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# Determination of the indoor air bacterial load and associated factors in primary schools in Hawassa City, Ethiopia, 2023. A comparative cross-sectional study

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ABSTRACT

**Background:** Indoor environments, particularly in schools, are a significant source of bacterial exposure, posing a public health risk. The aim of this study was to investigate the bacterial load and associated factors in the indoor environments of governmental and private primary schools in Hawassa, City, Ethiopia, 2023.

**Methods:** A comparative cross-sectional study design was used in March, 2023; in 56 randomly selected classrooms focusing in five governmental and five private primary schools in Hawassa, City. The bacterial load was estimated using the settle-plate method of passive air sampling. Differences in the mean bacterial load between government and private primary schools were evaluated using a non-parametric test. Multiple linear regression analysis using log-transformed data was performed.

**Result:** The average bacterial load in government primary schools was 8684.84 CFU/$m^3$; while in private schools, it was 4396.43 CFU/$m^3$. The isolated bacterial species included *coagulase-negative Staphylococcus species (CoNS), gram-negative Bacillus species, gram-positive Bacillus species, and Staphylococcus aureus*. A significant difference in the mean bacterial load (p=0.002) was observed between government and private primary schools with private primary schools showing lower levels. In government schools, the bacterial load was significantly associated with classroom cleanliness, occupant density, cleaning frequency, and classroom area. Conversely, the bacterial load in private schools showed strong association with the occupant density, cleaning frequency, relative humidity, and ventilation conditions.

**Conclusion:** In government and private primary schools, the level of bacterial loads exceeded the WHO criteria. This study revealed significant differences in indoor bacterial loads between government and private primary schools, with private schools showing lower levels. The correlation between bacterial load and environmental factors was distinct in each setting. As a recommendation; improving cleanliness, layout optimization, ventilation improvement, and hygiene education for both sectors, with regular air quality monitoring are crucial for tracking progress.

**Key words:** Bacterial Load, Classrooms, Contributing Factors, Indoor Air, Settle Plate

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**Availability of data and material:** Data will be made available upon request.

**Conflict of interest:** The authors declare that they have no conflicts of interest.

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## Introduction

Clean air is a fundamental need of life [1]. As individuals spend an increasing amount of time in indoor environments; indoor air pollution (IAP) has become a significant public and occupational health concern. It is estimated that people spend over 90% of their time indoors, encompassing various settings such as homes, workplaces, and educational institutions. On average, individuals inhale approximately 10–14 $m^3$ of air daily [2, 3]. Millions of people, including young individuals, spend approximately 24% to 30% of their waking hours in schools. For Humans to thrive, learn, and succeed, it is crucial to provide them with safe and healthy environments [2]. Primary education holds immense significance for children in every nation, as it serves as, the foundation for their educational journey. This phase of education typically involves seven to eight hours a day in school [4].

People inhale air filled with microorganisms, commonly referred to as bioaerosols [5]. These bioaerosols comprise a diverse range of airborne particles, including pollen, bacteria, fungi, viruses, and their byproducts. Their contribution to indoor air pollution can range from 5% to 62.34% [6]. Human activity is significantly influenced the presence of bacteria in indoor environments [7]. Within school premises, air can become increasingly contaminated with various pollutants, with fungi and bacteria being the most notable airborne microorganisms [8].

The bioaerosols compromise by normal activities in various environments, including schools. Infectious aerosols, which are characterized by their small size (less than 5 μm), can deeply penetrate the human respiratory system. These aerosols can remain suspended in the air for extended periods, posing a significant risk to airborne infections, particularly in confined spaces [5, 6]. Exposure to airborne germs can lead to allergic reactions, respiratory problems, and immunological toxic illnesses among students. Airborne bacteria are known to cause several infectious diseases, and their components have been associated with the development and exacerbation of chronic respiratory conditions, such as asthma, in schools and other indoor settings [2, 7]. Lower respiratory infections (LRIs) are a major cause of morbidity and mortality worldwide. Bacterial pathogens, including *Streptococcus pneumoniae (pneumococcus*), are significant causes of LRIs and can cause conditions such as meningitis, tuberculosis, septicemia, pneumonia, otitis, mastoiditis, cellulitis, and arthritis in infants and young children [9].

The normal flora present on students, as well as on their uniforms, bags, and sandals, along with their everyday activities such as sneezing, coughing, talking, walking, and washing, can potentially serve as sources of airborne microorganism infection or contamination within the classroom [10, 11]. Additionally, items such as filing cabinets, books, and sandals were identified as potential sources of contamination. Furthermore, household tasks like cleaning or using dry dust mops can aerosolize particles harboring bacteria [12]. In public schools, which accommodate thousands of students on a daily basis, the bustling environment contributes to elevated concentrations of bacteria and fungi in the air [2]. The most important methods for reducing indoor air pollution in schools are source control (educating building occupants, especially operations and maintenance of class), extraction (filtering and air cleaning of airborne contaminants), dilution (ventilating indoor air with outside air), and controlling the factors that lead to poor IAQ [13, 14].

According to the World Health Organization (WHO), indoor air pollution accounts for 2.7% of the global disease burden, ranking it as the eighth most significant risk factor [1]. Over the past 20 years, studies conducted by the U.S. The Environmental Protection Agency indicated that indoor air can be 70–100 times more polluted than outdoor air [7]. These findings highlight the critical importance of addressing indoor air quality to protect public health. Indoor air pollution is one of the primary preventable causes of illness and mortality [5]. In 2016, indoor air pollution accounted for 3.8 million deaths worldwide. Shockingly, more than 90% of these fatalities linked to air pollution occurred in low-income countries, primarily in Asia and Africa, with Eastern Mediterranean countries, Europe, and the Americas following [15, 16]. According to the 2016 Global Burden of Disease Report, lower respiratory infections (LRIs) were responsible for 652,572 deaths among children under the age of five worldwide. Globally, pneumonia due to *S. Pneumoniae* caused more deaths than all other etiologies combined [17]. These statistics underscore the urgent need to address and combat the harmful effects of indoor air pollution, particularly in low-income regions, to reduce disease burden and prevent avoidable mortality.

According to the WHO report, indoor air pollution (IAP) resulted in the loss of 41 million DALYs (disability-adjusted life years). Lower respiratory infections caused by IAP account for approximately 11% of all deaths in low-income nations [18]. In the United States, asthma is the leading cause of hospitalizations and school absences among children, as indicated by the US EPA. This condition leads to 10 million missed school days annually and 100,000 hospital visits each year, with an estimated cost of around $2 billion. School-aged children are particularly vulnerable to the adverse health effects of indoor air pollution for various reasons. Exposure to IAP in this age group has been associated with a range of health and cognitive problems [13]. These findings emphasize the significant impact of indoor air pollution on public health and highlight the need for effective measures to mitigate its effects, particularly in educational environments. By prioritizing air quality improvements, we can help reduce the disease burden and create healthier spaces for children to learn and thrive.

Exposure of school-age children to indoor air pollution (IAP) is associated with various health and cognitive problems that can affect pupils' academic performance. Indoor air pollution has been linked to both severe and mild health impacts, including headaches, fatigue, nausea, respiratory infections, and asthma [2]. Numerous studies have several causes of indoor air pollution in schools. These factors include high occupant density, elevated levels of physical activity, insufficient air exchange rates, and poor cleanliness. These conditions contribute to the accumulation of pollutants in the indoor environment, worsening the risk of health issues and hindering students' learning and overall well-being. Recognizing and addressing these contributing factors is essential for improving indoor air quality in schools. By implementing measures to reduce pollutant sources, enhance ventilation, and maintain cleanliness, educational institutions can create healthier learning environments that promote optimal academic performance and student health [11, 19].

