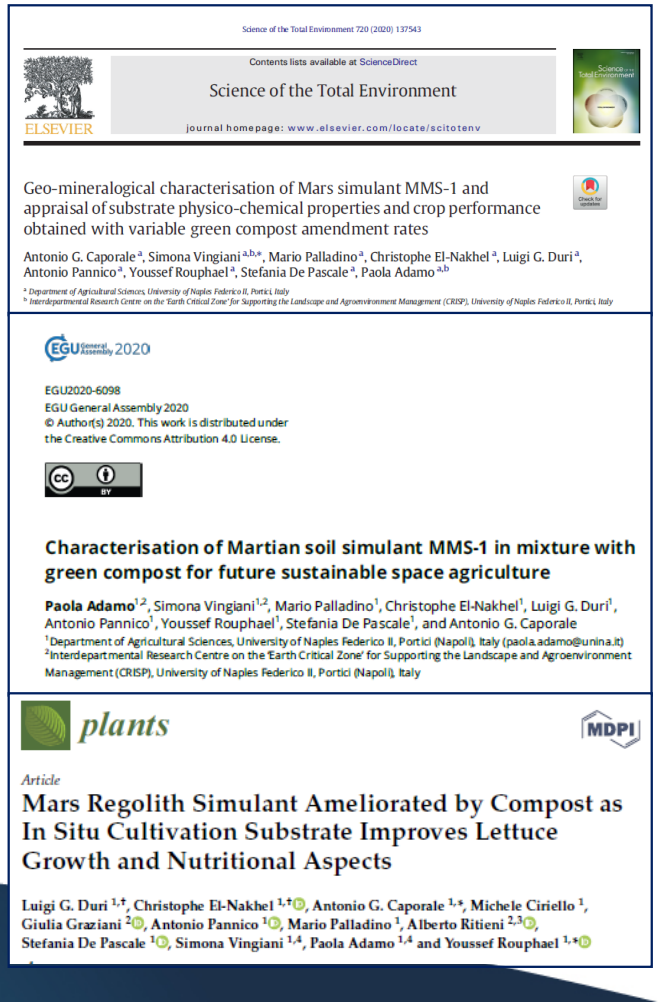
Roberta Paradiso

Simona Vingiani

Mario Palladino

WP 1200 – Objectives

- ✓ To define strategies to make the lunar and martian regolith simulants suitable substrates for growing crops for BLSSs
- ✓ To analyze the effects of adding compost of vegetable or human origin (obtained as part of the activities of WP 2000) to martian and lunar regolith simulants in terms of (i) physical, chemical and biological fertility of the substrates and (ii) growth of plants
- ✓ To add biostimulants and corrective agents to substrates with characteristics limiting plant growth
- ✓ To assess the maintenance of the fertility of cultivation substrates over time and the stability and sustainability of plant productions
- ✓ To provide the scientific requirements for the implementation of cultivation techniques in BLSSs



Science of the Total Environment 720 (2020) 137543

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Geo-mineralogical characterisation of Mars simulant MMS-1 and appraisal of substrate physico-chemical properties and crop performance obtained with variable green compost amendment rates

Antonio G. Caporale[a], Simona Vingiani[a,b,*], Mario Palladino[a], Christophe El-Nakhel[a], Luigi G. Duri[a], Antonio Pannico[a], Youssef Rouphael[a], Stefania De Pascale[a], Paola Adamo[a,b]

[a] Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy
[b] Interdepartmental Research Centre on the 'Earth Critical Zone' for Supporting the Landscape and Agroenvironment Management (CRISP), University of Naples Federico II, Portici, Italy

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Characterisation of Martian soil simulant MMS-1 in mixture with green compost for future sustainable space agriculture

Paola Adamo[1,2], Simona Vingiani[1,2], Mario Palladino[1], Christophe El-Nakhel[1], Luigi G. Duri[1], Antonio Pannico[1], Youssef Rouphael[1], Stefania De Pascale[1], and Antonio G. Caporale

[1]Department of Agricultural Sciences, University of Naples Federico II, Portici (Napoli), Italy (paola.adamo@unina.it)
[2]Interdepartmental Research Centre on the 'Earth Critical Zone' for Supporting the Landscape and Agroenvironment Management (CRISP), University of Naples Federico II, Portici (Napoli), Italy

plants

MDPI

Article

Mars Regolith Simulant Ameliorated by Compost as In Situ Cultivation Substrate Improves Lettuce Growth and Nutritional Aspects

