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# Overview of the International Space Station's Water and Cabin Air Quality: A Five-Year Status

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**Since the beginning of the International Space Station (ISS), water and air quality have been monitored to ensure crew health and verify the performance of the regenerative Environmental Control and Life Support (ECLS) systems. Over the last 25 years, the ISS has evolved greatly with significant changes to operations, crew complement sizes, visiting vehicles, payloads, and upgrades within the regenerative hardware, seen through Technology Demonstration integrations. In particular, better assessment and prevention of volatile organic releases from payloads and crew hygiene products, and implementation of advanced sorbents both on the air and water strings have been successful in reducing contaminant loads. Data on air and water quality for the last five years on ISS will be presented (nominal and contingency air grab samples, in-flight monitoring for air and water quality, and water samples from all segments of the ISS water system), including some notable events. The available data demonstrate the performance of existing ECLS systems and overall status of how the approach to air and water quality have evolved through the new ISS architecture baseline operations.**

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## Nomenclature

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| *AQM* | = | Air Quality Monitor |
| *AR* | = | Atmosphere Revitalization |
| *CHIPS* | = | Charcoal HEPA Integrated Particle Scrubbers |
| *CFU* | = | Colony Forming Units |
| *DMSD* | = | Dimethylsilanediol |
| *ECLS* | = | Environmental Control and Life Support |
| *FBCO2* | = | Four Bed Carbon Dioxide Scrubber |
| *HEPA* | = | High-efficiency particulate air |
| *ISS* | = | International Space Station |
| IMV | = | Intramodule Ventilation |
| *MMST* | = | Monomethylsilanetriol |
| *NDIR* | = | Non-dispersive infrared |
| *PDMS* | = | Polydimethylsiloxane |
| *PFU* | = | Protoflight Unit |
| *PWD* | = | Potable Water Dispenser |
| *SERFE* | = | SWME Express Rack Flight Experiment |
| *SMAC* | = | Spacecraft Maximum Allowable Concentration |
| *SWEG* | = | Spacecraft Water Exposure Guidelines |
| *THC* | = | Temperature and Humidity Control |
| *TOC* | = | Total Organic Carbon |
| *TOCA* | = | Total Organic Carbon Analyzer |
| *WRM* | = | Water Recovery and Management |
| *WRS* | = | Water Recovery System |
| *WPA* | = | Water Processor Assembly |

## I. Introduction

THE International Space Station (ISS) has celebrated over 23 years of continuous human occupation. Over the years, advancements in science and technology towards future exploration beyond Lower Earth Orbit have also been achieved. These celebrations are in part thanks to the regenerative systems aboard the ISS that ensure crew safe, breathable air and drinking water. These regenerative systems for environmental control and life support (ECLS) have been integrated since November 2008 and are continuously monitored for operational performance to ensure quality air and water are recycled, or generated, safely. Monitoring for system performance and for crew health perspectives are achieved through limited in-flight monitoring by ISS sensors/analyzers and return to ground samples for higher characterization analysis. These results offer crucial insights towards maintaining crew safety but also help identify opportunities for improvement within the ECLS systems such as how overall ISS operations may impact process sensitivities within the ECLS architecture. To that end, targeted advancements via select upgrades within the ECLS systems and overall improvements to managing releases of problematic contaminants within the ISS closed loop processes have been implemented to a high degree.

The ISS continues to support more science initiatives and commercial partnerships. This has brought on the increased crew counts and has also increased vehicle docking frequency onto the ISS. The upgraded ECLS systems described below highlight the current architectures and how the systems have been performing with respect to in-flight and return to ground sampling. The focus of this paper will be discussing the last five (5) years of ISS operations (i.e., 2019-2023, Increments 58-70). It is within this time period that the most significant changes within the ECLS architecture were implemented and thus presents an opportunity to capture what is considered the new ECLS baseline operations with respect to air and water quality.

## II. ECLSS Air Architecture Overview

The ISS ECLSS architecture was designed as required to support several key capabilities outlined by the ISS System Specification.[1] These capabilities include controlling atmospheric pressure, monitoring atmospheric constituents, atmosphere conditioning and contaminant control, providing water for consumption and hygiene, fire detection, and support of extravehicular activity. These specific functions are provided by the core ECLS subsystems which include Atmosphere Control and Supply, Temperature and Humidity Control (THC), Water Recovery and Management (WRM), and Atmosphere Revitalization (AR).[2,3] While many subsystems contain engineered interdependencies (e.g. water recovery and oxygen generation), their operation within the integrated environment of the ISS cabin generates new interrelated responses. Specifically, trends in cabin air and water quality have proven to be intimately coupled, especially in the case of water-soluble trace contaminant species by gas to liquid mass transfer via the THC Subsystem.[4,5]

