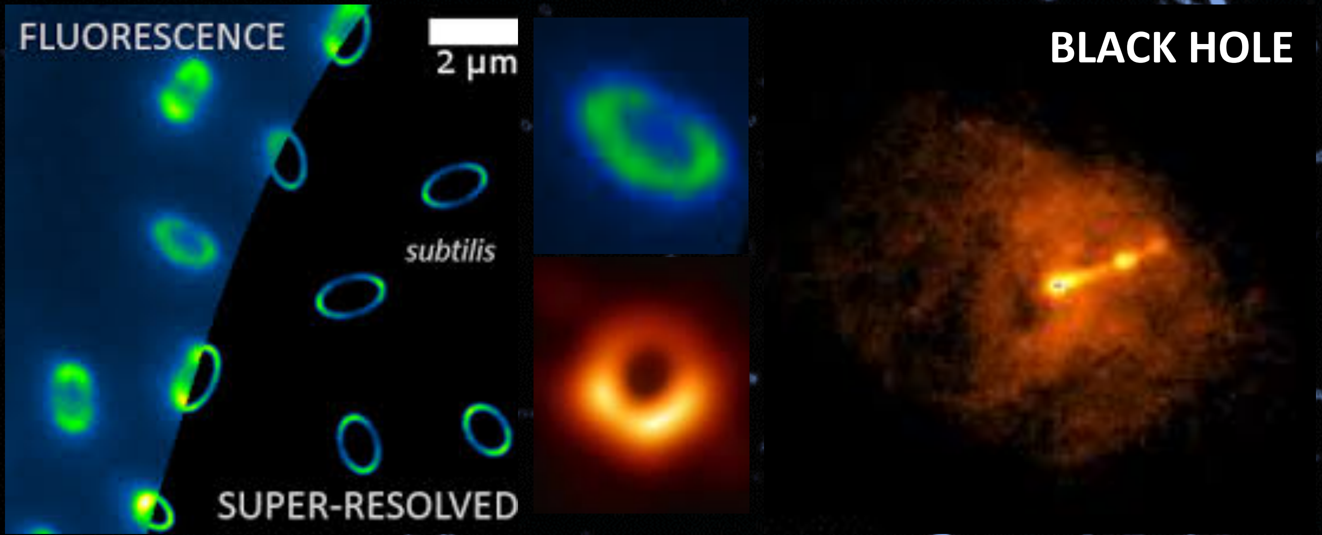
Citometria a flusso e qualità delle acque: novità dallo spazio

Dr. Stefano Amalfitano



Istituto di Ricerca Sulle Acque
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Abstract

Stefano Amalfitano

A differenza di ogni altro sistema acquatico terrestre, l'esplorazione dello spazio richiede lo sviluppo di metodi indipendenti dalla coltivazione per il monitoraggio della qualità delle acque, soprattutto sui veicoli spaziali per viaggi di lunga durata. Nell'ambito del progetto H2020 Biowyse, la citometria a flusso è stata selezionata tra le tecnologie più promettenti per la valutazione in tempo reale della carica microbica totale nelle acque spaziali. L'analisi di acque a diverso livello di contaminazione ha consentito di identificare delle nuove soglie di qualità basate su parametri alternativi al conteggio delle unità formanti colonia (CFU) su piastre di coltivazione. Questo nuovo approccio sarà di fondamentale importanza per migliorare la reattività dell'equipaggio in caso di imprevista contaminazione microbiologica.

1885 Escherich: Isolation of *Bacillus coli* (*Escherichia coli*)

1892 Schardinger: *E. coli* as "indicator" of fecal contamination of water (indicator concept) ①

1893/4 Koch/Franklands: Number of microbial cells in water as an indication for its pollution
② ----> heterotrophic plate count, HPC
First recommendations:
< 100/ml in marine, ground- and surface water, this can be reached by slow-sand filtration

1901 Group of coliforms as an indicator

1904 Improvement of thermotolerant (fecal) coliforms ③

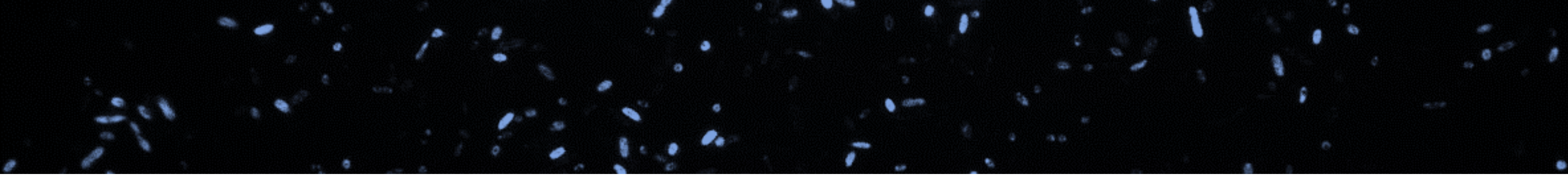
1977 Genospecies *E. coli* as best indicator