Several studies have been carried out in Ethiopia on the indoor air microbiological quality conditions in various building premises, such as public libraries, university dorms, residential residences, and hospital wards, [3, 16, 20-24]. However, studies assessing the IAQ status in schools, particularly in the study area, are scarce. Therefore, this study aimed to determine the indoor air bacterial load and isolate common bacterial species and associated factors in primary school classrooms in Hawassa City, Ethiopia. This information will be used to establish acceptable microbial population levels and develop appropriate guidelines for reducing the microbial density in indoor school air. Moreover, it can raise awareness regarding the issues related to bacterial indoor air quality and offer resources for more profound comprehension.

## Methods and materials

### *Study area*

The research was carried out in Hawassa City, situated 275 kilometers South of Addis Ababa on the side of Lake Hawassa of the Great Rift Valley at an

elevation of 1,700 meters above sea level [25]. During sampling, the city's outdoor average temperature was 25.6 °C, and its average humidity was 54.4%. According to the Hawassa City Educational Bureau 2023 report, there are 18 government and private elementary schools in the City, respectively. There were a total of 32,028 private and 65,705 government school students enrolled in Hawassa City who are distribute in to 98 private and 396 government classrooms [26].

### Study design and period

An institutional-based comparative cross-sectional study was conducted from March 01 to 30, 2023.

### *Population*

The source and study populations were all private and government primary schools in Hawassa City (Grades 1-8).

### *Eligible criteria*

### Inclusion criteria

All study classrooms in private and government primary schools were included in the study.

### Exclusion criteria

All classrooms those are not operational during data collection.

### Sample size determination

The sample size was determined based on environmental sampling and the sample size determination formula; equation 1 [27] by using the following equation [28].

$$n = \frac{(4 * \sigma 2)}{\delta 2} \quad [1]$$

Where n is the number of samples, σ is the standard deviation and δ is an acceptable error [half the width of a 95% confidence interval on the mean (X±δ)].

From 18 government and 18 private elementary schools, 30% of schools and 20% of classes were selected as study units using a simple random sampling technique. Using a 3% acceptable error (δ), the mean of five randomly selected government and private primary schools were 37.8 (σ=4.7) and 12 (σ=2.8), respectively).Therefore, based on equation 1, the sample size for government and primate primary schools were calculated as follows.

Government primary school

$$n = \frac{(4 * (4.76 * 4.76)}{1.5 * 1.5} = 42 \text{ classrooms}$$

Private primary school =

$$n = \frac{(4 * (2.82 * 2.82)}{1.5 * 1.5} = 14 \text{ classrooms}$$

Total sample size (**n)** = 42+14= 56

### *Sampling technique and procedures*

To compare the bacterial density between the public and private primary schools in Hawassa City, 168 air samples were collected from 56 classrooms (Figure 1). The settle plate method, also known as the passive air sampling method, was used to measure the bacterial load. This method involved placing the culture medium in standard Petri dishes with a diameter of 9 cm (equivalent to 63.585 $cm^2$] and exposing them for 1 hour. The 1/1/1 scheme was applied to determine the bacterial load. Specifically, this scheme involved counting the number of microbes that settled onto the media plates left open to the air for one hour, positioned one meter away from the floor, and at least one meter away from walls or other obstacles [20].

To inhibit fungal growth, bacteria were collected on blood agar medium and subsequently treated with an antifungal drug (glimeofulvin). The sampling process was carried out at three specific time intervals throughout the day, considering variations in environmental factors and occupant density. These time intervals were as follows: 6:30-7:30 am (before the start of the class session), 1:30-2:30 pm (during the class session), and 5:00-6:00 pm (shortly after the students left the classroom). After exposing the plate for 1 hour, it

was sealed and placed in a cold box for transport to the Microbiology laboratory at Hawassa University. Subsequently, the plate was incubated at 37 °C for 24–48 hours. Upon completion of the incubation period, the bacterial load was manually counted using a colony counter. This involved drawing a cross across the plate and counting only one-fourth of the colonies, which were then multiplied by four to obtain the total count. The count was then converted to colony-forming units per cubic meter (CFU/$m^3$] using the standard formula [29).

$$N = \frac{a \times 10000}{bt \times 0.2} = 5a \times 10^4 (bt)^{-1} \quad [2]$$

Where N = Microbial CFU/$m^3$ of indoor air; a = Number of colonies per Petri dish; b = Dish surface area ($cm^2$); and t = Exposure time.

After incubating the culture plate, each bacterium was isolated using conventional techniques, such as biochemical testing, colony morphology, microscopy, and Gram staining [30, 31].

*Operational definition*

Bacterial load

Less than 1000 CFU/$m^3$ is acceptable, whereas more than 1000 CFU/$m^3$ is unsuitable, according to the WHO expert group's bacterial load standard [10]. The European Commission has established sanitary standards for non-industrial premises regarding the bacterial load, with less than 50 CFU/$m^3$ considered "very low," 50–100 CFU/$m^3$ considered "low," 100–500 CFU/$m^3$ considered "intermediate," 500–2000 CFU/$m^3$ considered "high," and ≥2000 CFU/$m^3$ considered "very high." The WHO expert group's standard for bacterial load is as follows: less than 1000 CFU/$m^3$ is acceptable, whereas greater than 1000 CFU/$m^3$ is unacceptable [32]. WHO standards of Crowdness index ≤ (1.4 $m^2$/pupil) not crowded > (1.4 $m^2$/pupil) crowded [33]. Good: cleanliness: conditions with trash-free walkways and mopped and stainless floors; otherwise, it is poor [16]. Adequate ventilation: an open window that covers at least 10% of the room floor space is considered adequate; otherwise, it is considered inadequate [16]. Classroom cleanliness: absence of visible dust particles, litter, and spider webs in classrooms [34]. Latrine cleanliness: the pit was not full, no fecal matter was seen around the pit latrine, the area was properly swept, and there was no bad smell at the time of data collection [34].

*Data collection tools and procedures*

Blood agar was used for bacterial sample collection. Aero-Qual Series 500 temperature and humidity sensor was used to measure room temperature, nitrogen dioxide, carbon monoxide, humidity, and particulate matter ($PM_{2.5}$ & $PM_{10}$) in the sampling rooms [35]. An observational checklist was used to collect data on building-related factors (type of ventilation, condition of ventilation, type of ventilation with respect to air circulation, number of open windows per class), occupant density, classroom area ($m^2$), and cleanliness-related factors (classroom cleaning frequency, cleanliness of the classroom, cleanliness of the latrine, and WASH facilities).

*Data quality assurance*

Training was given to data collectors and laboratory technicians before data collection to ensure the quality of the data. A pre-test was performed to ensure the sampling procedure and check the functionality of equipment to reduce the potential for bias or errors. Before data collection sterilization of culture media, glassware, and other materials were performed by autoclaving at 121°C for 15 minutes to ensure the elimination of any potential contaminants [36]. To avoid contamination during air sampling and analysis protective gowns, masks, and sterile gloves were worn to avoid contamination of agar plates during air sampling and analysis. Moreover, during culture media preparation Laminar flow cabinet (Model: LCB-V3F Series) equipment was used to prevent microbial contamination and particles [37]. The culture media was also checked visually before use for any microbial growth.