Over the prior 5-year period, two modifications within the ISS ECLSS architecture have resulted in major perturbations of the annual ISS global air and water quality trends. First, the Charcoal HEPA Integrated Particle Scrubbers (CHIPS) were installed to protect the Condensing Heat Exchangers located within the Common Cabin Air Assembly throughout April to October 2019.[6] These filters contain activated carbon which prevents fouling of the hydrophilic Condensing Heat Exchanger surface coating by capturing heavy trace contaminants whose adsorption may undesirably render the coating surface hydrophobic.[7] The increased scrubbing air flow through the CHIPS also greatly decreased the cabin atmospheric dwell time of many volatile methyl siloxane species, which in turn prevents decomposition to recalcitrant reaction products such as dimethylsilanediol (DMSD).[8,9] Next, the Four Bed Carbon Dioxide Scrubber (FBCO2) was commissioned in September 2019.[10] The FBCO2 is a carbon dioxide ($CO_2$) removal technology demonstration which implements thermal and vacuum swing adsorption to scrub carbon dioxide utilizing a zeolite sorbent with water and air save capabilities. While operationally similar with the existing ISS Carbon Dioxide Removal Assembly design, the FBCO2 instead contains a 13X zeolite. Utilization of 13X effectively doubles the molecular sieve opening dimension within the $CO_2$ capture stage. This configurational change enables the FBCO2 to scrub an array of trace contaminants from the cabin atmosphere; a capability not previously observed with other ISS $CO_2$ removal systems.[11]

In addition to FBCO2, a second significant $CO_2$ Removal Technology was demonstrated recently on ISS. Fully activated in April 2019, the Thermal Amine Scrubber provides $CO_2$ removal by means of a regenerable solid amine-based sorbent technology. Featuring air and water save capabilities, the Thermal Amine Scrubber implements thermal and vacuum swing adsorption cycles to provide continuous $CO_2$ control. The concurrent operations of Thermal Amine Scrubber with FBCO2 has both provided and augmented U.S. $CO_2$ removal capabilities, allowing for operational flexibility in times of increased crew size and overlap. Both $CO_2$ removal technologies remain available for continuous or supplemental operations in 2024.

## III. ECLSS Water Architecture Overview

Early challenges within the ISS Water Processor Assembly (WPA) were related to management of small, water-soluble volatile organic contaminant species. The baseline WPA design and operations were tailored to an immature understanding of what upstream systems may impart to the downstream WRS, and therefore subassemblies were sized to accommodate projected levels based on limited data. Although these predictions were still considered high fidelity, the unknowns of what would be released, or possibly generated, within the operational ISS environment were not realized until returned samples were characterized and assessed. As alluded to above, an impactful contaminant species, DMSD, had proven challenging for removal within the WPA. In general, DMSD is not a major crew health concern, but it contributes to increased levels of total organic carbon (TOC) load within the WPA product water. These TOC levels are monitored via in-flight sample analysis and if levels are high enough, action is required to recover quality water, often requiring replacement of major components within the WPA. Thus, addressing the DMSD contamination into and through the WPA was high priority to minimize the frequency of these major component replacements. A three-tiered approach was proposed and almost fully implemented, by way of source reduction (via crew hygiene product selectivity), air scrubbing (via CHIPs filters), and enhanced sorbent and catalyst operational capability within the WPA.[6,12] Both the CHIPS filters and the enhanced sorbent found within the WPA Multifiltration beds have been in operation showing significant progress in managing the overall TOC levels, with better DMSD management.[12] Complete implementation of the final tier, by way of an upgraded WPA catalyst, is awaiting installation and will likely be completed within the next year.

## IV. Air Quality Summary (2019-2024)

Air quality on ISS is monitored using a combination of archival air samples and in-flight monitoring (ISS MORD 50260). Archival samples are collected in small cylinders called mini grab sample containers (mGSCs) every 45 days in the US Lab and at various locations around the station; our Russian colleagues collect their archival samples in sorbent-containing tubes which they refer to as AK-1M. Since 2008, the Air Quality Monitors (AQM) have provided in-flight monitoring for numerous compounds, and beginning in 2022, the second iteration of the Analyzing Interferometer for Ambient Air (ANITA-2) has been operated by ESA on ISS. After its performance was evaluated, the ISS Program has determined that it will use ANITA-2 for operational decision making on trace contaminants beginning in 2024. Data on ISS air (and water) quality are provided after the conclusion of every ISS Increment, and the data and interpretation are available at the public-facing website for Toxicology and Environmental Chemistry (https://www.nasa.gov/directorates/esdmd/hhp/toxicology-analysis-of-spacecraft-air/).

From the beginning of January 2019 through the end of calendar year 2023, air quality on ISS was generally deemed excellent. A summary of T-values from all nominal (n = 71) and contingency (n = 11) mGSCs (archival samples) is

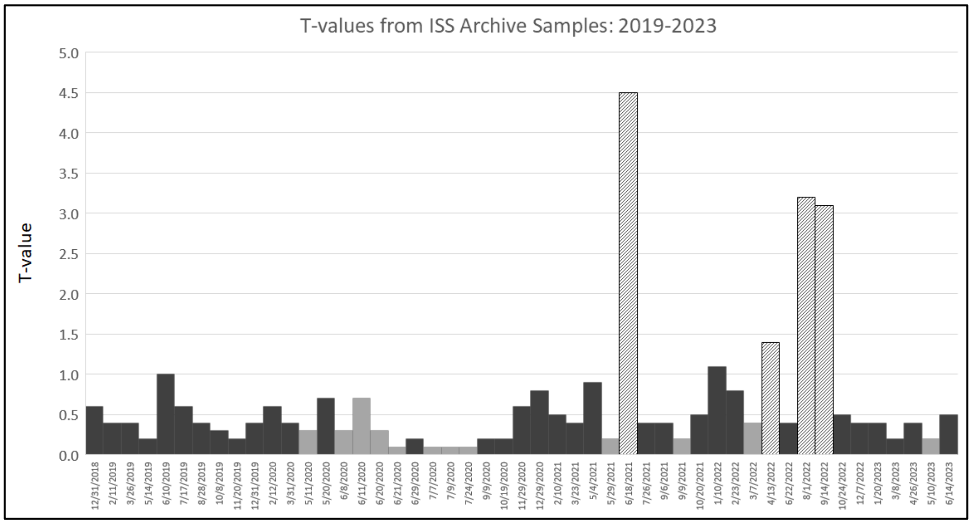


**Figure 1: T-values from ISS Archive Samples, Nominal and Contingency.**

Nominal archive samples (■), contingency samples (■), samples in which T-value exceeded 1 (▨).