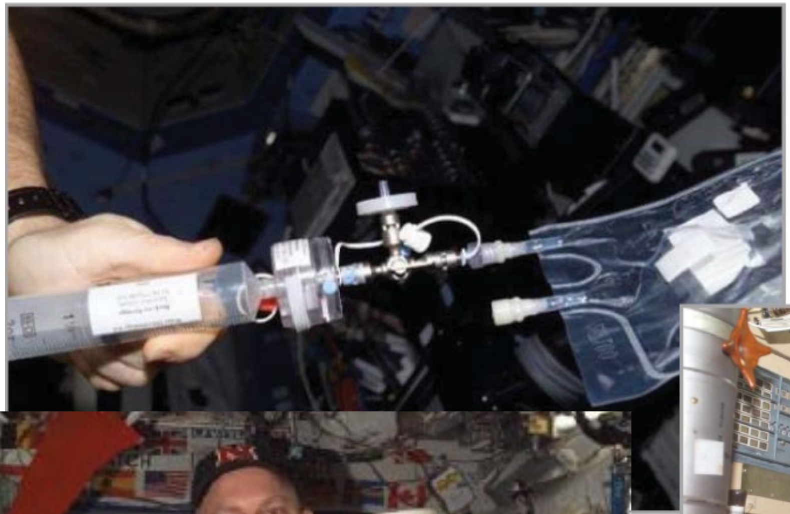
R. Koch

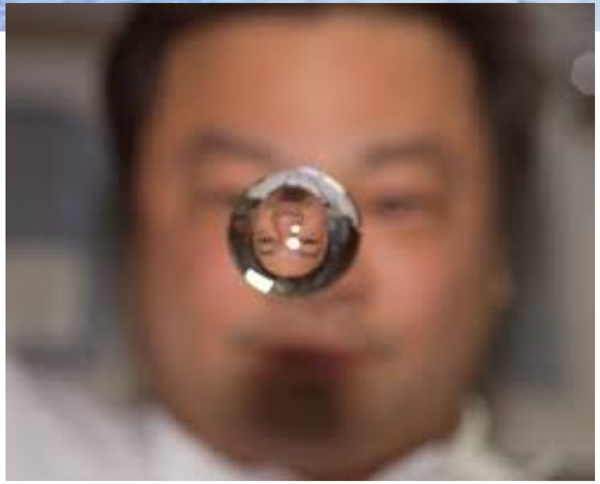
Heterotrophic plate counts
20-500 CFU/ml