*Data processing and analysis*

All collected bacterial load from air sampling and environmental factors like temperature, humidity,

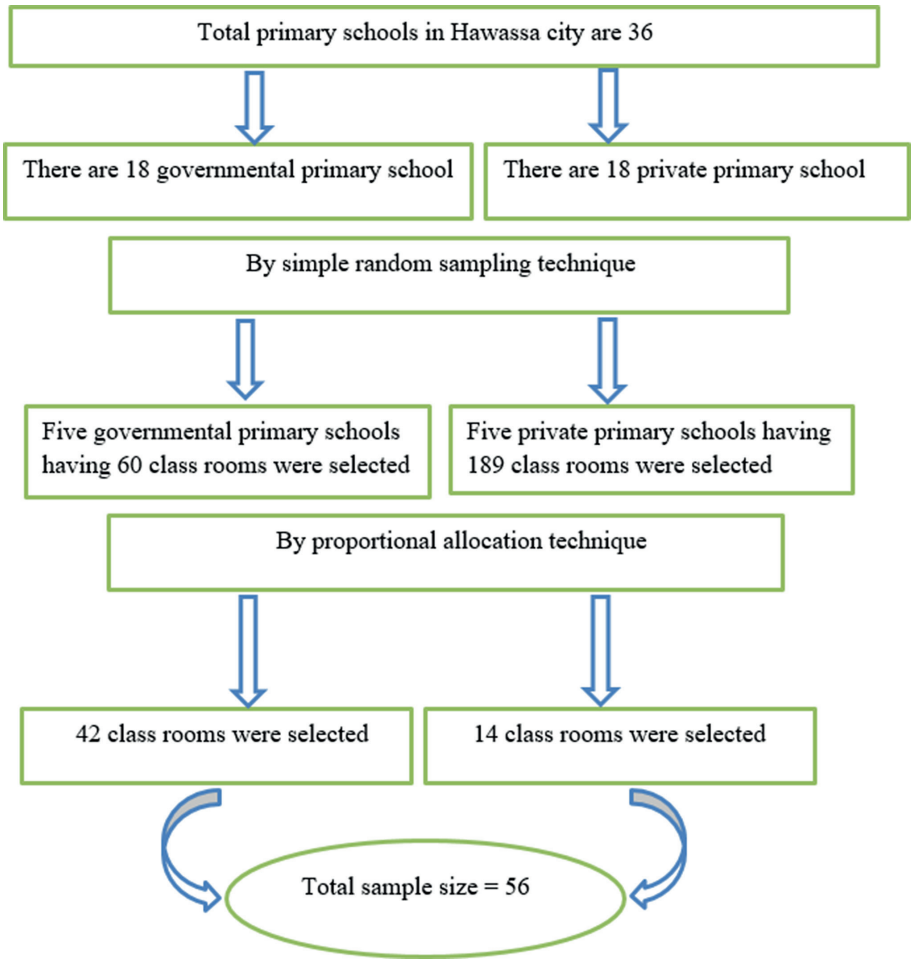


**Figure 1.** Sampling procedures in primary schools in Hawassa City, Ethiopia.

room cleanliness, ventilation condition, occupant density, carbon monoxide, nitrogen dioxide, WASH facility, and particulate matters were cleaned, coded, and entered into Epi-data version 3.1 then exported to SPSS version 26 for statistical analysis. Descriptive statistics such as the mean, median, standard deviation, frequency, and range were employed. To improve the accuracy of statistical analysis, log transformation of data was applied to normalize the distribution and reduce variance. The normality of the data was checked by Shapiro-Wilk normality test and the bacterial load differences between government and private primary schools were tested by the Mann–Whitney U test. Kruskal-Wallis H test was used to examine bacterial load differences among primary schools and significant variations across sampling times. Kendall's tau-b correlation test was applied to evaluate the influence of environmental factors on the indoor air bacterial load. Statistical significance was determined with a threshold of $p \leq 0.05$.

## Results

### *Bacterial loads in a school indoor air environment*

The mean indoor air bacterial load was 8684.84 CFU/$m^3$ in government primary schools and 4396.43 CFU/$m^3$ in private primary schools. Among the government primary schools, the highest mean bacterial load was recorded in School 5 in the afternoon (15931.87 CFU/$m^3$), and the lowest mean bacterial

load was recorded in School 1 in the morning (2855.3 CFU/$m^3$). Conversely, in private primary schools, the highest mean bacterial load was recorded in School 7 in the afternoon (10418.5 CFU/$m^3$), and the lowest bacterial load was recorded in School 6 in the morning (1172.5 CFU/$m^3$) (Table 1). Assessment of air quality sampling in classrooms at ten primary schools in Hawassa City according to the sanitary standard of the European Commission for nonindustrial premises was shown in Table S1.

The mean bacterial loads in government primary schools in the morning, afternoon, and midafternoon were 6742.6 CFU/$m^3$, 11242.3 CFU/$m^3$, and 8069.5 CFU/$m^3$, respectively. In private primary schools, the mean bacterial loads during the morning, afternoon, and midafternoon were 2724 CFU/$m^3$, 6203.2 CFU/$m^3$, and 4262 CFU/$m^3$, respectively (Figure 2).

According to the sanitary standards of the European Commission for non-industrial premises, the bacterial loads of government primary schools during the morning, afternoon, and midafternoon were classified as having a very high degree of pollution. However, in private primary schools, the bacterial loads were classified under high and very high pollution levels (Table 2).

*Bacterial load between the government and private primary schools*

Because the data did not follow a normal distribution, the Mann–Whitney U test was used to assess the mean bacterial load differences between government and private primary schools. The highest bacterial load was recorded in government primary schools (8684.8 CFU/$m^3$). There was a significant difference in the mean bacterial load between government and private primary schools at ($z = -3.103$, $p = 0.002$).

*Bacterial load of 10 primary schools*

In government primary schools, the highest (12727 CFU/$m^3$) and lowest (4110 CFU/$m^3$) bacterial loads were recorded in schools 5 and 1, respectively. The Kruskal-Wallis H test result revealed that a significant differences in the mean bacterial load among the five government primary schools was observed ($X2 = 20.10$, $DF = 4$, and $p = 0.000$). Based on the *post hoc* test results, a significant mean bacterial load difference was observed between schools 5 and 1 ($p = 0.000$). However, in private primary schools, the highest bacterial load was 5589 CFU/$m^3$ in school 7 and the lowest bacterial load was 1940 CFU/$m^3$ in school 6. The Kruskal-Wallis H test result indicated a significant mean bacterial load difference among 5 private primary schools ($X2 = 11.00$, $DF = 4$, $p = 0.027$). The *post hoc* test results indicated a significant mean bacterial load difference between schools 10 and 6 ($p = 0.001$).

*Isolated bacterial species in primary schools*

As figure out from (Table 2), in private primary schools, four bacterial species were isolated: Gram-positive Bacillus, *Staphylococcus aureus*, coagulase-negative Bacillus (CoNS), and Gram-negative Bacillus. In government primary schools, gram-positive Bacillus species, *Staphylococcus aureus,* coagulase-negative *Staphylococcus* (CoNS) species, and gram-negative Bacillus species were isolated.

*Staphylococcus aureus* and Gram-positive Bacillus species were found in all government and private primary schools. Nevertheless, CONS were not found in school 4 in the government and in schools 6 and 8 in private primary schools (Table 3).

*Factors associated with the bacteria load*

Physical parameters of the indoor air factors

Throughout the sampling period, the mean indoor temperature was ranged from 19.8˚C to 31.1˚C, and the relative humidity was ranged between 42.9 % and 65%. Likewise, the particulate matter concentrations ($PM_{2.5}$ and $PM_{10}$) were ranged between 17.5 μg/$m^3$ to 202.5 μg/$m^3$, and 18.5 μg/$m^3$ to 340 μg/$m^3$, respectively (Table S2).