provided in Figure 1. In this period, only 4 nominal mGSCs had a T-value greater than 1. The first of these occurred in Increment 65, and the T-value of 4.3 was overwhelmingly attributable to the presence of heptafluorobutanoyl fluoride, a rare, fluorinated compound for which little toxicology data are available. As such, it was assessed using the very low default SMAC value of 0.1 mg/m$^3$. Three samples exceeded T-values of 1 in Increment 67, and those exceedances were attributable to the presence of acrylonitrile (which has an interim SMAC value of 0.07 mg/m$^3$). In September 2022, acrylonitrile was measured at 0.19 mg/m$^3$ in the Russian SM but was not detected in the US Lab. Acrylonitrile was intermittently detected on ISS between early 2021 and Fall of 2022 but has not been detected since. Given that the T-value exceedances in all cases were brief (and no crew reported irritation, a symptom of acrolein exposure), none of these observations indicate a concern for crew health. Over this period, the average T-value was 0.38 (including the samples described above and contingency air samples), indicating excellent air quality on ISS overall.

Total alcohol content in ISS is tracked carefully, not because it represents a risk to crew health, but because levels greater than 5 mg/m$^3$ may pose concerns for the operational health of the WRS. The average level of total alcohols over this period was just above 5 mg/m$^3$; the maximum level observed was 10 mg/m$^3$ during Increment 65 (June 2021). In all cases, the primary contributor to the total alcohol content was ethanol. As described above, the WRS has been operating nominally and thus the numerous episodes in which total alcohols rose above our guidance value did not cause any hardware issues.

Data from the AQM units have generally agreed with data from the mGSCs, with the notable exception of the benzene anomaly in 2020 (this episode is discussed in detail in ICES paper 2024-157), and a complete report is available at the Toxicology and Environmental Chemistry website). Data from ANITA-2 will be represented elsewhere, but it is worth noting that the high sampling rate (every 6 minutes) of the unit over the past 2 years has yielded very interesting results on a number of compounds in ISS air, including Freon 218 (octafluoropropane), sulfur hexafluoride, volatile siloxanes, and methane.

Crew collect samples of the atmosphere of visiting vehicles on ingress, to provide retrospective data on the potential contribution of these vehicles to atmospheric concerns on the station. During this period, 24 ingress samples were collected and analyzed. The importance of these samples can be difficult to characterize, as the ISS atmosphere mixes notoriously well with the internal atmosphere after only a few minutes after Intermodular Ventilation (IMV) is established upon ingress. If crew collect the sample immediately, it can give useful insight; but beyond 10 minutes, the sample will reflect the ISS atmosphere in general. Only one sample gave rise had a T-value greater than 1: HTV-8 (September 2019). The HTV-8 ingress sample contained higher-than-usual levels of carbon monoxide and trimethylsilanol, which led to a T-value of 1.5 (compared against the 7-d SMAC; in this scenario, a T-value of < 3 is considered acceptable).

On occasion, crew will collect an mGSC in response to a concern for air quality (referred to as a contingency sample). The best example of this during the 5-year period is the benzene anomaly, in which 8 contingency mGSCs were collected for later analysis alongside the 4 planned nominal archive samples. Crew collected two contingency samples within Node 3 in 2021 in response to significant odors in that area, which were later determined to be attributable to the Brine Processor Assembly (BPA).[13] No unusual compounds were detected; this event is another in a familiar pattern wherein mGSCs are often collected in response to odors of unknown origin, but the results generally are not informative. This is not surprising, as the human nose is sensitive to volatile compounds at orders of magnitude below the detection limits of any analytical instrumentation. A similar event occurred in February 2022, in which crew collected a contingency sample in response to an odor described as "like sulfur or rotten eggs." Analysis of the sample revealed only slightly elevated concentrations of $CO_2$ and ethyl acetate, neither of which could have caused the odor described by crew. In real-time, the odor was attributed to operations of the Urine Processor Assembly (UPA). When UPA operations were terminated, the odor dissipated overnight, but could never fully pinpoint a direct cause to UPA. Crew collected another contingency sample in May 2023; after accessing an area that had been sealed for many years, a crewmember noted very strong odors and retreated from the area. The sample was collected later, and thus it is likely that the confined area had already equilibrated with the rest of ISS. Analysis of the sample did not provide any insight. Another contingency sample was collected in November 2023 (in response to strong odors in Node 3 following docking of 86P) but has not been analyzed at the time of writing.