Escherichia coli (fecal coliforms)
0-1 CFU/ml

Courtesy: T. Egli

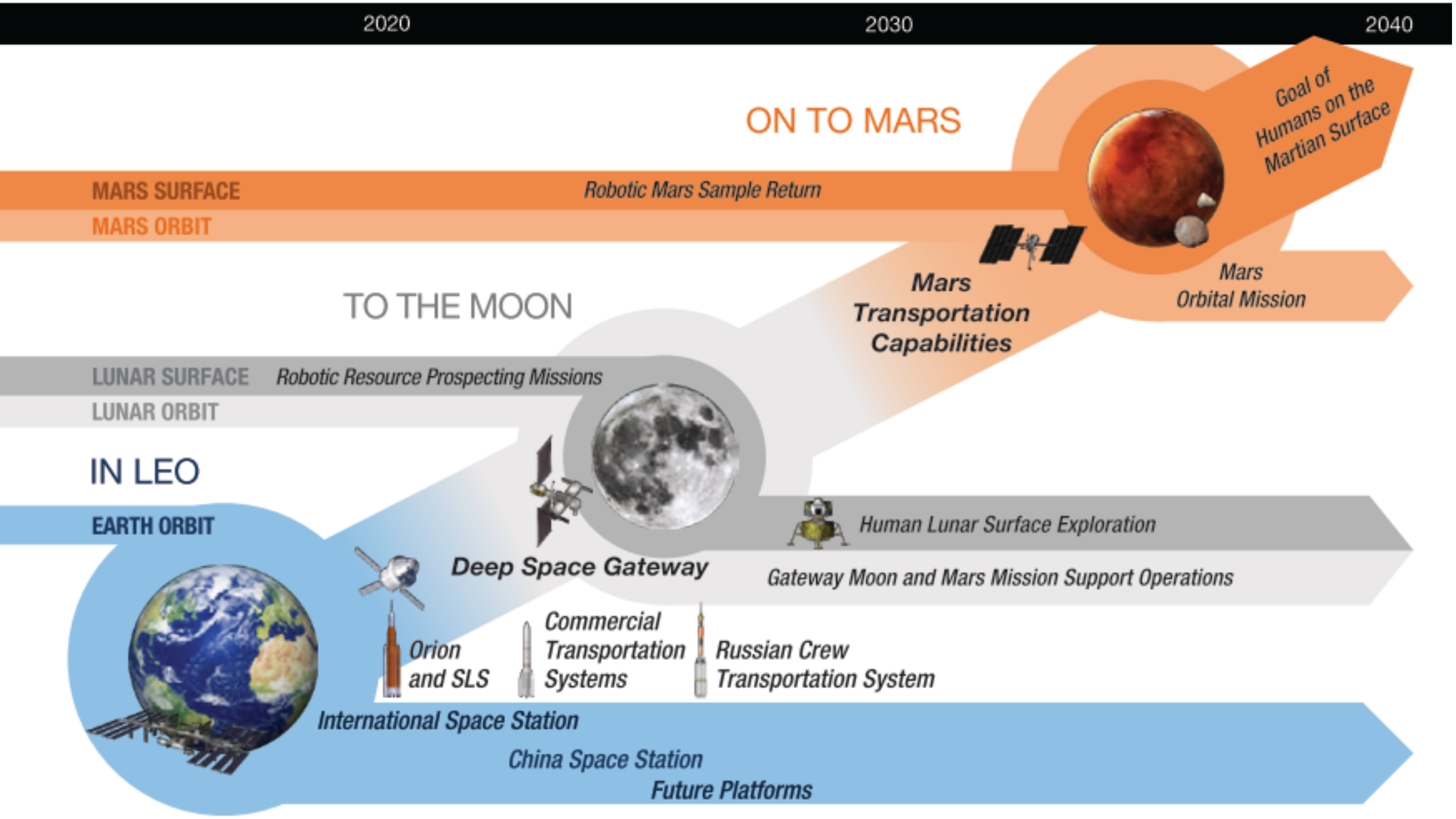






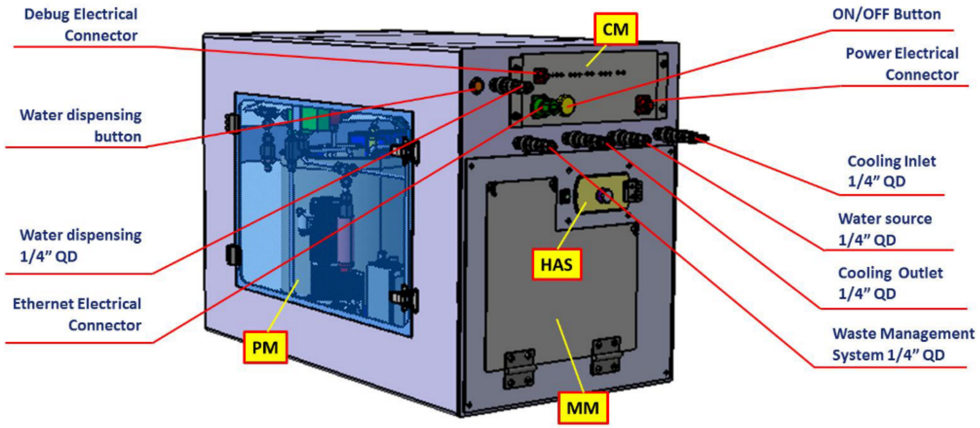
Courtesy: NASA

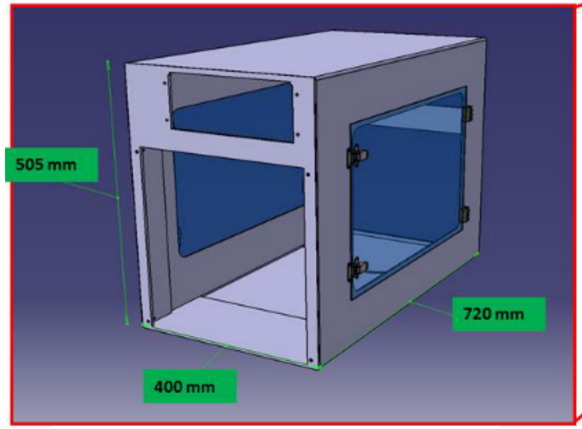
The Global Exploration Roadmap

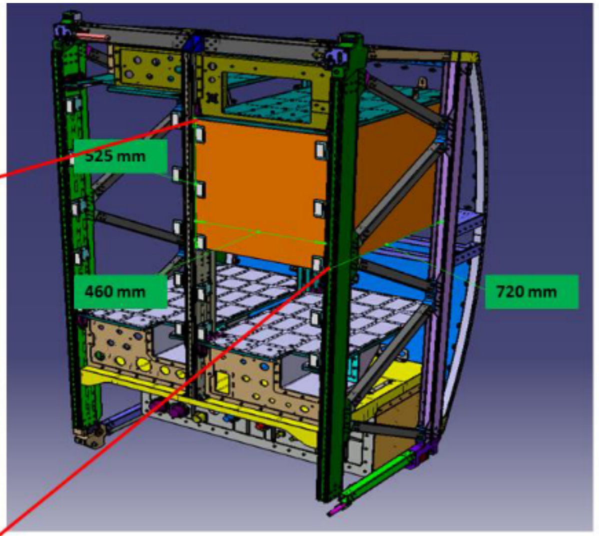


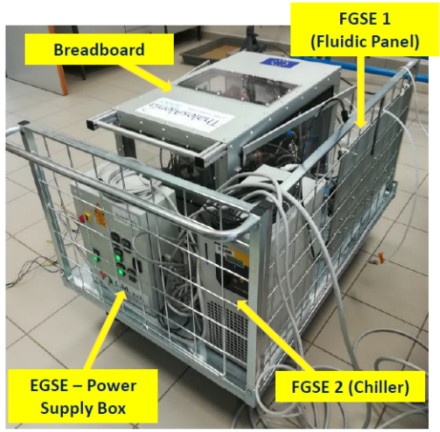
BIOWYSE http://www.biowyse.eu/

Biocontamination Integrated Control of Wet Systems for Space Exploration



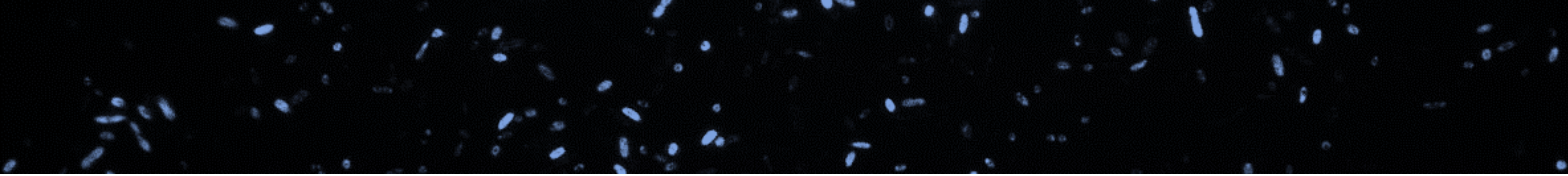






For Safe Water and Habitat Management on-board ISS

and Future Human Space Exploration Vehicles and Planetary Outposts.