Crowdedness, building, and sanitation-related factors

Out of the 42 classrooms in government primary schools, 18 (42.9%) had adequate ventilation, and 24 (57.1%) had inadequate ventilation. In contrast, in 14

**Table 1.** Bacterial load in government and private primary schools in Hawassa City, Ethiopia, 2023 (n = 56).

| **Morning bacterial Load (6:30 -7:30 am)** | | | | | | |
|---|---|---|---|---|---|---|
| **School** | **No Classrooms** | **Mean** | **SD** | **Min** | **Max** | **Median** |
| S1 | 9 | 2855.3 | 868.22 | 1572 | 4573 | 2621 |
| S2 | 7 | 10907.25 | 1703.65 | 6815 | 11467 | 9305 |
| S3 | 10 | 5082 | 2274.87 | 2359 | 8780 | 4757 |
| S4 | 8 | 6962.12 | 4379.44 | 393 | 14678 | 7666.5 |
| S5 | 8 | 9102.4 | 4280.36 | 3931 | 16382 | 12221 |
| S6 | 3 | 1172.5 | 732.92 | 834 | 2293 | 1441.0 |
| S7 | 2 | 1522.66 | 157.68 | 1061 | 1284 | 1172.5 |
| S8 | 3 | 1812.3 | 372.24 | 1507 | 2227 | 1703.0 |
| S 9 | 2 | 4119 | 821.65 | 3538 | 4700 | 4119.0 |
| S10 | 4 | 4387.25 | 1907.18 | 1598 | 5635 | 5158 |
| **Afternoon bacteria load (1:30-2:30 pm)** | | | | | | |
| **S. Name** | **No of class** | | **SD** | **Min** | **Max** | **Median** |
| S1 | 9 | 5988.55 | 2701.12 | 3066 | 11791 | 5504.0 |
| S2 | 7 | 15183.57 | 3014.04 | 11139 | 19003 | 16120 |
| S 3 | 10 | 9864.5 | 4554.88 | 1653 | 17430 | 10006 |
| S 4 | 8 | 10734.12 | 5619.43 | 2359 | 183480 | 11172 |
| S 5 | 8 | 15931.87 | 6746.03 | 5535 | 23066 | 18020 |
| S6 | 3 | 2516 | 120.12 | 2385 | 2621 | 2542.0 |
| S 7 | 2 | 10418.5 | 92.63 | 10353 | 10484 | 10418.5 |
| S 8 | 3 | 3450.66 | 200.10 | 3276 | 3669 | 3407.0 |
| S 9 | 2 | 7266 | 984.29 | 6570 | 7962 | 7266.0 |
| S10 | 4 | 8394 | 1366.57 | 6552 | 9856 | 8584 |
| **Mid Afternoon (5:00-6:00 pm) Bacterial load** | | | | | | |
| S1 | 9 | 3487.66 | 878.91 | 2096 | 4950 | 3328 |
| S2 | 7 | 11293.85 | 1741.67 | 8210 | 13118 | 12083 |
| S 3 | 10 | 7450 | 3803.99 | 2123 | 12581 | 7273 |
| S4 | 8 | 7902.75 | 5525.69 | 580 | 17299 | 6224.3 |
| S5 | 8 | 11343.87 | 4520.17 | 4757 | 17037 | 12043.5 |
| S 6 | 3 | 1781.66 | 787.8 | 969 | 2542 | 1832 |
| S 7 | 2 | 5176 | 92.63 | 5111 | 5242 | 5176.5 |
| S 8 | 3 | 2386.33 | 120.58 | 2254 | 2490 | 2415 |
| S 9 | 2 | 6074 | 1844.13 | 4770 | 7378 | 6074 |
| S10 | 4 | 6165.75 | 1585.32 | 4508 | 7535 | 6310 |

NB. From Schools 1-5 and 6-10, the government and private primary schools, respectively.

classrooms in private primary schools, 8 (57.1%) had adequate ventilation and 6 (42.9%) had inadequate ventilation. From the 42 classrooms in government primary schools, 8 (19%) classrooms had good cleanliness and 34 (81%) had poor cleanliness. In contrast, among the 14 classrooms in private primary schools, 11 (78.6%) had good cleanliness and 3 (21.4%) had bad cleanliness (Table S3).

### Frequency of school WASH-related factors

Out of the five government primary schools, only 1 (20%) had clean latrines, while 4 (80%) had not clean latrine. Similarly, in terms of water access, only 1 government primary school (20%) had adequate access, whereas 4 (80%) had not adequate access. In private primary schools, 3 (60%) had clean latrines, while 2 (40%) private primary schools had not clean latrine (Table S4).

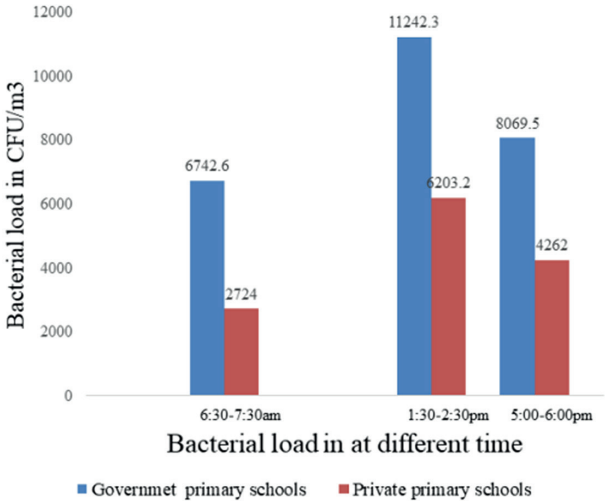


**Figure 2.** Average bacterial loads in private and government primary schools in Hawassa City.

*Correlation of bacterial load and contributing factors*

In government primary schools, temperature, $PM_{10}$, $PM_{2.5}$, and CO were positively correlated with bacterial load in the morning, afternoon, and mid-afternoon, but CO was negatively correlated in the mid-afternoon. Humidity was negatively correlated with bacterial load in the morning, afternoon, and midafternoon. However, $NO_2$ was negatively correlated with the bacterial load in the morning and midafternoon, but positively correlated in the afternoon, and classroom cleanliness was positively correlated (Table S5). In private primary schools, the number of students per classroom was strongly negatively correlated with the bacteria load; however, the cleaning frequency of the classroom and classroom cleanliness were strongly positively correlated with the bacteria load. Type of ventilation with respect to air circulation, number of open able windows, average T (°C), average $PM_{10}$ and average $PM_{2.5}$, ventilation condition, and occupant density positively correlated with bacteria load. However, the average relative humidity was negatively correlated with the bacterial load (Table S6).