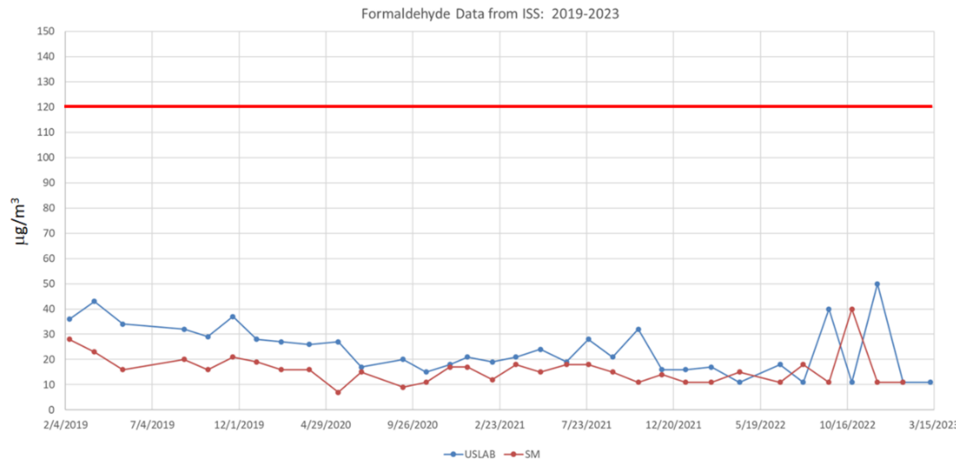


**Figure 2. Results of Formaldehyde Badge Samples.**

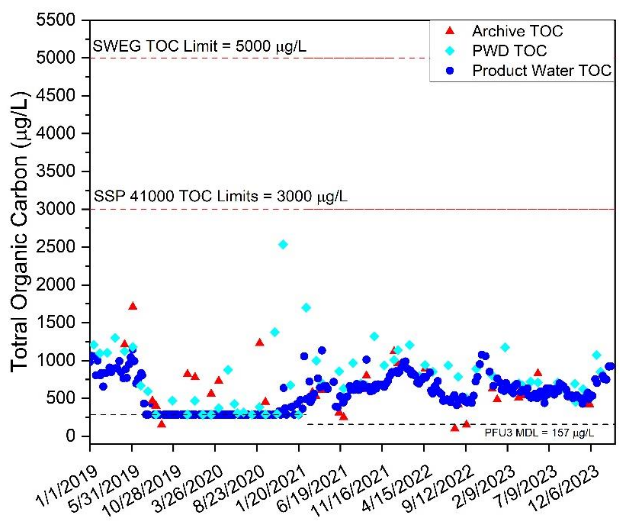


**Figure 3. Returned to Ground and In-flight Analysis for Total Organic Carbon of Potable Water.** TOC concentrations in WPA product water (●) and in samples collected from the PWD (▲) as determined by the US Total Organic Carbon Analyzer. Archival samples (▲) are generally collected the same day as a PWD TOCA analysis, though some of the data here also represents PWD Auxiliary port samples collected as a standard check of the product water or as a part of engineering investigations.

Our approach to monitoring formaldehyde has been the use of passive sorbent badges (bisulfate based), which return to Earth for analysis along with the mGSCs from ISS. Crew deploy the badges for a 48-hour period within the US Lab and the Russian SM in conjunction with nominal mGSC sampling. At the time of writing, 32 sets of formaldehyde badges had been analyzed, spanning up to January 2023 (Figure 2). In general, formaldehyde levels on ISS assessed using this method ranged from < 10 to 50 µg/m$^3$ (< 8 - 40 ppb). In comparison, NASA's 180-day SMAC for formaldehyde is 120 µg/m$^3$ (100 ppb).

Despite momentary events, the air quality on ISS has historically been excellent and that trend continued over the past 5 years. The effort dedicated to maintaining air quality is staggering, beginning with careful selection of materials, testing the off-gassing of vehicles and materials, and thorough assessment of all chemicals that fly to the ISS. The long history of good air quality is a testament to the success of the approach, and there is much to emulate in NASA's experience and successes as others take up the mantle of operating manned space stations in Low Earth Orbit (LEO).

## V. Water Quality Summary (2019-2024)

The ground-based results from total organic carbon (TOC) water samples collected from the ISS Potable Water Dispenser (PWD) between January 2019 and January 2024, along with TOC results provided by the in-flight Total Organic Carbon Analyzer (TOCA), are shown in Figure 3. All samples analyzed possessed TOC concentrations much lower than both the human health-related Spacecraft Water Exposure Guideline (SWEG)[14] and the more stringent SSP 41000[15] limits focused on system health. During this period, a single sample from the PWD analyzed by TOCA resulted in a TOC of ≈ 2500 µg/L (November 25, 2020). This result is clearly out of family with other samples in the same timeframe. Investigation of the operations occurring with TOCA immediately before this sample showed that samples from the SERFE technology demonstration with TOC in the ≈ 7000 µg/L range had been analyzed, but the testing had terminated during the third run. Additionally, the relative standard deviation of the PWD sample analysis was much higher than normally seen for TOC; therefore, it is likely that the first run of the PWD sample was contaminated by carryover from the previous run. For the archival samples collected in this period, a large percentage of TOC can be attributed to DMSD, which is to be expected as it accumulates in the MF beds and is then released as the beds become saturated and/or more strongly-bound species displace it.[12] However, for the relatively low levels of TOC in these samples, analytical reporting limits often make it difficult to identify the overall contribution of specific compounds. Interestingly, two recent samples collected from the PWD Ambient leg and PWD Aux port have shown the presence of monomethylsilanetriol (MMST), a further hydrolysis product of polydimethylsiloxanes (PDMS), which had not been present in the product water (and never in potable water) since 2015. The only other compound regularly observed in potable/product water samples in methyl sulfone.

