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An Environmental Microbiology Classroom For Hygienic Awareness Among High School Students

'Affaf Rijwany Mohd Rezal[1], Nur Umairah Syamina Mohd Sofian[1], Nur Fazidah Asmuje[1], Liyana Amalina Adnan[1], Ehwan Ngadi[1], Wan Abd Al Qadr Imad Wan-Mohtar[2], Rahayu Ahmad[1*]

[1] Al Razi Halal Action Laboratory, Kolej PERMATA Insan, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800, Nilai, Negeri Sembilan, Malaysia
[2] Functional Omics and Bioprocess Development Laboratory, Biotechnology Program, Institute of Biological Sciences, Faculty of Science, University Malaya, 50630, Kuala Lumpur, Malaysia

*Corresponding Author: rahayu@usim.edu.my

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Abstract: *Microbial contamination of the classroom environment is a worry since it can create a variety of health problems and negatively impact kids' and teachers' general well-being. To increase awareness of personal hygiene during a pandemic and post-pandemic, students can reveal to themselves the true state of their body parts, clothing and surrounding conditions that are exposed to the breeding of germs. Thus, an environmental microbiology classroom has been conducted with Biotechnology Club high school students for a screening of microbial growth and contaminations on students' body parts, attire, and school's selected area. Swabbing method of students' hands, collar of the shirt (boys) and scarf (girls) and doorknob (classes, laboratory, toilet & management office) was carried out and subsequently swabbing onto the nutrient agar (NA) plate for microorganisms' growth. After 24 hours incubation, the colonies of the growing microorganisms were observed, and their number of different colonies were counted. Through this experiment, it is suggested that the students can observe themselves how dirty their hands, clothes and even selected areas like doorknob can cause the spread of the microbial infections if not cleaned regularly. Several hygienic practices, mitigation activities and awareness were suggested to the students in preventing the spread of microbial infection by handing out pocket posters for students to read and paste in rooms or classes.*

Keywords: Environmental Microbiology, School Environment, Hygienic Practice, Education

1. Introduction

Microbial contamination in open areas refers to the presence of harmful microorganisms, such as bacteria, viruses, fungi, and other pathogens in outdoor environments. These contaminants can pose a risk to human health and environment. Microbial contamination of the classroom environment is one of the open areas which can cause various health issues and affect the overall well-being of students and teachers. Classrooms are shared spaces with high levels of human interaction, and they provide an ideal environment for microorganisms to thrive and spread. When the world was hit by Covid-19 pandemic, various awareness measures were announced to avoid

and prevent the spread of the Covid-19 viruses. Most schools were closed to reduce the transmission of the severe acute respiratory syndrome Coronavirus 2 (Sars-CoV-2). After the Covid-19 infection subsided, in mid-2021, schools were reopened to carry out their operations as usual. The school staff worked tirelessly for preparation to make sure that the school conditions comply with standard operation procedure (SOP). Students are also often reminded to take care of personal hygiene such as wearing face masks all the time, washing hands regularly and maintaining social distance. However, the order to maintain personal hygiene is sometimes taken for granted by school students because they do not see the germs spreading by their naked eye.

Teaching and learning environment play an important role and impact in motivate students' understanding (Vallori 2014). The relevance of meaningful learning and teaching approach could be established through showing how theory can be applied in practice. To further increase students' awareness of how important to take care of personal hygiene during pandemics or post-pandemic is to carry out theoretical learning along with practical to have a better understanding of the real situation in avoiding and reducing disease infections (Vallori, 2014; Melendez, 2019).

The findings of previous study have stated that teaching and learning through theory alone is not encouraging and does not provide motivation (Kember et al., 2008). Relevance teaching and learning should be established through showing how theory can be applied in practical life and relating material to everyday applications of finding applications in current newsworthy issues to give a high impact on students (Kember et al., 2008).

Environmental microbiology is the study of how microbes interact with the environment and with each other, including their effects on the landscape, the spread of viruses and bacteria, the distribution of algae, fungi, and parasitic organisms, and the implications for human health and the environment that are brought about by these interactions (Stephens et al., 2019). The environmental microbiology class is devoted to the advancement of students' understanding of microbial interactions and microbial processes in the school surroundings. Students must have a thorough awareness of the microbes that cause illnesses like germs and play a role in human health, such as the microbiome. Students will retain these ideas in this manner as adults, eventually assisting in their continued health. Understanding the broader bacterial community to which students are expose will help inform public health effort and contribute to our growing understating of the bacterial community associated with school environments (Beasley et al., 2022).

Therefore, an environmental microbiology classroom was conducted among Biotechnology students club to give the students the first-hand experienced and opportunity to observe for themselves on how microbes grows and multiple in the students' immediate environment if hygiene practice is not well-practiced, therefore it can lead to disease infection.