*Factors Associated with the mean indoor air bacterial load*

As shown in Table 4, this study revealed that the bacterial load was significantly associated with classroom cleanliness, classroom area, occupant density

**Table 2.** Frequency distribution of the isolated bacterial species in private and government primary schools.

| | Isolated bacterial species in private schools | | |
|---|---|---|---|
| **Isolated bacterial species name** | **Morning (%)** | **Midafternoon (%)** | **Afternoon (%)** |
| *S. aureus* | 50 | 64 | 50 |
| *CoNS* | 14.3 | 21.4 | 14.3 |
| *G+ve bacillus species* | 21.4 | 14.3 | 21.4 |
| *G–ve bacillus species* | 14.3 | Not detected | 7.1 |
| *S.aureus and G+ve bacillus species* | Not detected | Not detected | 7.1 |
| | **Isolated bacterial species in government schools** | | |
| *S.aureus* | 28.6 | 71.4 | 45.2 |
| *CoNS* | 7.1 | 4.8 | 28.6 |
| *G+ve bacillus species* | 28.6 | Not detected | 9.5 |
| *G–ve bacillus species* | 19 | Not detected | 2.4 |
| *S. aureus and G+ve bacillus species* | 16.7 | 23.8 | 14.3 |

**Table 3.** Isolated bacterial species in primary school classrooms in Hawassa City, Ethiopia, 2023.

| | | Isolated Bacterial species | | | | |
|---|---|---|---|---|---|---|
| **School ID** | **No of classrooms** | ***S. aureus*** | ***CONS*** | ***G+ve BA*** | ***G-ve BA*** | **G+ve *BA and S. aureus*** |
| S 1 | 9 | + | + | + | + | + |
| S 2 | 7 | + | + | + | _ | + |
| S3 | 10 | + | + | + | + | + |
| S 4 | 8 | + | - | + | + | + |
| S 5 | 8 | + | + | + | + | + |
| S 6 | 3 | + | - | + | + | + |
| S7 | 2 | + | + | + | + | + |
| S 8 | 3 | + | - | + | _ | + |
| S 9 | 2 | + | + | + | _ | + |
| S10 | 4 | + | + | + | _ | + |

N.B: + = Present, - = absent, *BA (bacillus species), CONS (coagulase-negative staphylococcus species).*

**Table 4.** Factors related to the average bacterial load in government primary school classrooms.

| | **Unstandardized coefficients** | | **Standardized coefficients** | | **(95% CI for β)** | |
|---|---|---|---|---|---|---|
| **Variables** | **β** | **Standard error** | **BETA** | **P** | **L** | **U** |
| ($m^2$/pupil) | –0.484 | 0.203 | –0.341 | **0.024** | –0.898 | –0.069 |
| Cleaning frequency of the classroom | –0.123 | 0.0573 | 0.361 | **0.029** | 0.015 | 0.231 |
| Classroom area | –0.023 | 0.009 | –0.378 | **0.020** | –0.042 | –0.004 |
| Classroom cleanness | 0.204 | 0.095 | 0.299 | **0.04** | 0.01 | 0.398 |
| Type of ventilation with respect to air circulation | 0.086 | 0.054 | 0.220 | 0.125 | –0.025 | 0.197 |
| No of openable windows per classroom | 0.036 | 0.035 | 0.146 | 0.313 | –0.036 | 0.108 |
| Average $PM_{10}$ | 0.022 | 0.622 | 0.008 | 0.972 | –1.249 | 1.293 |
| Average T(°C) | –0.015 | 0.053 | –0.053 | 0.785 | –0.123 | 0.094 |
| Average RH (%) | –0.037 | 0.033 | –0.202 | 0.264 | –0.104 | 0.029 |
| Average $PM_{2.5}$ | 0.150 | 1.269 | 0.26 | 0.907 | –2.442 | 2.743 |
| Ventilation condition | –0.047 | 0.071 | –0.086 | 0.516 | –0.191 | 0.098 |
| Constant | 6.979 | 2.756 | | 0.17 | 1.351 | 12.607 |

The bolded letter indicates the variable that is significantly associated at $p < 0.05$ level.

($m^2$/pupil), and classroom cleaning frequency. An unclean classroom had a 20.4% higher bacterial load than a clean classroom (β = 0.204,95% CI,0.01,0.398); a classroom area increase by one unit the bacteria load decreased by 2.3% (β = –0.0203,95% CI –0.042, –0.004), cleaning frequency increase by one unit the bacteria load decreased by 12.3% (β = –0.123, 95%, CI,0.015,0.231), when occupant density ($m^2$/pupil) increase by one unit, the bacteria load decreased by 48.4% (β = –0.484,95%, CI,–0.898, –0.069).

**Table 5.** Factors associated with the mean bacterial load in private primary school classrooms in Hawassa City, Ethiopia, 2023.

| | Unstandardized coefficients | | Standardized coefficients | | (95% CI for β) | |
|---|---|---|---|---|---|---|
| **Variables** | **β** | **Standard error** | **BETA** | **P** | **L** | **U** |
| ($m^2$/pupil) | 2870.20 | 483.57 | 0.478 | **0.027** | 789.5 | 4950.8 |
| Cleaning frequency of the classroom | –2689.5 | 374.338 | –0.465 | **0.019** | –4300.16 | –1078.87 |
| No of openable windows per classroom | –1147.35 | 169.9 1 | 0.316 | **0.021** | 416.25 | 1878.44 |
| Average humidity of the classroom | –701.8 | 97.36 | –0.318 | **0.019** | –1120.7 | –282.86 |
| Ventilation condition | –1690.98 | 257.72 | –0.413 | **0.022** | –2799,8 | –582.01 |
| Type of ventilation | 2018.81 | 197.49 | 0,822 | **0.009** | 1169.07 | 2868.54 |
| Classroom area | –82.514 | 23.524 | –0.283 | 0.071 | –183.729 | 18.700 |
| Average PM10 | 3726.253 | 1604.415 | 0.133 | 0.146 | –3176.48 | 10629.491 |
| Average T(°C) | 756.462 | 196.735 | 0.232 | 0.061 | –90.2 | 1602.743 |
| Average PM2.5 | 2871.155 | 1734.854 | 0.072 | 0.240 | –4593.33 | 10335.642 |
| Classroom cleanness | –959.527 | 258.468 | –0.195 | 0.066 | –2071.62 | 152.570 |
| Constant | 24991.5 | 9088.99 | | 0.111 | –14115 | 126408 |

The bolded letter indicates the variable that is significantly associated at $p < 0.05$ level.

In the current study, a multiple linear regression model predicted the total indoor air bacteria load in private primary school classrooms, as shown in Table 5, and the bacteria load was significantly associated with cleaning frequency, relative humidity, ventilation condition, and type of ventilation with respect to air circulation, occupant density, and number of openable windows per classroom.

For each additional unit increase in occupant density, there was a corresponding increase in bacterial load of 2870 units in bacterial load (β=2870.95% CI 789.5, 4950.8). With each increment of one unit in cleaning frequency, the bacterial load decreased by 2689.5 units (β= –2689.5, 95% CI–4300, 1078.8). Few open able windows per classroom increased the bacteria load by 1147 .unit (β=1147, 95% CI 416, 1878). The mean bacteria load decreased by 701 units (β= –701, 95% CI–1120.7, 282.8) with one unit increase in indoor humidity, and adequate ventilation increased by one unit, and the bacteria load had decreased by 1690.9 unit (β= – 1690.9, 95% CI–2799, 8, –582) (Table 5). Student density increased by one unit, and the bacterial load increased by 2870 unit (β=2870.95% CI 789.5, 4950.8). Cleaning frequency increased by one unit, and the bacterial load had decreased by a-2689.5 unit (β= –2689.5, 95% CI–4300, 1078.8). A low number of openable windows per classroom increased the bacteria load by 1147 unit (β=1147, 95% CI 416, 1878). The mean bacteria load decreased by 701 units (β= –701, 95% CI–1120.7, 282.8) with one unit increased in indoor humidity, and an increase of one unit in adequate ventilation resulted in a decrease of -1690.9 units in the bacteria load (β= – 1690.9, 95% CI –2799.8, –582) (Table 5).