One item to note in the WPA product water TOC data is the offset in the results provided by the archival samples versus the results from the TOCA in late 2019 into early 2021. The most likely explanation for this offset was degraded TOCA performance. Results from calibration check standards performed every 90 days showed a clear decreasing trend in the TOCA response over time. The exact cause of the decreasing response was believed to be related to a decrease in the intensity of the non-dispersive infrared (NDIR) source in the TOCA. The TOCA in use at that time (PFU2) was originally deployed in 2013 and successfully operated beyond its 5-year certified operational lifetime. As investigations into the performance of the NDIR source in PFU2 showed signs of significant degradation, the decision was made to activate the spare TOCA (PFU3). Activation of this instrument occurred in February 2021, and the archival samples began to agree much better with the inflight analyses. This new hardware also possessed a decreased minimum detection limit (157 µg/L v/s 285 µg/L for PFU2), though the results for water produced by the WPA since the activation of PFU3 have generally been well above this level.

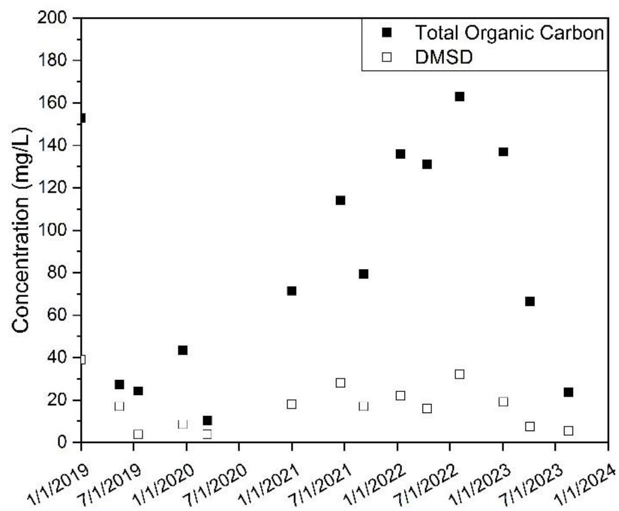


**Figure 4. Total Organic Carbon and DMSD Results for Humidity Condensate.** TOC and DMSD concentration in humidity condensate collected from the WPA condensate sample port. Note that the DMSD concentration has remained stable even as the overall condensate TOC has increased.

The TOC of humidity condensate collected from the ISS atmosphere is presented in Figure 4 along with the concentration of DMSD present in the samples. At the beginning of 2019, a significant drop in the condensate TOC can be observed, along with a corresponding drop in the DMSD present. These decreases correlate with the installation of Charcoal/HEPA Integrated Particle Scrubbers (CHIPS) on the ISS in April 2019; these filters aimed to reduce the dwell time and mass of various PDMS and silanols circulating within in the cabin atmosphere and limit the species mass reaching the heat exchanger surfaces. Interestingly, even as the condensate TOC began to increase in 2021, the DMSD concentration remained relatively stable. This is likely a result of other operational changes on the ISS aimed at reducing the potential for PDMS to be volatilized (e.g more focus on scrutinizing crew hygiene items).[16]

Other standard, major contributors to the condensate TOC are shown in Figures 5 and 6. Here, it can be seen that some human metabolic products, methanol and acetone, are generally present in the 6-10 mg/L and 2-5 mg/mL ranges, respectively. Efforts are made to minimize the presence of 2-propanol to protect the WPA, requiring alternative cleaning methods to be used in-flight. However, cargo arriving on visiting vehicles inevitably add 2-propanol to the stack resulting from their cleaning prior to, and offgassing during, transit. Even so, the 2-propanol levels are generally in the 0 to 3 mg/L range in the condensate. Interestingly, several condensate samples in 2019 showed elevated levels of 2-propanol. This time-period coincided with the docking of SpaceX-Demo-1 in March 2019 and Northrup Grumman-11 in April 2019. An investigation of anomalously high 2-propanol readings from the Air Quality Monitor (AQM) following the SpaceX docking determined that the MegaHEPA filter in the Dragon vehicle had adsorbed significant quantities of 2-propanol because of cleaning prior to flight which was subsequently being released back into the atmosphere, where it eventually was collected in the condensate. Subsequent elevated AQM readings in April/May 2019 further led this investigation to the conclusion that the CHIPS filters arriving on NG-11 had been cleaned similarly to the SuperHEPA prior to flight, also had adsorbed a large quantity of 2-propanol, and were releasing this compound into the atmosphere following installation.[17] As these filters were semi-permanently installed, more time was required for the 2-propanol to be removed, explaining the continued high condensate concentrations over the next several months.