Study aims

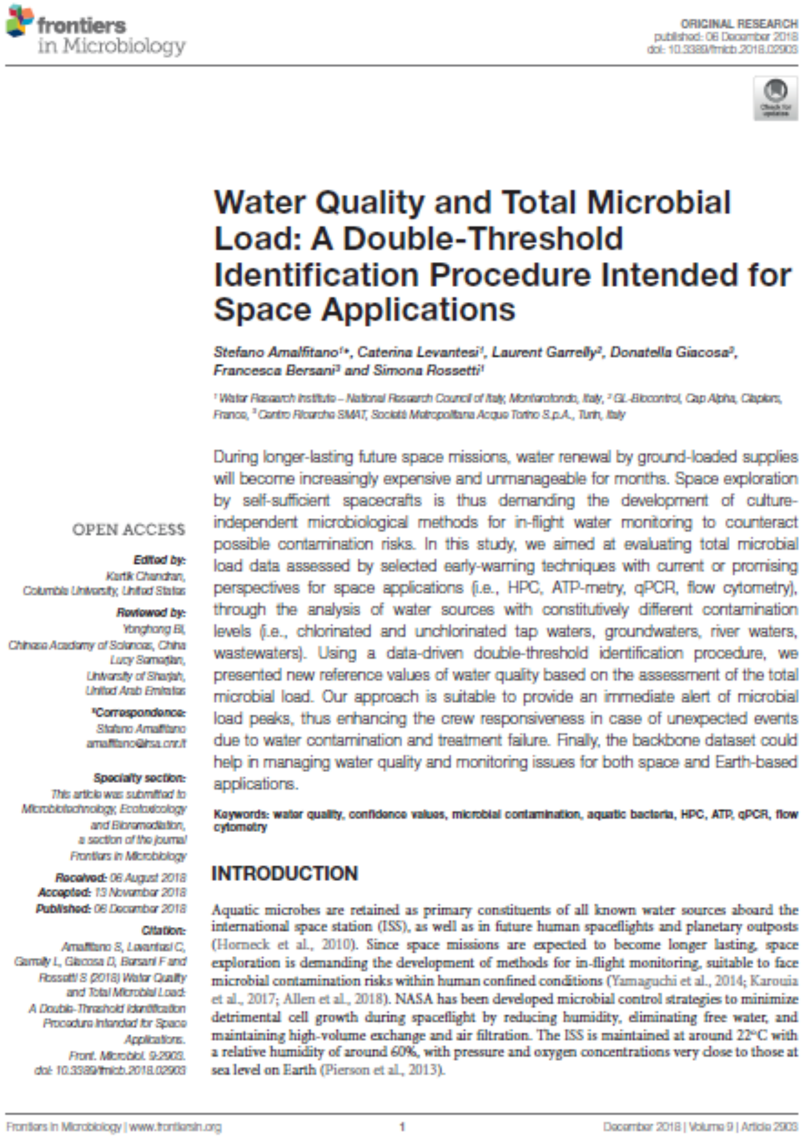
- to cross-validate reliable measurement methods to assess microbial contamination and cell densities in waters with different quality
- to provide insights on microbial community composition in space waters

Methods

Water samples with different levels of biological contamination included:

7 drinking waters (potable tap waters)
7 groundwaters from wells and piezometers
7 treated waters (drinking water treatment plant effluents before disinfection)
7 surface waters from streams and rivers (natural and engineered sources)
7 secondary effluents of wastewater treatment plants
2 drinking space waters after 5-years exposure to microgravity

- ATP-metry (total cell content)
- Heterotrophic Plate Counts - HPC (AWWA and EPA standard methods)
- Flow cytometry - FCM (Sybr Green I and Live/Dead double staining)
- Total cell count - EFM (DAPI staining and epifluorescence microscopy)
- qPCR (16S rDNA primer sets)
- NGS (16S rDNA Illumina profiling)



frontiers in Microbiology

ORIGINAL RESEARCH
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Water Quality and Total Microbial Load: A Double-Threshold Identification Procedure Intended for Space Applications

Stefano Amalfitano[1]*, Caterina Levantesi[1], Laurent Garrelly[2], Donatella Giacosa[3], Francesca Bersani[3] and Simona Rossetti[1]

[1] Water Research Institute – National Research Council of Italy, Monterotondo, Italy, [2] GL-Biocontrol, Cap Alpha, Clapiers, France, [3] Centro Ricerche SMAT, Società Metropolitana Acque Torino S.p.A., Turin, Italy

During longer-lasting future space missions, water renewal by ground-loaded supplies will become increasingly expensive and unmanageable for months. Space exploration by self-sufficient spacecrafts is thus demanding the development of culture-independent microbiological methods for in-flight water monitoring to counteract possible contamination risks. In this study, we aimed at evaluating total microbial load data assessed by selected early-warning techniques with current or promising perspectives for space applications (i.e., HPC, ATP-metry, qPCR, flow cytometry), through the analysis of water sources with constitutively different contamination levels (i.e., chlorinated and unchlorinated tap waters, groundwaters, river waters, wastewaters). Using a data-driven double-threshold identification procedure, we presented new reference values of water quality based on the assessment of the total microbial load. Our approach is suitable to provide an immediate alert of microbial load peaks, thus enhancing the crew responsiveness in case of unexpected events due to water contamination and treatment failure. Finally, the backbone dataset could help in managing water quality and monitoring issues for both space and Earth-based applications.

Keywords: water quality, confidence values, microbial contamination, aquatic bacteria, HPC, ATP, qPCR, flow cytometry

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Reviewed by:
Yonghong Bi,
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University of Sharjah,
United Arab Emirates

*Correspondence:
Stefano Amalfitano
amalfitano@irsa.cnr.it

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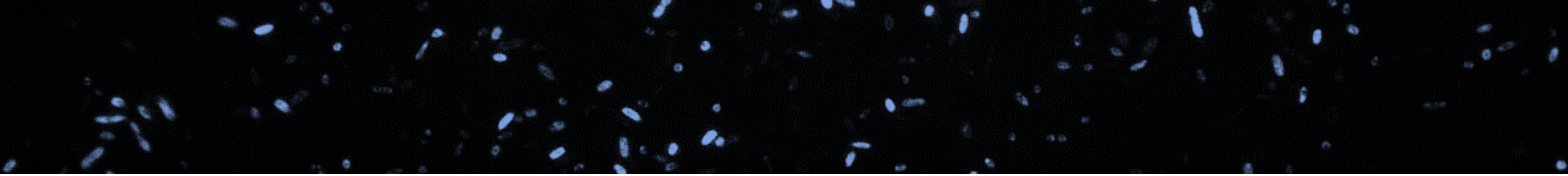
INTRODUCTION