## Discussion

In this study, the mean indoor air bacterial load was 8684.84 CFU/$m^3$ in government primary schools and 4396.43 CFU/$m^3$ in private primary schools. The mean bacterial load observed in government primary schools in this study exceeded that reported in previous studies conducted in Portugal (2373 CFU/$m^3$) [38], Poland (2205 CFU/$m^3$) [6], and Egypt (3073.6 CFU/$m^3$) [19]. Additionally, a systematic literature review from

Indonesia reported varying bacterial loads across different regions, such as Europe (3490 CFU/$m^{3)}$, North America (293 CFU/$m^3$), Asia (632.383 CFU/$m^3$), and Africa (2523.58 CFU/$m^3$) [39] while in Gondar, Ethiopia it was (3670.49 CFU/$m^3$) (3670.49 CFU/$m^3$) [40]. Discrepancies in these findings could be attributed to differences in the study season, physical parameters (as observed in Poland), differences in the number of occupants, physical environmental factors, building conditions, and sanitation standards (as noted in Portugal and Egypt). Moreover, distinct physical environmental parameters and building conditions may contribute to variations, such as the temperature disparity between Gondar and Hawassa. In contrast, the mean bacterial load in private primary schools was closely aligned with a study conducted in Nigeria (4378.82 CFU/$m^3$ [12].

When comparing the mean bacterial load in the classrooms before and after lessons in both the government and private primary schools, the mean bacterial load was highest during class time. This finding is consistent with research conducted in Saudi Arabia [2] and studies conducted in the United States [41]. The difference might be due to factors such as high occupant density and students' activities, which result in the release of bacteria and increased disturbance of bacteria aerosol in the classroom air. In this study, there was a significant difference in the mean bacterial load between government and private primary schools ($p = 0.002$).This difference attributed to several factors observed in the government primary schools, including poor classroom cleanliness, low cleaning frequency, limited classroom space, and findings from observational checklists indicating inadequate water access for hand washing, poor latrine cleanliness, absence of hand washing facilities with soap near the toilet, a large number of students using a single faucet, and a large number of students using one set hole in the latrine.

There was a significant difference in the mean bacterial load among different government primary schools ($p = 0.000$). This finding was similar to the study done in Gondar [40]. In addition, a significant mean bacterial difference was observed among private primary schools ($p = 0.023$). This result agreed with the study done in Nigeria [12]. The significant differences among government primary schools might be due to differences in occupant density, classroom area, classroom and latrine cleanliness, ventilation condition, WASH facility, classroom cleaning frequency, and latrine cleanliness. However, in private primary schools, some classrooms had a low number of openable windows, low occupant density, low classroom cleaning frequency and latrine, inadequate ventilation, high relative humidity, and inadequate ventilation. This study showed that the mean bacterial loads of government and private primary schools were 8684.84 CFU/$m^3$ and 4396.43 CFU/$m^3$, respectively, which were above the acceptable value of the WHO expert group's recommendation (≤ 1000 CFU/$m^3$) [1). In the current study, the mean bacterial load in government and private primary schools was above the permissive limit of the European Commission for non-industrial premises (≤ 500 )CFU/m3**)** in indoor environments [32).

In this study, in government and private primary schools, *S. aureus*, *CONS*, and gram-positive *bacillus* were the predominant isolated bacterial species. However, gram-negative *bacillu*s species were found in small numbers. This study was in line with the study conducted in Poland [6], Turkey [42], India [11], Nigeria [12], and Indonesia [39], and harmonized with the study conducted in Gonder [40]. In this study, *Staphylococcus aureus* was the predominant isolated bacterial species in both government and private primary schools. This finding was similar to that of studies conducted in Saudi Arabia and India [2, 43]. However, there was a disparity with the study conducted in Egypt [19]. This might be because *Staphylococcus aureus* bacteria can stay longer in the form of aerosols and can easily live in different body parts of humans, like skin and mucous membranes, which are released easily when they perform any activity.

The results of this study indicated that the indoor air temperature was positively correlated with the total bacterial load in both government and private primary schools during the morning (r = (0.044, 0.384), afternoon (r = (0.177, 0.285), and mid-afternoon (r = (0.006, 0.294)) periods, respectively. In this study, the temperature ranged from (19.8-22.1°C) in the

morning, (28.8-31.5°C in the afternoon) and (24.4-26.6°C in the midafternoon), respectively. The findings of this study are similar to those of a study conducted in Gondar town [40]. This is because the temperatures in most classrooms fell within the ranges conducive to the survival and proliferation of bacteria. This could have contributed to the high bacterial loads observed in this study. In this study, relative humidity was negatively correlated with the bacterial load in the morning (r = (-0.078, -0.047)), afternoon (r= (↑-0.215, -0.248), and midafternoon (r = (-0.262, 0.094), respectively, in government and private primary schools. This finding was in line with the study conducted in Gondar [40]. However, it is contrast to studies conducted in Poland [44] and Portugal [38]. This disparity may arise from variations in environmental factors; for instance, temperature differences and relative humidity are inversely related to temperature. In Poland and Portugal, the average winter temperatures are considerably lower than in Hawassa City, Ethiopia.

In the current study, in government primary schools, $PM_{10}$ (r = 0.005) morning (r = 0.055) afternoon, (r = 0.174) midafternoon, and $PM_{2.5}$ (r = 0.209 morning, r=0.150 afternoon, r=0.085) midafternoon were positively correlated with bacterial load. This finding was in line with a study conducted in Malaysia [45]. The possible justification for the positive correlation might be that the $PM_{10}$ concentration increases the bacterial load because the bioaerosols are attached to the coarse solid particles. In private primary schools, $PM_{10}$ morning (r = 0.294), afternoon (r = -0.212), midafternoon (r = 0.206), and $PM_{2.5}$ morning (r = 0.389), afternoon (r = -0.389), midafternoon (r = -0.278) were correlated with the bacteria load. This finding of a positive correlation of $PM_{10}$ was consistent with the studies done in Portugal [46], Malaysia [45], and Gondar [40]. The possible reason for the positive correlation might be that when $PM_{10}$ concentrations increase, the bacterial load increases because bacterial aerosols are attached to coarse solid particles, and the negative correlation of this study is in line with a study conducted in Poland [44]. In this study, $PM_{2.5}$ was positively correlated with the bacterial load in the morning. This finding was consistent with the study conducted in Portugal [46] and Gondar in the morning [18]; the negative correlation of $PM_{2.5}$ in the afternoon and midafternoon in this study was similar to the study conducted in Poland [44] and Gondar in the afternoon [40].

In this study, occupant density was positively correlated with the bacterial load in government and private primary schools. This was in agreement with studies conducted in Malaysia [45], systematic literature reviews conducted in Indonesia [12], and studies done in Egypt [24] and Nigeria [12]. Specifically, in governmental primary schools, classrooms with poor cleanliness exhibited higher bacterial counts than clean classrooms, and this was significantly associated with the bacterial load. This finding followed conducted in Arbaminch [24] and Jimma town [16]. A possible reason for the significant association might be that poor classroom cleanliness facilitates the growth and survival of bacteria. This study revealed that in private primary schools, the mean bacterial load was significantly associated with classroom ventilation. This finding is consistent with studies conducted in Portugal [38] and Jimma [16]. The possible reason might be that inadequate ventilation plays a crucial role in the increase in the bacterial load in the indoor air environment [47]. On the other hand, in private primary schools, there was a significant difference in bacterial load between classrooms, which might be due to the difference in the number of openable windows, which contradicted the study conducted in Harar [48]. This difference might occur when the number of open windows increases; fresh air may enter the classroom, allowing for better ventilation. In the present study in government primary schools, the classroom area was significantly related to the mean bacteria load. This result was in line with studies conducted in Egypt [49] and Gondar [14] It might be due to the fact that a large area of the classroom allows for more air circulation and better ventilation in the room.