Luigi G. Duri [1,†], Christophe El-Nakhel [1,†], Antonio G. Caporale [1,*], Michele Ciriello [1], Giulia Graziani [2], Antonio Pannico [1], Mario Palladino [1], Alberto Ritieni [2,3], Stefania De Pascale [1], Simona Vingiani [1,4], Paola Adamo [1,4] and Youssef Rouphael [1,*]

WP 1300 - Effect of ionizing radiation on plants

Veronica De Micco

Carmen Arena

Chiara Amitrano, Ermenegilda Vitale, Sara De Francesco, Giulia Costanzo

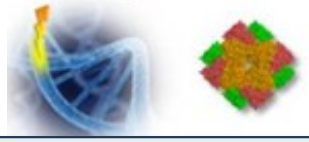
Radiation Procedure Consultants

- Maria Gabriella Pugliese (Dip. Fisica, UNINA)
- Cecilia Arrichiello, Gianluca Ametrano (Istituto Pascale)
- Marco Durante, Walter Tinganelli (GSI)

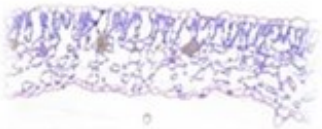
WP 1300 - Objectives

Effect of ionizing radiation on higher plants

- **Genomic material and proteins**



- **Functional and anatomical traits**



- **Photosynthesis and growth**

- **Food quality**

In terms of growth and productivity

To unravel the mechanisms for radioresistance in plants

Arena et al (2014) *Acta Astr* 104: 419-431
Arena et al (2017) *Biol Plant 61: 305-314.*
Arena et al (2019) *Plant Biol* 21: 615-626.

To evaluate the impact of radiations on plants in terms of resource regeneration capacity in BLSSs

De Micco et al (2011) *Rad. Env Biophys 50: 1-19.*
De Micco et al (2014a) *Plant Biol* 1: 187-193
De Micco et al (2014b) *The Scientific World Journal* 1-136

WP 1400 – Effects of μg on plant reproduction

Giovanna Aronne Luigi Gennaro Izzo Maurizio Iovane

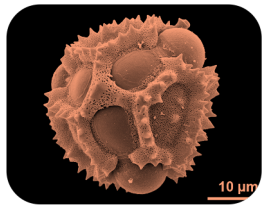
WP1400 – Objectives

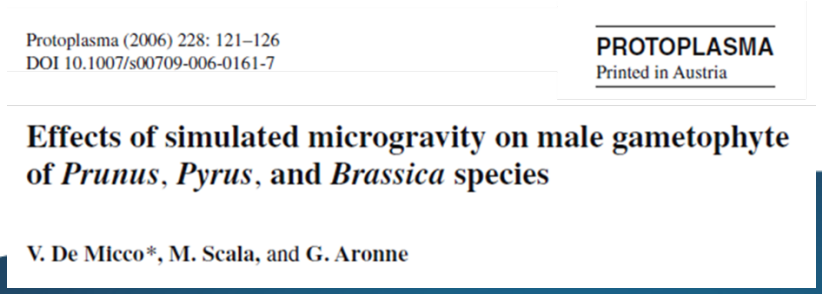
Considering that:

Micrograv ity affects successful reproduction in plants interfering with pollen functionality and the fulfillment of the seed-to-seed cycle

Objectives are:

- ✓To analyze the effects of simulated microgravity on pollen germination and subsequent gametogenesis on plant species to be used in long time space missions and in BLSS.
- ✓To assess scientific requirements for experiments onboard the ISS on plant reproduction in microgravity

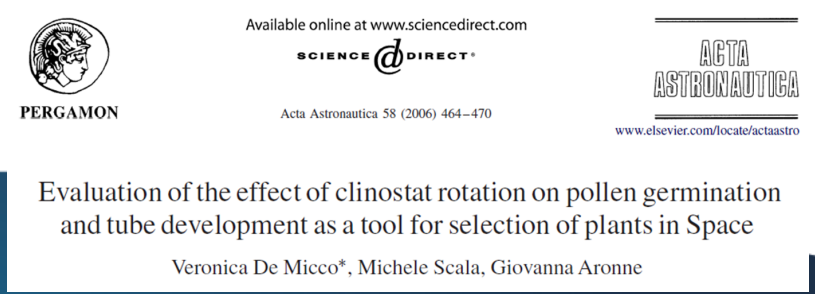



Protoplasma (2006) 228: 121–126
DOI 10.1007/s00709-006-0161-7

PROTOPLASMA
Printed in Austria

Effects of simulated microgravity on male gametophyte of *Prunus*, *Pyrus*, and *Brassica* species