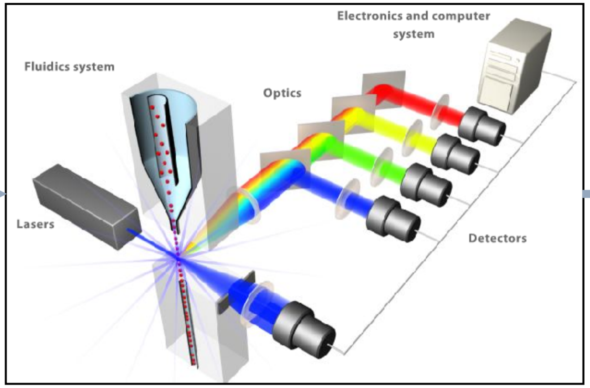
Aquatic microbes are retained as primary constituents of all known water sources aboard the international space station (ISS), as well as in future human spaceflights and planetary outposts (Horneck et al., 2010). Since space missions are expected to become longer lasting, space exploration is demanding the development of methods for in-flight monitoring, suitable to face microbial contamination risks within human confined conditions (Yamaguchi et al., 2014; Karouia et al., 2017; Allen et al., 2018). NASA has been developed microbial control strategies to minimize detrimental cell growth during spaceflight by reducing humidity, eliminating free water, and maintaining high-volume exchange and air filtration. The ISS is maintained at around 22°C with a relative humidity of around 60%, with pressure and oxygen concentrations very close to those at sea level on Earth (Pierson et al., 2013).



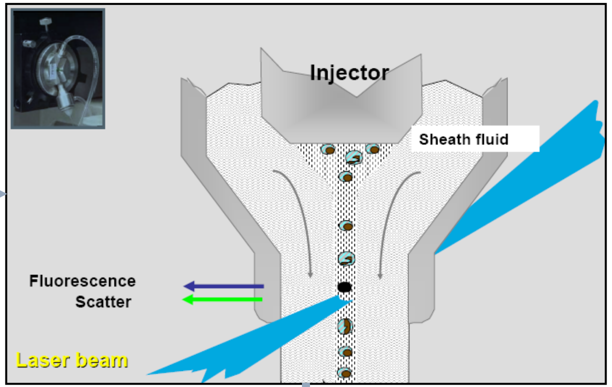
➢ Flow cytometry

- Multiparametric technology to count and measures the optical properties of individual particles in a flow stream
- The analysis of several thousands of cells in a few seconds (up to 100.000 particles/s) is more accurate, statistically significant, and independent from the operator.

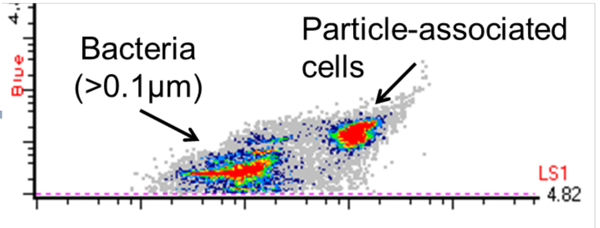
Portable systems (Apogee A50-micro)



