

Microbiological monitoring of scuba cylinders under routine filling and seasonal diving conditions

Recep Özkan¹, Osman Turkmen², Elif Okumuş³, Sukran Özhan⁴, Hasan Sivrikaya², Eşe Başbulut⁵, Kübra Ozgok Kangal⁶

¹ Department of Hyperbaric and Diving Medicine, Ataturk University Erzurum, Türkiye

² Department of Hyperbaric and Diving Medicine, Samsun Training and Research Hospital, Samsun, Türkiye

³ Department of Medical Microbiology, University of Health Sciences, Van Training and Research Hospital, Van, Türkiye

⁴ Department of Medical Microbiology, Erzurum City Hospital, Erzurum, Türkiye

⁵ Department of Medical Microbiology, Samsun Training and Research Hospital, Samsun, Türkiye

⁶ Department of Hyperbaric and Diving Medicine, University of Health Sciences, Gülhane Training and Research Hospital, Ankara, Türkiye

Corresponding author: Dr Recep Özkan, Department of Hyperbaric and Diving Medicine, Ataturk University, 25240 Erzurum, Türkiye

ORCID: [0000-0001-7342-6473](https://orcid.org/0000-0001-7342-6473)

recepzkn@hotmail.com

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Abstract

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Introduction: The aim of this study was to monitor the microbiological status of the inner surfaces of scuba cylinders under routine air filling conditions and to assess whether microbiological contamination emerges or persists over time.

Methods: A prospective longitudinal microbiological study was conducted using two complementary sampling models. Serial sampling of a steel scuba cylinder and its associated compressor air filter was performed at three-day intervals over 45 days. To assess reproducibility under operational conditions, additional microbiological sampling was performed from 27 scuba cylinders actively used during the diving season. Samples were analysed using standard culture methods.

Results: No microbial growth was detected in bacterial cultures obtained from any of the cylinder inner surface or compressor air filter samples throughout the 45-day monitoring period. No temporal changes or cumulative contamination trends were observed. Similarly, no microbial growth was detected in cultures obtained from the additional 27 scuba cylinders sampled during the active diving season.

Conclusions: Under routine operating conditions with standard maintenance and air filtration practices, the internal environment of scuba cylinders may remain microbiologically stable over time, suggesting a low risk of microbiological contamination in properly maintained compressed air systems.

Introduction

Infections associated with scuba diving have most commonly been attributed to direct exposure to aquatic environments, contact with moist surfaces, and variations in environmental hygiene. In particular, the inadvertent ingestion of water that may contain fecal contamination during diving has been linked to an increased risk of gastrointestinal infections, as well as infections involving the ear, skin, eyes, and respiratory tract.¹ Natural water environments, which may vary considerably in temperature, humidity, and microbial load, have also been reported to facilitate the development of infections during diving activities.

In contrast, the contribution of diving equipment to infection risk has received relatively limited attention. Although shared components such as regulators, mouthpieces, masks,

and other parts of the breathing circuit are often considered potential vectors for microbial transmission, available data on their microbiological contamination remain scarce and inconsistent. Notably, the microbiological safety of compressed breathing air within scuba cylinders has not been adequately explored in the existing literature.

From a theoretical standpoint, both the internal surfaces of scuba cylinders and the compressor systems used for filling may be exposed to several factors that could influence microbiological contamination. Moisture introduction, ingress of ambient air during filling, filter performance, and maintenance practices are all variables that may affect the microbiological quality of breathing air. Despite these theoretical considerations, data regarding the microbiological stability of scuba cylinders and associated compressor systems over time remain limited.

Evaluating the microbiological stability of a scuba cylinder and its associated compressor filter over time is therefore relevant not only for diving safety but also for assessing the effectiveness of routine equipment maintenance protocols. A longitudinal monitoring approach allows for the detection of potential cumulative contamination and subtle microbiological changes that may otherwise go unnoticed.

Accordingly, the present study prospectively and longitudinally monitored a single steel scuba cylinder and the air filter of the compressor used to fill it under routine operating conditions. The primary objective was to generate data addressing this underexplored aspect of microbiological safety in compressed air systems used in scuba operations and to provide a methodological foundation for future studies involving larger sample sizes and multicentre designs.

In addition, to assess whether the findings observed in the longitudinal monitoring model were reproducible under real-world conditions, a single-time-point microbiological assessment was performed in 27 scuba cylinders actively used during the diving season at an independent diving centre.

Methods

A. EXPERIMENTAL SETUP

A scuba cylinder that had completed routine maintenance and cleaning protocols was chosen as the representative sample for the investigation. A single 15 L steel scuba cylinder (Aqualung, working pressure 230 bar) fitted with a Coltri Sub valve (EN 144-1, M25×2 thread) and a portable petrol-driven high-pressure breathing air compressor

(CompTrade Kompressoren, Austria) were used throughout the study under routine operating conditions (Figure 1).