V. De Micco*, M. Scala, and G. Aronne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Acta Astronautica 58 (2006) 464–470

ACTA ASTRONAUTICA

www.elsevier.com/locate/actaastro

Evaluation of the effect of clinostat rotation on pollen germination and tube development as a tool for selection of plants in Space

Veronica De Micco*, Michele Scala, Giovanna Aronne

WP 2000 – Composting and digestion of organic waste

Leader: Eugenio Benvenuto

Team: Angiola Desiderio  Luca Nardi  Maria Elena Villani 

Objectives:

- Formulation of an organic waste mixture based on the actual composition of waste produced on board the ISS
- Preparation of a common mixture as a substrate for all the biodegradation processes scheduled in the REBUS project (bacteria, micro-fungi, insects)
- Definition of degradative processes (single or combined) useful for the production of fertilizer, compost, soil conditioner to promote the growth of plants in a space environment

Formulation and preparation of waste mixture

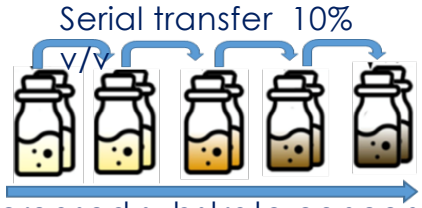
WP 2100 – Anaerobic digestion of organic wastes

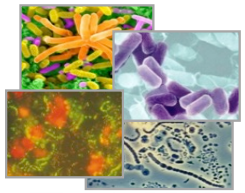
Team: Silvia Tabacchioni  Luigi Chiarini 

Objective: Optimization of anaerobic digestion of food wastes, to be reused as fertilizer/soil conditioner for *in-situ* plant cultivation, through selection of specific microbial consortia

Microbial consortia development

Space organic waste





Microbial consortia

- Evaluation of degradative ability and stability of selected microbial consortia
- Taxonomic assessment of the microorganisms composing the selected consortia

WP 2200 – Insect composting of organic wastes

Team: Maurizio Calvitti  Elena Lampazzi 

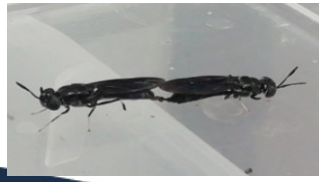
Objective: Development of *Hermetia illucens* (Insecta, Diptera) breeding system feasible in space environment, for the production of compost and/or soil conditioner of martian/lunar soils

Principal activities

- *Adaptation on Space organic waste*

- *Adaptation to captivity in confined/controlled systems*

- *Development of degradation processes optimized for space mission conditions*
- *Study of the bio-ethological characteristics in the perspective of use in space environment*

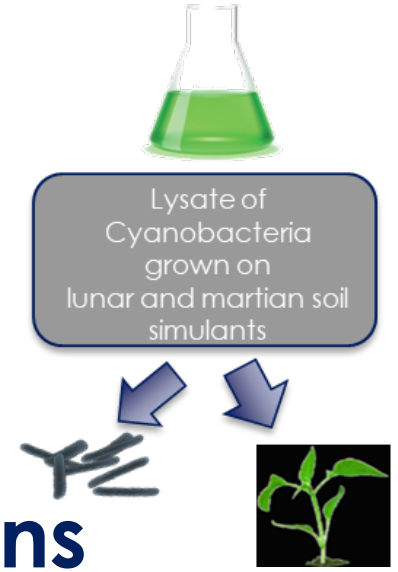


WP 3100 - Cyanobacteria for ISRU

Daniela Billi

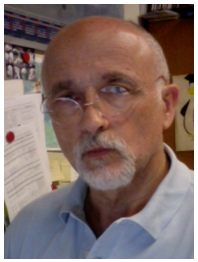
- Growth of desert cyanobacteria on lunar and martian soil simulants in order to use their lysate as growth medium to:

1) support bacterial growth*
2) support hydroponic plant
3) amend lunar and martian soil simulants



*Billi et al. (2020) Exploiting a perchlorate-tolerant desert cyanobacterium to support bacterial growth for *in-situ* resource utilization on Mars. Int. J. Astrobiol. *in press*

WP 3200 - Effects of Radiations



Livio Narici

- Radiation in relevant mission scenarios
- State of art in the literature of radiation measurements relevant to human-space exploration of deep space

WP 4100 - Waste degradation by microfungi

Team:

Solveig Tosi, Elena Savino, Chiara Nugnes

UNIVERSITÀ DI PAVIA
Department of Earth and Envirnmental Sciences

Objectives:

Treating waste produced in a ISS-like system using microfungi

Materials & Methods

9 fungal strains have been selected and artificially combined in a consortium.