Basic principles and system internal functioning

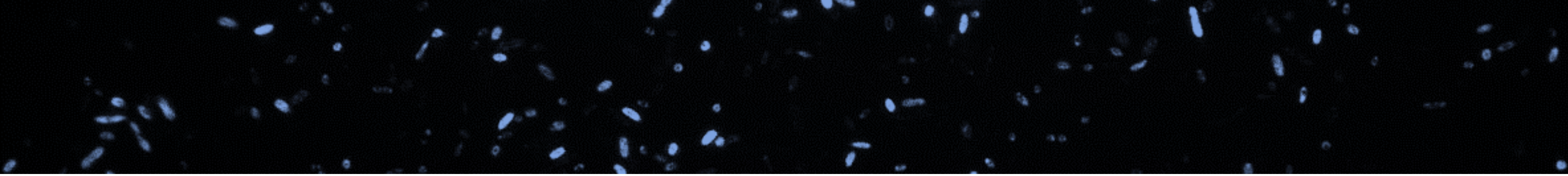


Quantitative assessment of water microbiological contamination

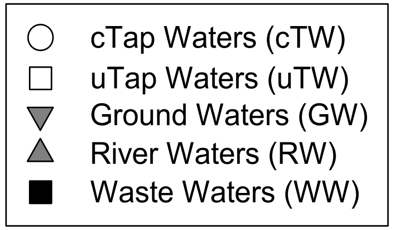


Spaceflight-compatible applications by FCM

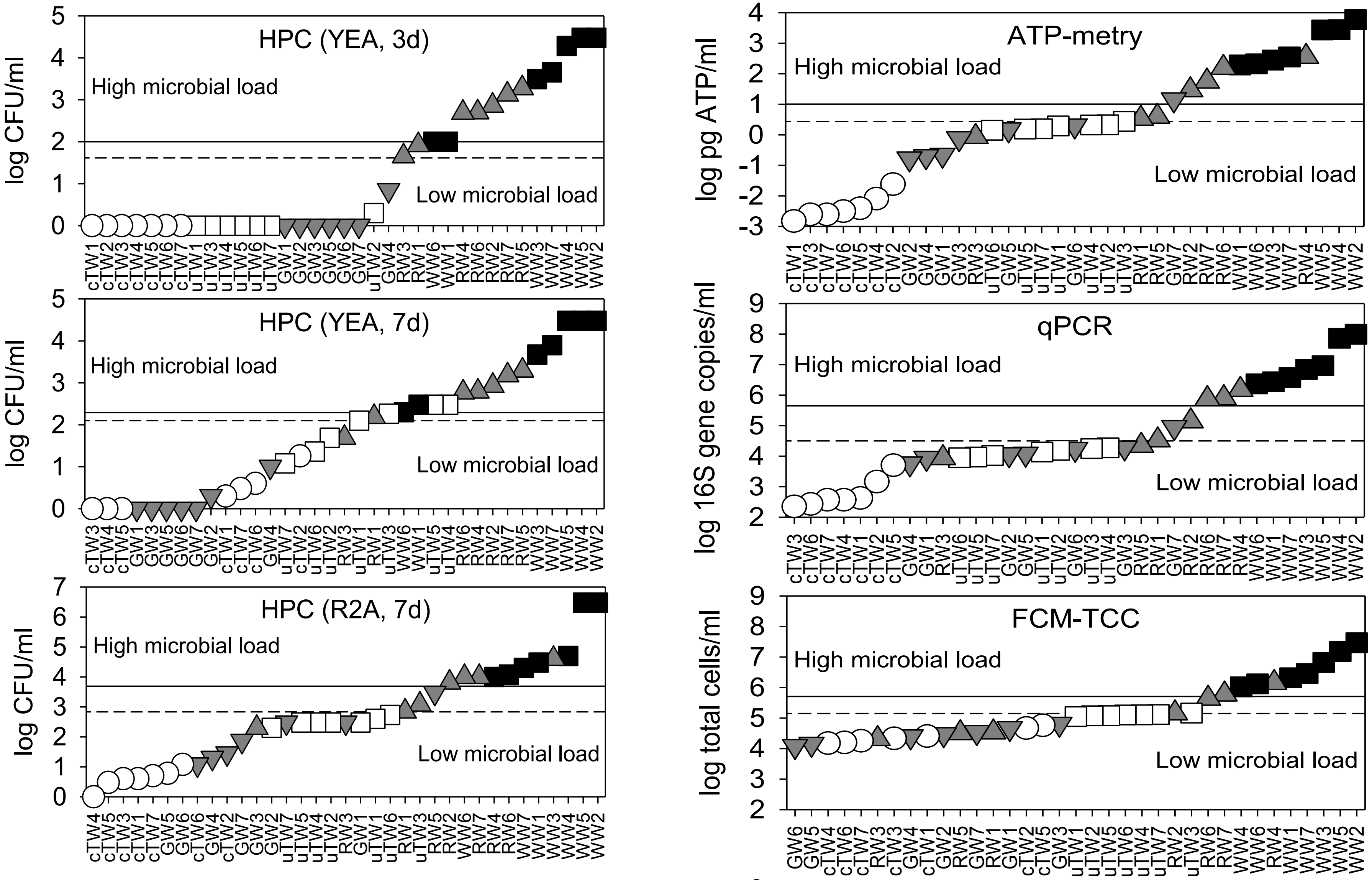
Crucian & Sams, 2005
Balestrieri et al., 2010
Cohen et al., 2011
Dubeau-Laramee et al., 2014