At the beginning of the experimental period, a swab sample was obtained from the cylinder valve outlet using a sterile swab and placed in an appropriate transport medium. In addition, to ensure contact with the entire inner surface of the cylinder, approximately 50 mL of sterile physiological saline was introduced into the cylinder. The cylinder was gently rotated to allow the fluid to contact all internal surfaces, after which the lavage fluid was transferred into a sterile, capped container.

The swab and lavage samples were assessed for bacterial contamination through inoculation into 5% sheep blood agar and chocolate agar medium. The inoculated plates were incubated at $35 \pm 1^\circ\text{C}$ for 48 hours. No microbial growth was detected during the incubation time; hence, the scuba cylinder was considered suitable for inclusion in the experimental study.

B. CYLINDER FILLING AND TEMPERATURE MEASUREMENT

In the primary longitudinal monitoring model, microbiological sampling was performed at three-day intervals over a 45-day period. A total of 15 sampling events were conducted, during which samples were collected from both the scuba cylinder and the associated compressor air filter. During each filling procedure, temperature measurements were obtained using a non-contact infrared thermometer (Bosch, Germany) directed at the external surface of the cylinder near its midpoint. Measurements were recorded at 10-bar increments as cylinder pressure increased from 0 to 200 bar.

The 27 scuba cylinders included in the supplementary sampling had been filled during the active diving season at an air filling station in accordance with the requirements for breathable compressed air defined in the EN 12021:2014 standard (European Committee for Standardization. EN 12021:2014. Respiratory protective devices – Compressed gases for breathing apparatus. Brussels: CEN; 2014). These cylinders were subsequently depressurised prior to microbiological sampling.

C. THE EXPERIMENT

Sampling of the scuba cylinder inner surface was performed using a sterile lavage technique. Sampling of the scuba cylinder inner surface was performed using the sterile lavage procedure described above. The collected lavage fluid was subsequently used for microbiological analysis. Following each sampling procedure, the internal surface of the cylinder was rinsed with distilled water to remove residual sampling fluid and was allowed to dry completely at room temperature. No chemical sterilisation procedures were applied. After drying, the cylinder was refilled according to standard air-

Figure 1

The steel scuba cylinder and the portable air compressor system used throughout the longitudinal monitoring period. The same cylinder and compressor were used for all filling procedures and microbiological sampling under routine operating conditions



filling procedures and stored under routine indoor conditions until the next scheduled sampling interval. This standardised handling procedure was applied throughout the study period.

Sampling of the compressor air filter was performed using sterile swabs. Microbiological sampling was carried out exclusively from the actively used compressor air filter (Figure 2, right), while the previously used filter (Figure 2, left) is presented solely for illustrative comparison. Swab samples were placed in Stuart transport medium to preserve microbial viability during transport and delivered to the microbiology laboratory without delay.

To exclude potential contamination originating from the physiological saline, negative control cultures were performed prior to sampling by inoculating the saline onto 5% sheep blood agar and chocolate agar and incubating under appropriate conditions; no microbial growth was observed.

Both the scuba cylinder inner surface lavage samples and the compressor air filter swab samples were inoculated onto 5% sheep blood agar and chocolate agar plates. All plates were incubated aerobically at $35 \pm 1^\circ\text{C}$. Cultures were initially evaluated at 24 hours, and plates showing no growth or requiring further assessment were incubated for a total of 48 hours.

Supplementary microbiological sampling of the 27 scuba cylinders was performed as a single-time-point assessment during the active diving season. These cylinders were sampled following routine air filling procedures using the same microbiological culture methods to assess whether the findings obtained from the primary longitudinal model were consistent under real-world operational conditions. After sample collection, the internal surfaces of the cylinders were rinsed with distilled water and allowed to dry at room temperature. No repeated sampling was performed in these cylinders.

Results

During repeated air-filling procedures, temperature measurements were recorded at regular pressure intervals from 0 to 200 bar across a total of 15 separate filling events. In all filling cycles, the cylinder surface temperature increased progressively with rising pressure, reaching a maximum of approximately 35°C at 200 bar. The most pronounced temperature increase consistently occurred during the early phase of pressurisation, followed by a gradual reduction in the rate of temperature rise as filling continued.

Throughout the study period, no bacterial growth was detected in any of the 30 samples collected over 45 days, including 15 scuba cylinder inner surface lavage samples and 15 compressor air filter swab samples. Representative blood agar plates obtained from both sample types demonstrated no bacterial growth after 48 hours of incubation (Figure 3A–D).