The consortium has been inoculated on a mix which mimics the waste from ISS also enriched with different concentrations of urine

Expected results

- Artificial microfungal consortium as a component of waste management
- Compost/substate/fertilizer for plant cultivation in BLSSs

Fungal strains tested for combing the artificial consortium
Chaetomium like 2
Chaetomium like 3
Chaetomium like 4
Micelia sterilia
Nigrospora like 2
Bjerkandera adusta
Ganoderma lucidum
Pleorotus ostreatus
Schizofillum comune

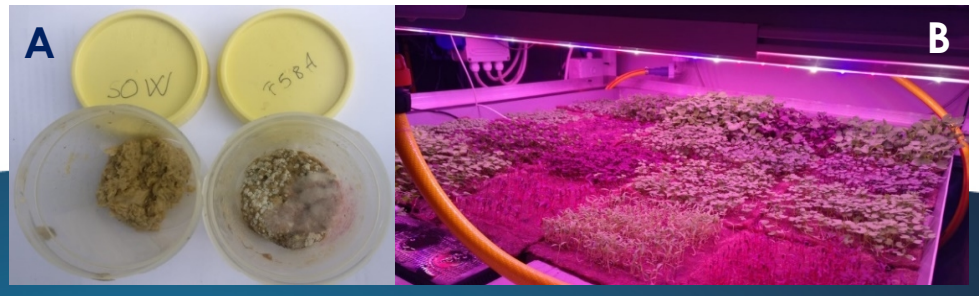


Figure A, waste with the consortium growing on it (on the right); control waste (on the left).

Figure B, growing microgreens

WP 5000 – Food Quality and Safety

CNR Team

CNR-IRET - Research Institute on Terrestrial Ecosystems - Porano

Alberto Battistelli, Stefano Moscatello, Simona Proietti, Michele Mattioni

WP 5100 – Food quality and safety and waste characterization

CNR-ISA - Institute of Food Science - Avellino

Filomena Nazzaro, Florinda Fratianni, Maria Nave Ombra, Antonio d'Acierno

WP 5200 – Prebiotics in plants and waste materials

WP 5100 Food quality and safety and waste characterization - Simona Proietti

Task 1 - analytical tests for food quality and safety on different plant species
Task 2 - qualitative analysis (QDA) of plant food samples
Task 3 - analysis of the presence of toxic and / anti-nutritional compounds in plant food samples
Task 4 - analysis of degree of microbial contamination in plant food samples
Task 5 - evaluation of prebiotics accumulation in plant samples (from partners)
Task 6 - analysis of waste materials and their derivatives after regenerative treatments

WP 5200 Prebiotics in plants and waste materials - Filomena Nazzaro

Task 1 – effects of environmental control on the content of prebiotics and their precursors
Task 2 - prebiotics production from their precursors
Task 3 - prebiotics purification from plant tissues
Task 4 - prebiotics supply to WP 6100
Task 5 - cultivation of probiotics with FOS, and microbial viability determination
Task 7 - determination of biochemical characteristics of microorganisms
Task 8 - determination of biological activities of probiotics

WP 6100 - Prebiotics and wellbeing: animal model and study proposal on board the ISS

Simone Macrì & Francesca Zoratto
Centre for Behavioral Sciences and Mental Health
Istituto Superiore di Sanità (ISS), Roma

Rationale

→Support the hypothesis that the prebiotic properties of fructans, extractable from vegetables grown in BLSS, have a positive effect on individual psychophysical well-being

WP 6100 - Objectives

1. **To assess whether the administration of 2 prebiotic substances can promote the psychophysical well-being in a murine experimental model**
   - Behavioral tests to evaluate emotional responses, executive functions and sociability
   - Test to evaluate the physiological reactivity to stress