## Conclusion and recommendation

In comparison with private primary schools, government primary schools exhibited a significantly higher bacterial load. Both government and private

elementary school classrooms exceeded the WHO criteria for bacterial contamination. Gram-positive and gram-negative *Bacillus* species, as well as coagulase-negative *Staphylococcus* and *Staphylococcus aureus*, were prevalent in the majority of classrooms in government and private primary schools. Factors such as classroom area, cleaning frequency, occupant density, and cleanliness were predictive of the bacterial load in government primary schools. However, the number of open windows per classroom, relative humidity, occupant density, cleaning frequency, ventilation condition, and type of ventilation to air circulation were predictive of the bacterial load in private primary schools. To mitigate indoor air pollution, the Hawassa City Education Bureau must implement appropriate prevention measures. Furthermore, building more classrooms is necessary to lessen crowding and maintain the relative humidity of the classrooms. The school management should also improve the WASH facilities and the cleanliness of the classrooms and latrines. Researchers should conduct further studies in areas such as the staff office, library, restroom, and outdoors. In addition, drug susceptibility testing for the identified bacterial species, as well as other harmful bacterial and fungal species, combined with other factors not included in this study.

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## Appendix

## Supplementary files

**Table S1.** Assessment of air quality sampling classrooms in ten primary schools in Hawassa City according to the sanitary standard of the European Commission for nonindustrial premises.

| | Bacteria load | | | | | |
|---|---|---|---|---|---|---|
| Sampling time | 6:30-7:30 am | | | | | |
| Range of Values (CFU/m$^3$) | | <50 | 50-100 | 100-500 | 500-2000 | >2000 |
| Degree of pollution | | Very low | Low | intermediate | High | Very high |
| Government primary schools | S1 | _ | _ | _ | _ | √ |
| | S2 | _ | _ | _ | _ | √ |
| | S3 | _ | _ | _ | _ | √ |
| | S4 | _ | _ | _ | _ | √ |
| | S5 | _ | _ | _ | _ | √ |
| Private primary schools | S6 | _ | _ | _ | √ | _ |
| | S7 | _ | _ | _ | √ | _ |
| | S8 | _ | _ | _ | √ | _ |
| | S9 | _ | _ | _ | _ | √ |
| | S10 | _ | _ | _ | _ | √ |
| | Bacteria load | | | | | |
| Sampling time | 1:30-2:30 pm | | | | | |
| Range of values (CFU/m$^3$) | | <50 | 50-100 | 100-500 | 500-2000 | >2000 |
| Degree of pollution | | Very low | Low | Intermediate | High | Very high |
| Government primary schools | S1 | _ | _ | _ | _ | |
| | S2 | _ | _ | _ | _ | |
| | S3 | _ | _ | _ | _ | |
| | S4 | _ | _ | _ | _ | |
| | S5 | _ | _ | _ | _ | |
| Private primary schools | S6 | _ | _ | _ | √ | |
| | S7 | _ | _ | _ | _ | √ |
| | S 8 | _ | _ | _ | _ | |
| | S9 | _ | _ | _ | _ | |
| | S10 | _ | _ | _ | _ | |
| | Bacterial load | | | | | |
| Sampling time | 5:00-6:00 pm | | | | | |
| Range of values (CFU/m$^3$) | | <50 | 50- 100 | 100-500 | 500-2000 | >2000 |
| Degree of pollution | | Very low | Low | Intermediate | High | Very high |

Table S1(*Continued*)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Government primary Schools | S1 | _ | _ | _ | _ | | |
| | S2 | _ | _ | _ | _ | | |
| | S3 | _ | _ | _ | _ | | |
| | S4 | _ | _ | _ | _ | | |
| | S5 | _ | _ | _ | _ | √ | |
| Private primary schools | S6 | _ | _ | _ | √ | | |
| | S7 | _ | _ | _ | _ | | |
| | S8 | _ | _ | _ | _ | √ | |
| | S9 | _ | _ | _ | _ | √ | |
| | S10 | _ | _ | _ | _ | | |

**NB:** ≤ 500 CFU/$m^3$ is the permissive standard, (√) in the range, and (- not in range)

**Table S2.** Mean value of a physical parameter of indoor air Factors related to the indoor air quality of the classrooms in primary schools in Hawassa City, Ethiopia, 2023 (n=56).

| **Physical indoor air Parameter factors** | | | | |
|---|---|---|---|---|
| **Schools** | **Average T (°C)** | **$PM_{10}$ (µg/$m^3$)** | **$PM_{2.5}$ (µg/$m^3$)** | **RH (%)** |
| **Morning (6:30 Am)** | | | | |
| S1 | 21.6 | 716.7 | 60.3 | 65.0 |
| S2 | 21.4 | 163.6 | 110.9 | 64.8 |
| S 3 | 21.8 | 171.1 | 115.4 | 63.9 |
| S4 | 21.0 | 135.0 | 74.6 | 65.0 |
| S5 | 21.9 | 68.3 | 71.9 | 64.0 |
| S6 | 19.8 | 340.0 | 63.7 | 61.6 |
| S7 | 19.9 | 72.5 | 63.5 | 65.0 |
| S8 | 21.5 | 56.33 | 54.3 | 64.5 |
| S9 | 21.5 | 53.0 | 49.5 | 62.0 |
| S10 | 22.1 | 69.5 | 202.5 | 62.5 |
| **Afternoon (1:30 pm)** | | | | |
| S1 | 29.1 | 18.7 | 25.8 | 45.0 |
| S2 | 31.5 | 32.3 | 23.0 | 43.3 |
| S3 | 29.0 | 29.3 | 24.2 | 44.4 |
| S4 | 29.7 | 37.0 | 28.9 | 44.8 |
| S5 | 31.1 | 28.0 | 30.3 | 44.0 |
| S6 | 29.2 | 26.0 | 29.0 | 43.6 |
| S7 | 29.9 | 25.0 | 25.5 | 43.3 |
| S8 | 28.8 | 29.3 | 29.0 | 44.5 |
| S9 | 29.0 | 21.5 | 24.0 | 43.7 |
| S10 | 29.5 | 21.8 | 17.5 | 42.9 |
| **Mid Afternoon (5-6 pm)** | | | | |
| S1 | 26.6 | 42.7 | 41.9 | 55.3 |
| S 2 | 26.2 | 168.3 | 54.3 | 55.0 |
| S3 | 25.1 | 152.4 | 93.6 | 55.5 |
| S4 | 24.8 | 66.4 | 61.4 | 55.6 |
| S5 | 26.6 | 85.9 | 52.9 | 53.0 |
| S6 | 24.4 | 46.3 | 49.7 | 54.3 |
| S7 | 25.1 | 40.0 | 45.0 | 53.5 |
| S8 | 24.9 | 44.o | 43.0 | 55.0 |
| S9 | 25.0 | 48.5 | 40.5 | 53.3 |
| S10 | 25.6 | 49.0 | 41.8 | 55.0 |

**Table S3.** Frequency distribution of crowding, building, and cleanliness-related factors in government and private primary school classrooms in Hawassa city, Ethiopia, 2023 (n = 56).