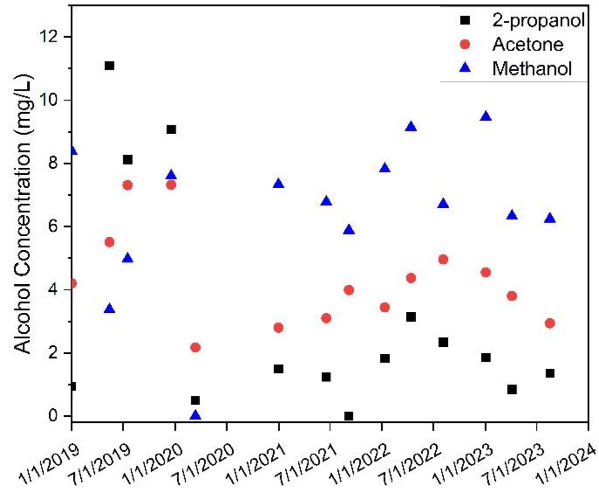


**Figure 5. Concentrations of Primary Alcohols Found in the US Condensate.** The concentrations of these compounds are generally stable with a few exceptions related to ISS atmospheric disturbances.

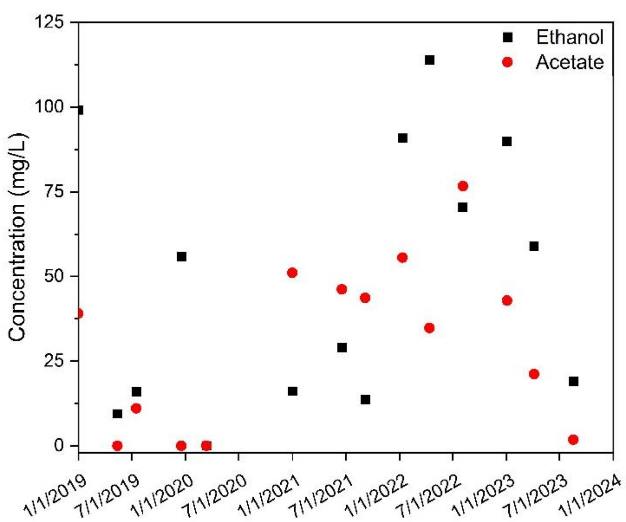


**Figure 6. Concentrations of Ethanol and Acetate in the US Condensate.**

Other major compounds of interest in the condensate have included ethanol and acetate. Ethanol has been said to account for greater than 70% of the total atmospheric alcohol concentration (where alcohols historically have been responsible for over 80% of the non-methane volatile organic compounds in the ISS atmosphere).[5] Figure 6 shows the concentrations for these compounds since the beginning of 2019. The ethanol concentration seems extremely high when compared to the other small alcohols (Figure 5) unless one considers its contribution to the atmospheric alcohols described above. The values shown here are much less than the maximum seen in the previous 5 years, in which the ethanol concentration in a November 2014 condensate sample approached 300 mg/L, with a subsequent sample possessing 175 mg/L ethanol. Unsurprisingly, these elevated levels corresponded with an increase in the atmospheric ethanol concentration.[18] Since early 2016, however, condensate ethanol levels have not surpassed 114 mg/L (April 2022) and have generally been much lower, with several recent samples being at or below 25 mg/L.

Acetate provides an interesting case-study with regards to condensate samples. In the period of interest, acetate recovered from these samples has ranged from 75 mg/L to below analytical reporting limits. This variation is striking when considering the relatively stable ranges of the other non-silanol species. However, recent work showed that the concentration of acetate (and other carboxylates) in archival samples can depend dramatically on the conditions under which the samples are collected, the length of time between collection and analysis, and the microbial load in the sample.[19] In that study, it was shown that, under some conditions, bacteria in the samples would catabolize acetate and other small carboxylates. As such, the values obtained for these compounds must be carefully considered, understanding that different sample collection procedures may need to be used if the final use of the concentration information is meant for comparison to ground testing or to provide insight into on-orbit conditions.

Changes in in-flight operations and hardware (e.g. single-bed MF bed usage, demonstration catalytic reactor, Exploration PWD-xPWD) could have effects on the overall water quality in the coming years, though initial results from both in-flight and archival sampling have shown that the water produced by the WPA remains suitable for human consumption. It remains to be seen if any of these changes will affect the ability for the water to be used for other operational purposes, such as EVAs. Additionally, new instrumentation may provide further insight into the quality of the water in flight, as the next-generation TOCA (miniTOCA)[20] is likely to be tested in the next several years, and efforts are underway to supply a suite of instruments to provide complete in-flight water analysis.[21]

## VI. Microbial Sampling Summary (2018 - 2024)

Mechanisms of microbial control are in place within the ECLSS WRS and air revitalization system (e.g., Trace Contaminant Control System, Oxygen Generation Assembly, and Carbon Dioxide Removal Assembly). Microbial control during water reclamation is first achieved through pre-treatment of the urine with a phosphoric acid/chromic acid solution (US segment). The product water (distillate) from urine processing is later joined by humidity condensate where further microbial control occurs from sterilization from the WPA by thermal catalytic oxidation.[22,23] Subsequent filtration and the dosing of iodine complete the final steps for microbial control within the water recovery system. The ISS atmosphere experiences high-volume exchange through HEPA filters, which are regularly inspected, vacuumed, and maintained.[24,25] While these controls provide exceptional microbial control, they do not eliminate all risk. To assess the efficacy of these controls, detect potential process escapes, and define areas where greater control may be needed, routine microbial monitoring is performed for the ISS air and water.[26,27] The