2. **To plan an experiment to be conducted on board the ISS to evaluate the effect of prebiotic substances on the psychophysical well-being of astronauts**
   - Neuropsychological tests designed to test executive functions
   - Psychometric scales to provide information on anxiety, personality, individual attachment styles, perceived conflict within the group

Caputo et al. *Translational Psychiatry* (2020)10:185
https://doi.org/10.1038/s41398-020-00869-4

Translational Psychiatry

ARTICLE Open Access

Genomic and physiological resilience in extreme environments are associated with a secure attachment style

Viviana Caputo[1], Maria Giuseppina Pacilli[2], Ivan Arisi[3,4], Tommaso Mazza[5], Rossella Brandi[6], Alice Traversa[7], Giampietro Casasanta[8], Edoardo Pisa[9], Michele Sonnessa[6], Beth Healey[10], Lorenzo Moggio[8,11], Mara D'Onofrio[4,6], Enrico Alleva[9] and Simone Macrì[9]

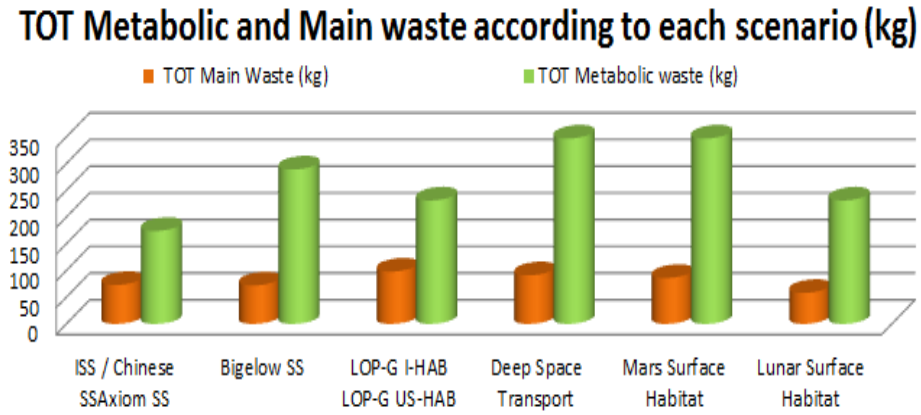
WP 7100 - Space context definition and analysis <

Team

- Giorgio Boscheri and Giovanni Marchitelli

Objective

- Definition of the reference exploration space frame and associated parameters for the development of bio-regenerative systems



Expected results

1) Study of on-site resource utilization (surface and in the transport scenarios) for the main exploration objectives (vehicles, systems and habitats, related life and habitability support systems)
2) Classification and quantification of wastes and resources available on ISS-like scenarios and projection on transport and orbital systems as well as planetary surface permanent outposts
3) Classification and quantification of the resources available on lunar and martian soil as well as CO_2, H_2O, O_2, and solar light
4) Resources availability assessment toward the needed amounts to cover the crew needs, within transport, orbital and surface systems

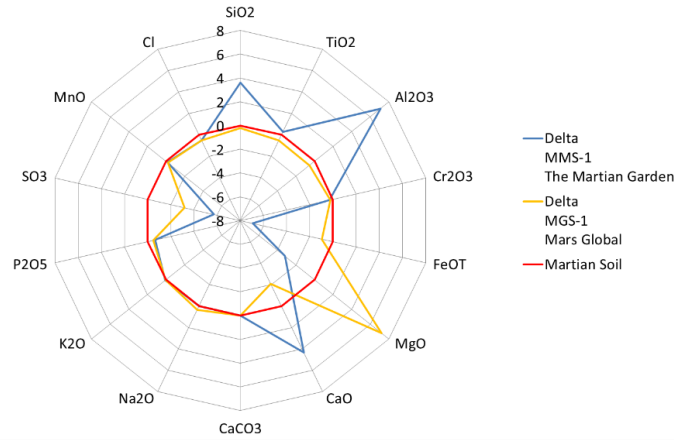
WP 7200 - Space environment analogue research

Objective

- Development of the space research platform for BLSSs in relevant environment

Expected results

- Study of possible modification of martian and lunar regolith simulants to make them representative of real soil and, at the same time, useful for the experiments planned
- Definition of the availability, needs and quality of water, technical identification of the necessary pre- and post-treatments and the related needed devices and tools
- Ground tests of innovative substrates generated by scientific WPs, in a controlled environment, within an existing growth chamber adapted for the purpose: EDEN ISS rack (after mission to Antarctica) or EDEN prototype located at TAS Torino Recyclab laboratory