| | | Government school | | Private schools | |
|---|---|---|---|---|---|
| **Variable** | **Category** | **Frequency** | **Percent (%)** | **Frequency** | **Percent (%)** |
| Classroom area($m^2$) | <56$m^2$ | 42 | 100 | 14 | 100 |
| | ≥56$m^2$ | | | | |
| Floor area per no of students ($m^2$/pupils) | ≤1.4 | 6 | 14.3 | 6 | 42.9 |
| | >1.4 | 36 | 85.7 | 8 | 57.1 |
| Ceiling type | Wood false | 9 | 21.4 | 5 | 35.7 |
| | Cheepwood | 28 | 66.7 | 14 | 100 |
| | Gypsum | 4 | 9.5 | | |
| | Other | 1 | 2.4 | | |
| Type of ventilation with respect to air circulation | Cross-ventilation | 32 | 76.2 | 10 | 71.4 |
| | Through ventilation | 5 | 11.9 | 1 | 7.1 |
| | One-sided ventilation | 5 | 11.9 | 3 | 21.4 |
| Condition of ventilation | Adequate | 18 | 42.9 | 8 | 57.1 |
| | Inadequate | 24 | 57.1 | 6 | 42.9 |
| Cleanness condition of the classroom | Good | 8 | 19.0 | 11 | 78.6 |
| | Bad | 34 | 81.0 | 3 | 21.4 |
| Cleaning frequency of the classroom | Two times per day | | | 12.0 | 85.7 |
| | Two times a week | 10 | 23.8 | | |
| | Once a day | 24 | 57.1 | 2.0 | 14.3 |
| | Three times per week | 8 | 19.1 | | |

**Table S4.** Frequency distribution of WASH-related factors in government and private primary School classrooms in Hawassa city, Ethiopia, 2023 (n = 56).

| | | | Government primary school | | Private primary schools | |
|---|---|---|---|---|---|---|
| **Variables** | **Category** | | **Frequency** | **Percent (%)** | **Frequency** | **Percent (%)** |
| Is there any type of water? supply for schools | Yes | | 5 | 100 | 5 | 100 |
| Is the water adequate | Yes | | 1 | 20 | 4 | 80 |
| | No | | 4 | 80 | 1 | 20 |
| The Proportion of single tap per students | <50 | | 2 | 20 | | |
| | >50 | | 10 | 100 | 8 | 80 |
| | | | | 100 | 14 | 100 |
| Type of latrine | Improved | | 5 | 100 | 5 | 100 |
| | unimproved | | | | | |
| Is there hand washing facility near to the latrine | Yes | | 1 | 20 | 4 | 80 |
| | No | | 4 | 80 | 1 | 20 |
| Proportion of one seat hole of the latrine to students | Boys | >15-20 | 5 | 100 | 5 | 100 |
| | Girls | >10-20 | 5 | 100 | 5 | 100 |
| Is there anal cleaning material on the floor | Yes | | 4 | 80 | 1 | 20 |
| | No | | 1 | 20 | 4 | 80 |
| Is there faeces around the pit floor | Yes | | 4 | 80 | 1 | 20 |
| | No | | 1 | 20 | 4 | 80 |
| Latrine cleanness | Good | | 1 | 20 | 3 | 60 |
| | Bad | | 4 | 80 | 2 | 40 |

**Table S5.** Correlation between indoor air bacterial load & physical indoor air quality parameters, in government primary schools in Hawassa city, Ethiopia (n = 56).

| Variables | Bacteria | T (°C) | $PM_{10}$ | $PM_{2.5}$ | RH (%) | CO | $NO_2$ |
|---|---|---|---|---|---|---|---|
| **Moring** | | | | | | | |
| Bacteria | 1.00 | | | | | | |
| T (°C) | 0.19 | 1.00 | | | | | |
| $PM_{10}$ | 0.12 | -0.35[aa] | 1.00 | | | | |
| PM2.5 | 0.17 | -0.20 | 0.47[aa] | 1.00 | | | |
| RH (%) | -0.01 | -0.32[aa] | 0.32[aa] | 0.18 | 1.00 | | |
| CO | 0.23 | 0.11 | 0.07 | 0.07 | 0.04 | 1.00 | |
| $NO_2$ | -0.06 | -0.02 | 0.00 | 0.03 | 0.04 | 0.00 | 1.00 |
| **Afternoon** | Bacteria | T (°C) | $PM_{10}$ | PM2.5 | RH (%) | CO | $NO_2$ |
| Bacteria | 1.00 | | | | | | |
| T (°C) | 0.24 | 1.00 | | | | | |
| $PM_{10}$ | 0.07 | -0.12 | 1.00 | | | | |
| PM2.5 | 0.07 | -0.08 | 0.06 | 1.00 | | | |
| RH (%) | -0.14 | -0.34[aa] | 0.25 | 0.07 | 1.00 | | |
| CO | -0.10 | -0.21 | 0.08 | 0.17 | 0.07 | 1.00 | |
| $NO_2$ | 0.15 | -0.02 | 0.02 | 0.04 | -0.17 | 0.09 | 1.00 |
| **Midafterno on (5:00 6:00pm)** | Bacteria | T (°C) | $PM_{10}$ | PM2.5 | RH (%) | CO | $NO_2$ |
| Bacteria | 1.00 | | | | | | |
| T (°C) | 0.18 | 1.00 | | | | | |
| $PM_{10}$ | 0.25 | 0.03 | 1.00 | | | | |
| PM2.5 | 0.16 | 0.26 | 0.13 | 1.00 | | | |
| RH (%) | -0.14 | -0.19 | 0.03 | 0.10 | 1.00 | | |
| CO | 0.27 | 0.02 | 0.18 | 0.08 | 0.02 | 1.00 | |
| NO2 | -0.01 | -0.05 | 0.16 | -0.02 | -0.03 | 0.10 | 1.00 |

**Table S6.** Correlation coefficients between indoor bacterial load &building, physical parameters and cleanness factors, in government and private primary schools in Hawassa city, Ethiopia, 2023 (n = 56).

| Variables | Bacteria | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 | X9 | X10 | X11 | X12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteria | 1.000 | | | | | | | | | | | | |
| X1 | -0.38** | 1.00 | | | | | | | | | | | |
| X2 | -0.26* | 0.35 | 1.00 | | | | | | | | | | |
| X3 | 0.22 | -0.09 | -0.08 | 1.00 | | | | | | | | | |
| X4 | 0.07 | 0.18 | 0.08 | 0.32** | 1,00 | | | | | | | | |
| X5 | 0.460** | 0.136 | -0.059 | 0.15 | 0.08 | 1.00 | | | | | | | |
| X6 | 0.337* | 0.03 | -0.04 | 0.32 | -0.20 | 0.08 | 1.00 | | | | | | |
| X7 | 0.28 | 0.13 | 0.04 | -0.03 | 0.09 | 0.43 | 0.22 | 1.00 | | | | | |
| X8 | -0.10 | 0.17 | 0.08 | 0.11 | 0.20 | 0.05 | -0.10 | -0.3** | 1.00 | | | | |
| X9 | 0.19* | -0.14 | 0.11 | 0.21 | -0.04 | 0.19 | 0.18 | -0.07 | 0.11 | 1.00 | | | |
| X10 | 0.14 | -0.10 | 0.02 | 0.11 | -0.10 | 0.16 | 0.12 | -0.13 | 0.07 | 0.3* | 1.00 | | |
| X11 | 0.12 | 0.16 | -0.03 | 0.20 | 0.37 | -0.03 | 0.12 | 0.08 | 0.05 | 0.10 | 0.20 | 1.0 | |
| X12 | 0.124 | 0.44** | -0.33* | 0.08 | 0.09 | 0.02* | 0.08 | 0.03 | 0.08 | -0.02 | -0.10 | 0.2 | 1.00 |

**N.B** =[aa] Correlation is significant at the levels of 0.01 and =[a] Correlation is significant at the levels of 0.05

**Key**: X1-X12; No of students per classroom, Classroom area, Type of ventilation with rest to air circulation, No of openable window, Cleaning frequency, Classroom cleanness, average T (°C), average RH(%), average $PM_{10}$(μg/$m^3$) and average $PM_{2.5}$(μg/$m^3$) respectively, ventilation condition and classroom area per pupil.