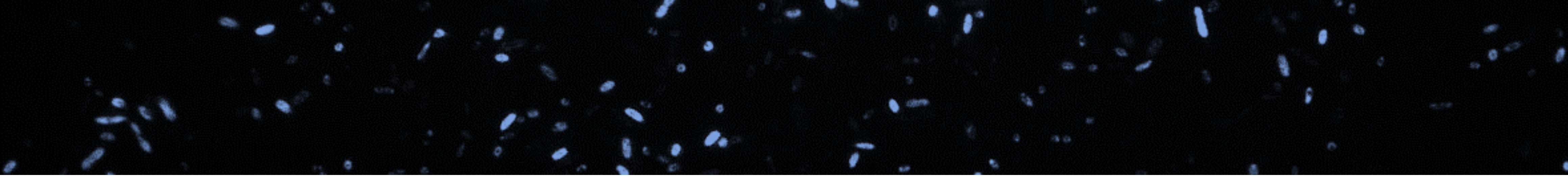
Results



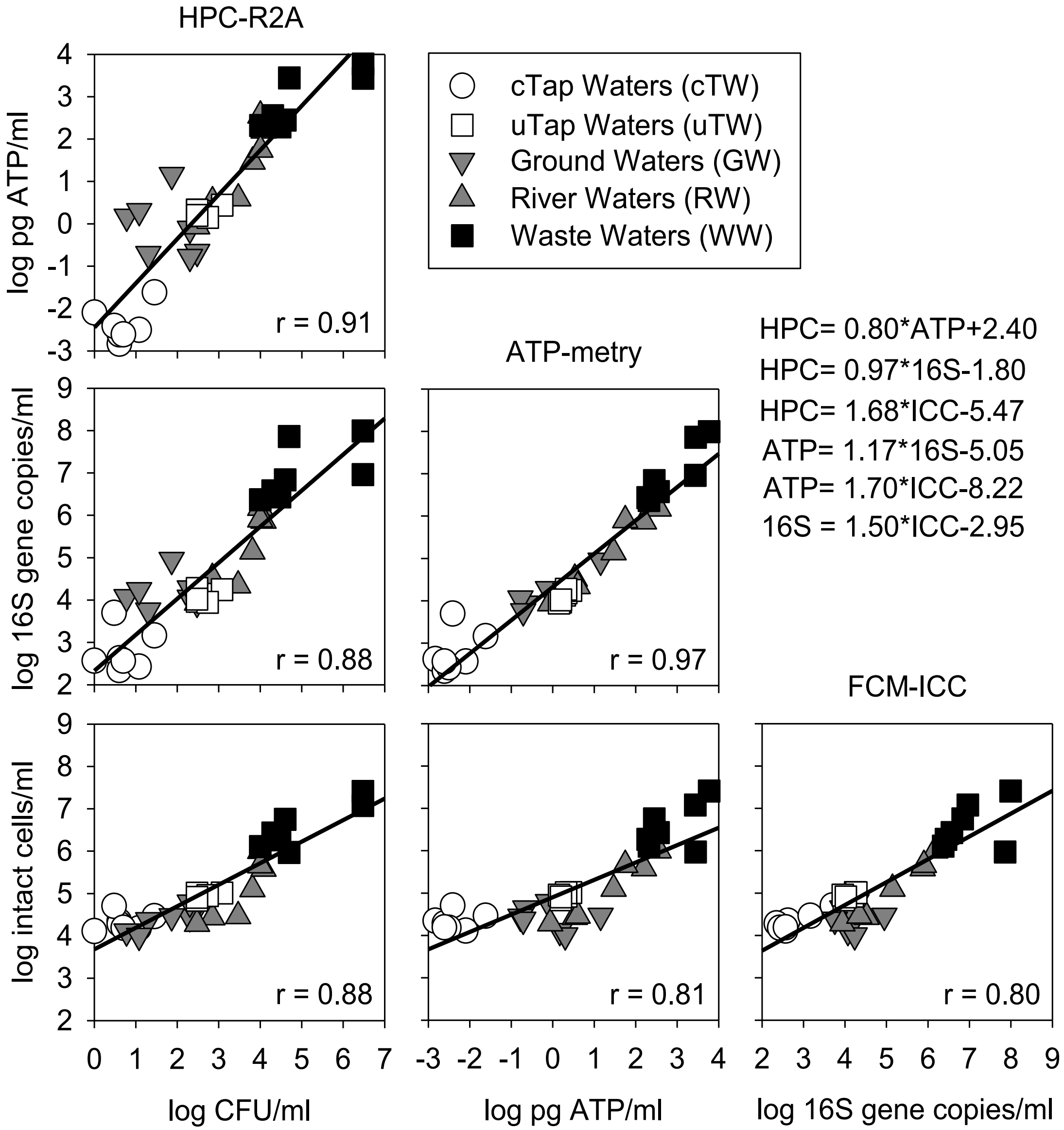
cTap Waters (cTW)
uTap Waters (uTW)
Ground Waters (GW)
River Waters (RW)
Waste Waters (WW)



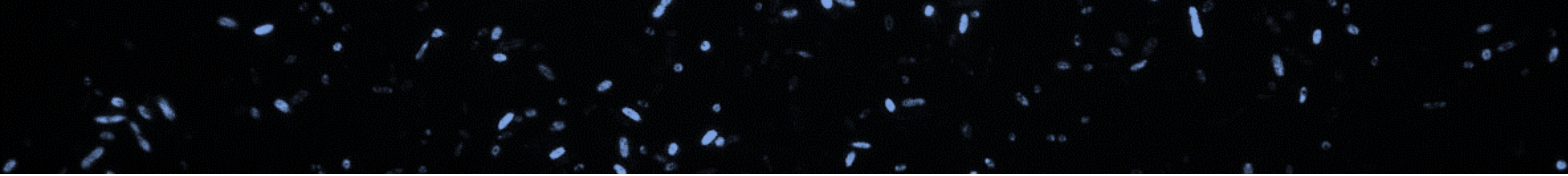
HPC (YEA, 3d)
log CFU/ml
High microbial load
Low microbial load
HPC (YEA, 7d)
log CFU/ml
High microbial load
Low microbial load
HPC (R2A, 7d)
log CFU/ml
High microbial load
Low microbial load
ATP-metry
log pg ATP/ml
High microbial load
Low microbial load
qPCR
log 16S gene copies/ml
High microbial load
Low microbial load
FCM-TCC
log total cells/ml
High microbial load
Low microbial load