Figure 2

Photographs of the compressor air filter elements. The filter shown on the right represents the actively used filter from which microbiological sampling was performed throughout the study period. The filter shown on the left represents a previously used and discarded filter and is included for illustrative comparison only; no microbiological samples were obtained from this filter



Similarly, no bacterial growth was observed on either 5% sheep blood agar or chocolate agar at 24 or 48 hours of incubation for any sample. In addition, sterile physiological saline used as a negative control showed no evidence of bacterial growth following incubation.

In addition, microbiological cultures obtained from 27 compressed air cylinders actively used during the diving season also demonstrated no bacterial growth following incubation under the same culture conditions.

Discussion

This longitudinal investigation found no microbial growth in serial samples collected over a 45-day period from the inner surface of a steel compressed air cylinder and from the air filter of the compressor used for air filling. Thus, under the conditions of the present study, bacterial contamination was not detected in the monitored scuba cylinder and compressor air filter throughout the monitoring period. The lack of observable microbial growth may be mainly linked to the utilisation of diving apparatus that undergoes proper maintenance and efficient filtration systems. In particular, efficient filtration in diving compressors can limit the passage of particulate matter and microorganisms, thereby reducing the risk of microbial contamination within compressed air systems.

Similarly, regular maintenance and cleaning practices play an important role in eliminating potential sources of contamination.

Temperature and pressure are established physical parameters that affect bacterial growth, survival, and viability.^{2–4} Prior research indicates that increased pressure and temperature may exert inhibitory effects on specific microbes; however, the effectiveness of these factors is primarily dependent upon the intensity and length of exposure.^{2–6} The present

study demonstrated that repeated air-filling methods led to a gradual rise in cylinder surface temperature, reaching a peak of around 35°C at 200 bar during 15 distinct filling events. Despite this increase, no bacterial colonisation was observed in any samples from the inner surfaces of scuba cylinders or compressor air filters collected throughout the 45-day monitoring period.

The temperature range recorded during normal filling stayed within ranges typically tolerated by several environmental and opportunistic bacteria.²⁻⁴ These data indicate that the alterations in pressure and temperature experienced during routine scuba cylinder filling are inadequate to induce bacterial inactivation or inhibit potential microbial survival.^{2,3,5} The obtained thermal profile aligns with the adiabatic compression of air, succeeded by partial heat transfer to the surrounding environment during the filling procedure.

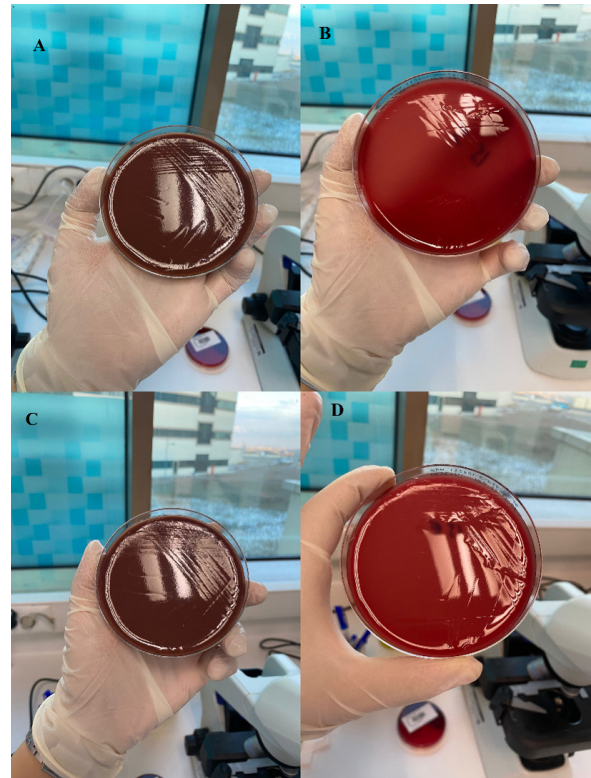
Importantly, the absence of bacterial colonisation throughout repeated sampling under natural operating conditions highlights the potential effectiveness of routine maintenance, proper cylinder handling, and standard air filtration practices in preventing microbial contamination of compressed air cylinder and filling systems. These findings indicate that, under appropriate operational conditions, the internal environment of a steel scuba cylinder can remain microbiologically stable over time. The absence of bacterial growth across all sampled time points, as illustrated in Figure 3A–D, supports the microbiological stability of the system.