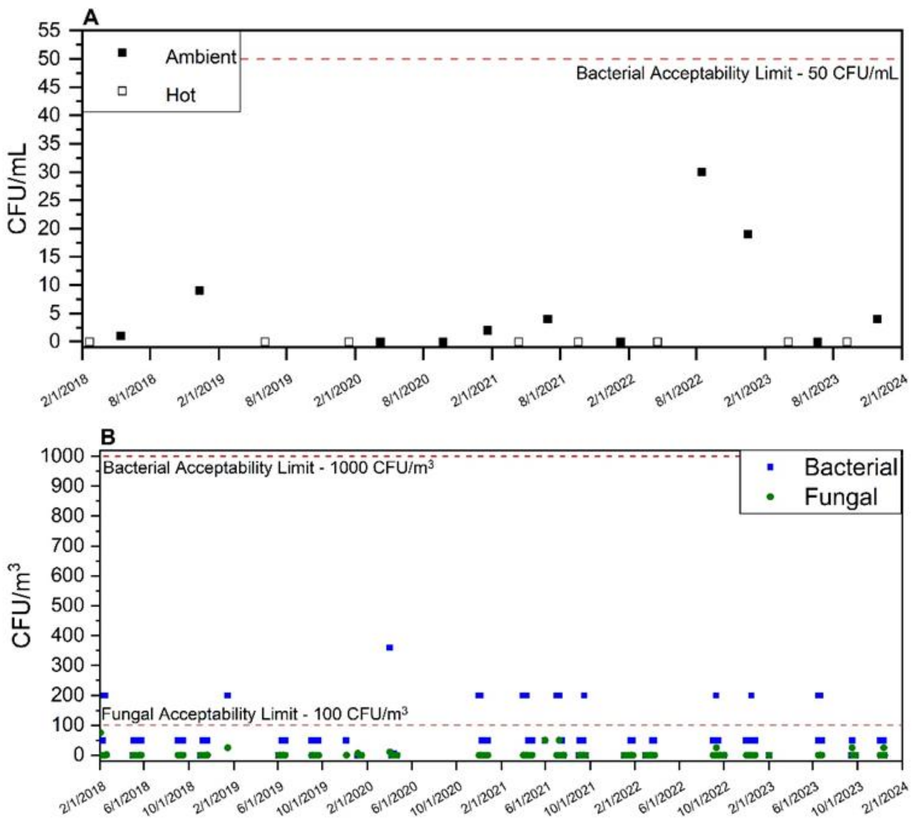


**Figure 7. Microbial Counts in Potable Water and ISS Cabin Atmosphere**. A) Levels of bacteria (CFU/mL) observed in samples from the ISS PWD Hot and Ambient ports since 2018. B) Levels of bacteria and fungi (CFU/m$^3$) noted in ISS atmospheric samples since 2018.

acceptability limit for potable water is 50 colony forming units (CFU)/mL of bacteria and no coliforms detected in 100 mL. For ISS air, bacteria must be below 1,000 CFU/m$^3$ and fungi below 100 CFU/m$^3$. Additionally, for both water and air, data are assessed for the presence of medically-significant microorganisms. Should the limits be reached, or medically-significant microbes be present, remediation is required. While the presence of coliforms is evaluated monthly from ISS potable water, monitoring for the levels and types of organisms present occurs on a quarterly basis for both air and water.[26,27]

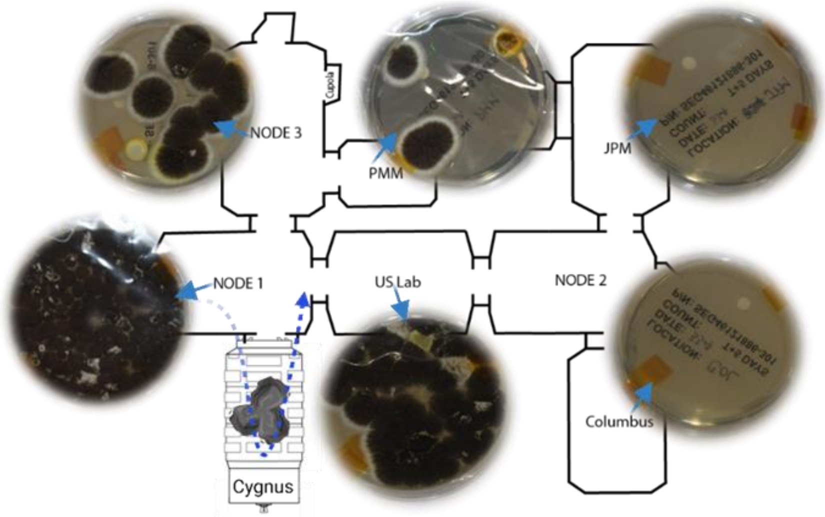


**Figure 8. Downlinked Images of Fungal Media Plates Following Air Sample Collection Across Six ISS Modules in November 2017.** Gross fungal contamination is noted on the plates from Node 1 and the Lab, followed by less contamination on plates from Node 3 and the PMM, and with no growth noted on plates from the JPM or Columbus. At the time of sampling, HEPA filters had been removed from Node 1 and trash was stowed in Cygnus. With HEPA filters reinstalled in December 2017, no fungal growth was observed following additional sampling.