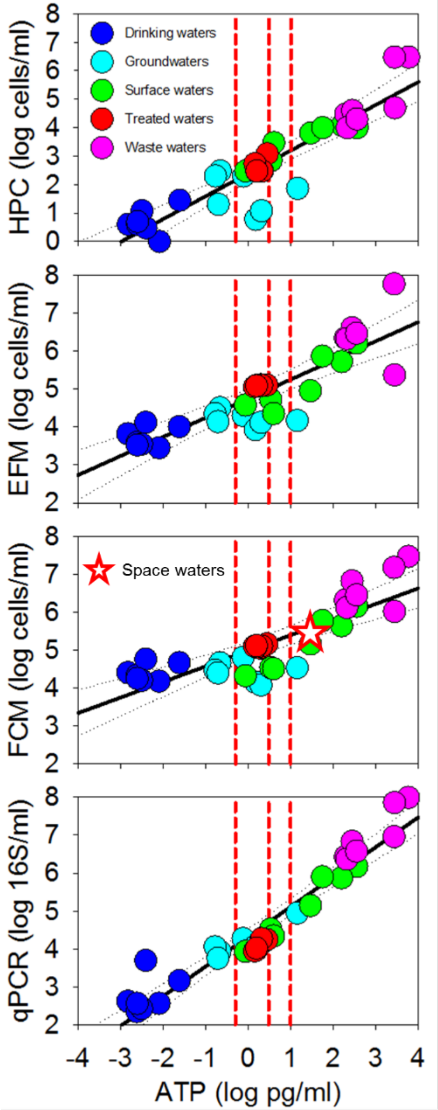


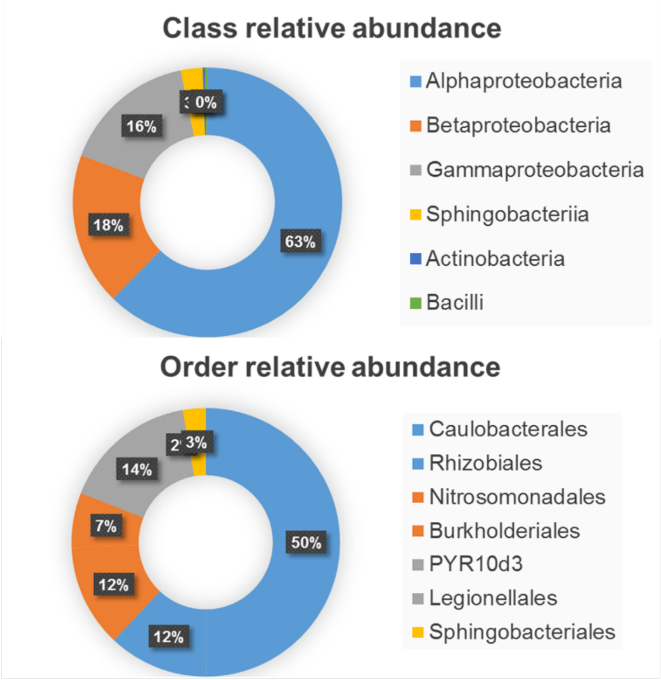
Results



Model	Parameter	Warning threshold	Alarm Threshold
FDM	HPC-YEA3d (CFU/ml)	41	100
FDM	HPC-YEA7d (CFU/ml)	126	195
FDM	HPC-R2A7d (CFU/ml)	684	4898
LRM	HPC vs ATP	557	1604
LRM	HPC vs qPCR	381	4980
LRM	HPC vs FCM	428	4145
	Reference values (mean ±*sd*)	512 ±*137*	3907 ±*1580*
FDM	ATP (pg/ml)	2.7	10.2
LRM	ATP vs HPC	3.3	26.1
LRM	ATP vs qPCR	1.7	37.9
LRM	ATP vs FCM	2.0	22.2
	Reference values (mean ±*sd*)	2.4 ±*0.7*	24.1 ±*11.5*
FDM	16S rDNA (10^4 copies/ml)	3.2	44.4
LRM	16S vs HPC	5.5	29.4
LRM	16S vs ATP	4.7	13.2
LRM	16S vs FCM	3.6	29.9
	Reference values (mean ±*sd*)	4.2 ±*1.0*	29.2 ±*12.8*
FDM	ICC (10^5 cells/ml)	1.0	4.2
LRM	ICC vs HPC	1.3	3.6
LRM	ICC vs ATP	1.2	2.1
LRM	ICC vs qPCR	1.0	4.1
	Reference values (mean ±*sd*)	1.1 ±*0.2*	3.5 ±*1.0*



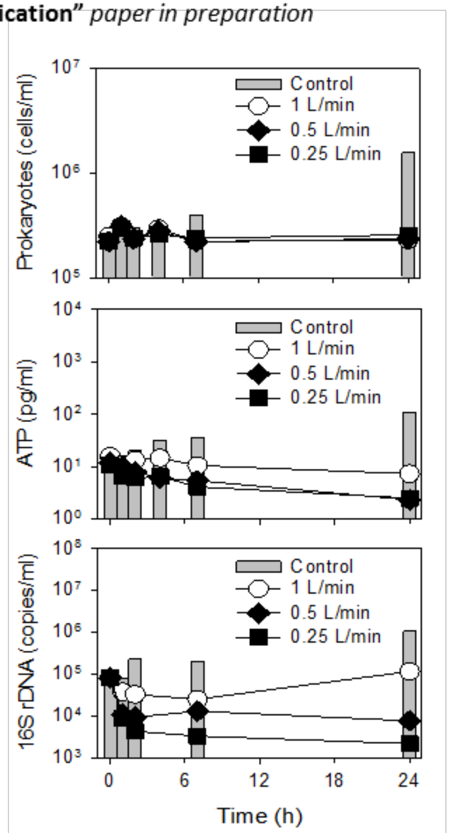




Bacterial community composition in waters after exposure to microgravity.

BIOWYSE SYSTEM: Efficacy of UV treatment to reduce the biological contamination under the conditions defined for the Biowyse system and ATP response

"Effect of UV-LED treatment on cell cultivability and viability for water quality control in space application" *paper in preparation*



VIABLE ISS: effect of decontamination treatments on microbial colonization of water and surfaces

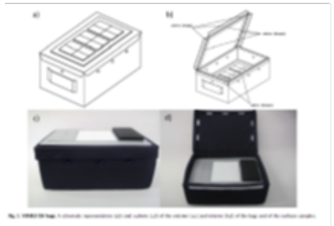

Research in Microbiology

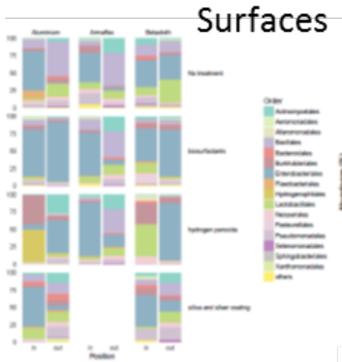
Original Article

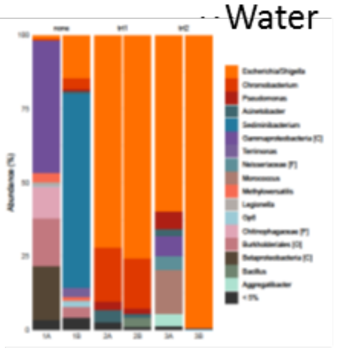
Furnishing spaceship environment: evaluation of bacterial biofilms on different materials used inside International Space Station

Elena Perrin, Giovanni Bacci, Laurent Garrelly, Francesco Canganella, Giovanna Bianconi, The Biowyse Consortium, Renato Fani, Alessio Mengoni

VIABLE-ISS samples







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