Previous studies have emphasised microbial contamination risks associated primarily with external diving equipment and communal water sources. Washburn et al.⁷ demonstrated that rinse tanks used communally by divers may harbor a wide range of microorganisms, including opportunistic pathogens, originating from both environmental and human sources. Similarly, investigations by the Divers Alert Network⁸ reported that bacterial contamination in rinse tanks increased throughout the day due to repeated use and inadequate hygiene practices. While these investigations concentrated on external equipment surfaces, they highlighted the significance of appropriate handling and maintenance methods, as external pollution could potentially operate as a source of secondary contamination for internal air systems during filling or handling. Collectively, these findings suggest that contamination risks reported in diving environments are predominantly associated with external equipment surfaces and shared-use facilities rather than the internal environment of compressed air systems.

The inside environment of scuba cylinders often comprises a dry, high-pressure, and nutrient-deficient microenvironment. The physical and environmental circumstances are typically unfavorable to the survival and reproduction of numerous bacterial and fungal species. In comparison to ambient air, humidity levels within scuba cylinders are significantly

Figure 3

Representative blood agar culture plates obtained during the longitudinal monitoring period. Panels A and B show cultures from scuba cylinder inner surface lavage samples, and panels C and D show cultures from compressor air filter swab samples. All plates were incubated aerobically at 35 ± 1°C and demonstrated no bacterial growth after 48 hours. The images shown are representative of all samples collected during the study period



reduced. Low humidity facilitates evaporation, resulting in heightened solute concentrations and increased osmotic pressure, which can harm or inactivate microorganisms, hence diminishing microbial activity and growth.⁹⁻¹¹

Moreover, elevated hydrostatic pressure has demonstrated efficacy in inactivating several vegetative bacteria, yeasts, and moulds.^{12,13} The simultaneous occurrence of elevated pressure and diminished humidity constitutes a substantial stressor for microorganisms, hence decreasing the probability of successful colonisation. The physical characteristics of scuba cylinders, specifically low humidity and high pressure, produce an inhospitable habitat for microbial survival and sustained colonisation. Collectively, these physical attributes offer a credible rationale for the microbiological stability noted during the monitoring period and support the lack of detectable bacterial colonisation in this investigation.

The study by van Rijen et al. investigating MRSA (methicillin-resistant *Staphylococcus aureus*) transmission associated with shared scuba cylinder use suggests that the sharing of scuba diving equipment may be linked to community-acquired MRSA carriage, most likely through direct contact with contaminated equipment surfaces

rather than via airborne transmission originating from the tank itself.¹⁴ In contrast, in the present study, no microbial growth was detected in any of the serial samples obtained from the inner surfaces of scuba cylinders or from compressor air filters. This finding supports the notion that contamination risks reported in the literature are predominantly associated with external equipment surfaces and that, under appropriate maintenance and filtration conditions, the internal environment of scuba cylinders can remain microbiologically stable.

In addition to the primary longitudinal monitoring model, supplementary microbiological cultures obtained from 27 compressed air cylinders actively used during the diving season also demonstrated no detectable microbial growth. The absence of bacterial colonisation in these real-world operational cylinders further supports the consistency of the longitudinal findings and suggests that microbiological stability of the internal cylinder environment may be maintained even under active underwater use and repeated refilling conditions.

This study has several limitations. The primary longitudinal monitoring component was conducted using a single compressed air cylinder and a single compressor system, while additional sampling was performed in multiple cylinders under real-world conditions; therefore, the generalisability of the findings to other cylinder materials, capacities, compressor types, or filling environments may be limited. The microbiological assessment exclusively utilised culture-based bacterial approaches, without the application of molecular detection techniques. Temperature data were recorded solely during filling activities, excluding storage and discharge phases. Furthermore, certain fastidious, slow-growing, or non-cultivable microorganisms may not have been detected using the culture-based methods employed in this study. In addition, temperature data were recorded solely from the external cylinder surface during filling activities and did not directly measure internal gas temperatures. Therefore, it cannot be excluded that transient internal temperatures may have exceeded the recorded surface temperatures and potentially influenced microbial viability.

Conclusions

This longitudinal investigation found no bacterial colonisation in serial samples obtained from the inner surfaces of a compressed air cylinder or from the associated compressor air filter over a 45-day monitoring period. In addition, no microbial growth was detected in samples collected from 27 compressed air cylinders actively used during the diving season, supporting the consistency of the primary longitudinal findings. Collectively, these results indicate that, under routine operating conditions and with appropriate maintenance and air filtration practices, the internal environment of scuba cylinders used in scuba systems can remain sterile and microbiologically stable over time.

Although the study employed a culture-based approach and a focused longitudinal model, it provides a structured methodological framework for future investigations involving larger sample sizes, different operational settings, and complementary molecular diagnostic techniques which would be necessary to achieve more thorough and generalisable understanding of the microbiological safety of scuba diving systems.

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