WP 7300 - Space research platforms on-board the ISS

Objective

- Conceptual design of study-derived relevant flight experiments for the ISS

Expected results

- Identification of Space infrastructures necessary for the collection and processing of selected *in-situ* resources, and of the main associated ISRU processes
- Preliminary definition of relevant experiments on ISS (e.g. experiment of production of substrate from waste, or experiment of growth of microgreens with substrate produced on the ground similar to what can be produced on ISS, according to what was scientifically defined in the course of the study):
  - definition of scientific needs and requirements (e.g. of the production process)
  - conceptual design of the experiment, with the support of the WP 8200

WP 8000 - Support for future Space experiments

KAYSER ITALIA

Michele Balsamo Alessandro Donati Liyana Popova Wioleta Pawlak

Kayser Italia activities are related to broader activities linked to support the technology to be developed and the basic research activities as well as the design of experiments onboard ISS

WP 8000 - Tasks and goals

WP 8100: To study the interactions between the astronaut and the BLSS, considering various aspects and parameters both psico-physiological and environmental

GOAL: To define the main components that will contribute to the well-being of the crew onboard ISS or future spacecrafts and orbital systems

WP 8200: To support the activities of WP 7300 for the definition of a preliminar experimental cultivation facility

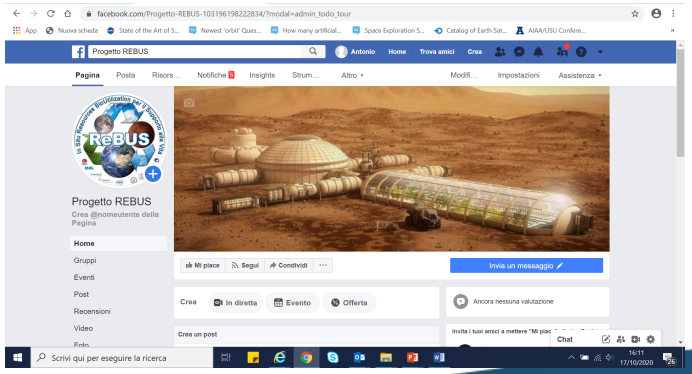
GOAL: To define aspects related to the spatialization of the facility, both in terms of operations and logistic

WP 8300: To support the definition of scientific experiments in μg proposed by the ReBUS scientic team

GOAL: To pave the way to the definition of scientific experiments and payloads to be performed onboard ISS or on future platforms as output of the ReBUS project

WP 9100 - Dissemination and Spin-off

Raimondo Fortezza **Antonio Ceriello**



Project Data Management

- Creation and management of a secure and reliable platform for project data storage and distribution

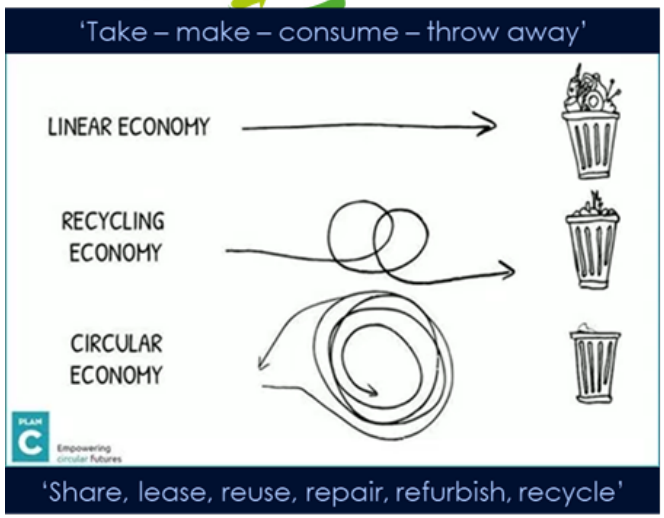
Support to Dissemination of Project Data

- Through scientific events and journals
- Through traditional publishing and television media
- Through the social media
- School Engagement
- Organisation of two tematic workshops

Spin-Off

- Monitoring of Announcement of Opportunity for Space experiments and support to preparation of proposal
- Identification of terrestrial application of the ReBUS results
- Identification of possible collaborations for ReBUS Data exploitation

From Space to Earth & back …



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THANK YOU.

Stefania De Pascale
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UCA UNIVERSITÉ Clermont Auvergne

UNIVERSITY of GUELPH

UMONS Université de Mons

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