The WRS has been providing microbially-clean water for crew consumption (Figure 7) since 2008. Water sampled form the PWD Hot Port has historically been free of any microbial growth. While PWD Ambient samples routinely demonstrate bacterial counts, they are well below the acceptability limit. The level of bacteria did reach 30 CFU/mL in August 2022 but trended downward afterwards, as noted by the 19 CFU/mL in December 2022 and 0 CFU/mL in June 2023. The bacteria associated with PWD Ambient samples include common waterborne bacteria that pose little-to-no risk to crew, with *Ralstonia pickettii* as the most common, followed by *Burkholderia* species, *R. insidiosa*, and *B. kururiensis*. It is also common to isolate Gram-negative bacteria that cannot be identified by the JSC Microbiology Laboratory standard methods. However, these bacteria have been investigated further and are closely related to those previously listed. Since the activation of the WRS, there had only been one sample that revealed the positive presence of coliform bacteria, but it was immediately attributed to crew contamination. With this one exception, all tests for coliforms have been negative.

The ISS atmosphere consistently displays low levels of bacteria and fungi. Of the samples collected within the last five years, 44% were negative for bacterial growth, while 92% were negative for fungal growth (Figure 8). The bacteria that have been detected are consistent with that of the human microbiome, specifically the skin and oral cavity. These bacteria are likely recently liberated from the crew and have not yet been collected by the HEPA filters. It is also probable that the counts provided by the crew are biased high. For water, the crew counts individual colonies, but, for air, the crew compares the growth on the plate to a colony density chart, which provides an over estimation of the true number of CFUs. The most commonly occurring bacteria are *Staphylococcus epidermidis*, followed by *S. hominis* and *S. capitis*, while the most commonly detected fungi are *Penicillium* spp. and *Aspergillus* spp.. Bacterial diversity is greater, with *Bacillus* spp., *Corynebacterium* spp., *Micrococcus* spp., and *Streptococcus* spp. having also been isolated. While these organisms are not of concern from a crew health perspective, *S. aureus*, *S. lugdunensis*, *Klebsiella aerogenes*, and *Enterococcus faecalis* have all also been isolated from ISS air samples. These are considered medically-significant and result in remediation when found on ISS surfaces. However, there is not a simple remediation strategy for air, and it is assumed that these bacteria will be trapped by HEPA filters. Should significant levels of bacteria or fungi be noted, an investigation will occur to locate and address the source. An example of this occurred in late November 2017, when substantial levels of *A. niger* fungus were noted on samples from Node 1 and the Lab, with less growth being detected on samples from Node 3 and the PMM (Figure 8). Repeat sampling was performed to determine if this was an isolated instance that had been cleared by the HEPA filters or was a larger issue. Samples were collected from Node 1 and the Lab in early December 2017 and again revealed significant fungal growth. Upon investigation by the JSC Microbiology team, it was determined that these elevated microbial levels were due to the HEPA filters in Node 1 being replaced with charcoal-only filters to remove problematic polydimethylsiloxanes from the ISS atmosphere. At that time, a Cygnus vehicle was docked to Node 1 and had been filled with trash. The IMV for Cygnus draws air from Node 1 and delivers it to the endcone of Cygnus, where it then returns to Node 1, passing through, in this case, trash. HEPA filters were reinstalled in Node 1 and sampling was again performed on December 22, 2017, at which point all microbial levels returned to normal, and no fungi were detected.

While the current monitoring strategy has served to protect crew and vehicle health, it has limitations in that real-time assessments are not possible, and the data are often biased. Determination of a colony forming unit requires cultivation of the bacteria and fungi in-flight, followed by sample return and laboratory-based identification. This process delays remediation, can potentially expose the crew to high levels of pathogens, and excludes important non-culturable microorganisms. Due to these limitations, a true assessment of wastewater within the WRS is not possible. While archive samples are collected, neither the microbial level or identities are a true representation of the community within the WRS, as the time between sample collection and receipt in the lab leads to a few types of bacteria outcompeting others.[19] These bacteria, commonly *Burkholderia* spp., are seen in high levels, typically $10^5 – 10^7$ CFU/mL, skewing the data and concealing the true diversity within the system. A new method based on nanopore sequencing has been validated for microbial monitoring of ISS surfaces,[26] air,[24] and, recently, potable water. This method removes both the need to culture the microbes and for sample return. Performing near real-time and comprehensive microbial monitoring of the ISS environment will provide greater insight toward ECLSS performance and increase risk assessment capabilities in support of crew and vehicle health.

## VII. Conclusion

Over the last five years of ISS operations, the air revitalization and water recovery systems have proven robust and capable systems to maintaining quality, safe drinking water and breathable air. The complexities of increased vehicle docking frequency and elevated crew sizes could have challenged these systems but taking the lessons learned from early operations and strategically targeting areas for improvements and upgrades have played a key role in this success. The implementation of limiting volatile organic release into the cabin atmosphere has effectively maintained levels of alcohols (both in air and in condensate) at lower baseline conditions, which helps maintain quality water production within the WRS. There have been challenges in assessing in-flight and return to ground results and finding immediate causes to transient disruptions given the complexities and unknown sensitivities due to limited real-time monitoring and subsequent sample degradation or biases; however, the ISS approach in maintaining sampling frequency and the flexibility in the increased monitoring rates, when necessary, have allowed critical insights to finding cause or confirming effective mitigation efforts. This assessment of the air revitalization and water recovery system of strategic sample analysis aboard the ISS validates these systems are exemplary regenerative technologies to help sustain human presence in space.

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