2. Experimental

Personnel involved

Biotechnology Club of Kolej PERMATA Insan, Universiti Sains Islam Malaysia enrols 40 students aged from 13 to 17 years old which are divided into 5 groups. Environmental microbiology is introduced through the screening of microorganisms from students' body parts (palm), attire (boy shirt's collar and girl's scarf) of school uniform and school area which

frequently touch areas like doorknob. Students are asked to carry out a swab on the particular-chosen subject and subsequently swabbing on the nutrient agar (NA) plate to allow the growth of microorganisms before observing after 24 hours incubation. For the swabbing on students' body parts and attire, only 10 students were chosen to be the samples and the process of the swabbing is demonstrated in front of all the students of the Biotechnology Club. The rest of the students were involved in counting and observing the viable plate. Apart from swabbing method, a representatives of Biotechnology Club students (3 students) were demonstrated the serial dilution method if the samples were collected from environment like soil and water which subsequently using viable count technique same as swabbing method.

Preparation of agar plate

Nutrient agar (NA) is essential in supporting the growth of microorganisms. Nutrient agar was prepared by suspending 20 g of nutrient agar powder in 1 L of distilled water, stirred until dissolved and autoclave at 121°C for 15 minutes at a pressure of 1 atm. The mixture is then set to be cooled but not solidified. The nutrient agar is poured into a sterile petri dish until the agar solidifies and is stored 4°C for long-term use (Ahmad et al., 2021).

Viable count

The viable count method was conducted to observe and measure the number of viable cells grown onto the NA plate. The students' unclean hands (palm), attires (shirt collars for boys and scarf for girls), and doorknob were swabbed with sterilized cotton buds. After swabbing, the cotton buds were swabbed onto the NA plate. The swabbed NA plates were incubated at room temperature, observed, and counted the bacterial colonies after 24 hours incubation. The characteristics of the grown microbes on the NA plates were observed and recorded (Madigan et al., 2003).

Safety Precautions

To avoid any contamination on the agar plates, the plates were sealed using parafilm before and after swabbing. Sample preparations were carried out in a laminar flow cabinet to reduce the exposure of bacteria and fungi from the surrounding air.

Raising Awareness

To increase the awareness of high school students about the importance of maintaining personal hygiene during pandemic and/or endemic, several mitigation activities have been suggested so that students always comply with the Standard Operation Procedure (SOP) that has been set. Among the measures that students need to practice in curbing the spread of infectious disease are always wearing face masks, washing their hands frequently, not sharing food utensils with friends and maintaining social distance. Pocket posters are prepared and distributed to the students to read or paste in the classroom or in the dormitory room.

3. Results and Discussion

Viable count

Microbial contamination in classroom can results from various sources including human activities which is the presence of students and teachers in classrooms may introduce a variety of microorganisms through coughing, sneezing, talking, and touching surfaces. The poor hygiene practices where the inadequate handwashing or improper sanitation method may also contribute

to the spread of microorganisms. Inadequate ventilation also can lead to the accumulation of airborne contaminants including bacteria, viruses, and fungi. In classroom environment, students always moving and the contaminated surfaces where students' frequent touches such as desks, chairs, door handles, and shared classroom materials can become reservoirs for microbes.

Direct microscopic counting counts both live and dead cells. In the learning of environmental microbiology classroom, we are interested in counting only live cells and for this purpose viable cell counting methods are very suitable (Madigan et al., 2003). A viable cell is one that can divide and produce offspring. The standard method to do a viable count is to count the number of cells in the sample that can form colonies on an appropriate agar medium. In this kind of counting procedure, it is assumed that each viable cell may produce one colony. A plate count can be done using either the spread plate or the pour plate technique.

In this study, the spread plate technique has been carried out with slight modification where the liquid sample is replaced with swabbing samples using sterilized cotton buds. Normally, in spread plate technique, a volume of an appropriately diluted sample is spread over the surface of an agar medium, but in this study swabbing samples at selected areas using sterilized cotton buds were spread onto the NA plates. The plate is incubated until the colonies appear and the number of colonies is counted, and the characteristics of the appeared colonies is observed.

There were three different areas were selected as the potential area for microorganisms' transmission which were students' palm, students' attire (collar shirt and scarves) and door- knob of selected places such as classroom, general office (frequently visit by the students) and toilet. As for the counting colonies, the average number of colonies appearing after 24 hours incubation were in the range of 20 to 100 colonies for all selected potential area. It was observed that the colonies appeared to have different characteristics. Based on the shape of the colonies, most of the colonies appeared as small round, medium round, and irregular shapes.

Figure 1 shows the technique on how to carry out the viable count of microorganisms after 24 hours incubation. Throughout this viable count technique, students will have their first experience of isolating, incubating, observing, and counting the viable microorganisms from their immediate environment. This experiment could enhance the theory of understanding on the essential elements for bacterial growth as the students also prepared the agar media before conducting the swabbing activities. This practical activity also helps the students to relate the theory of growth of microorganisms with the real daily life situation.

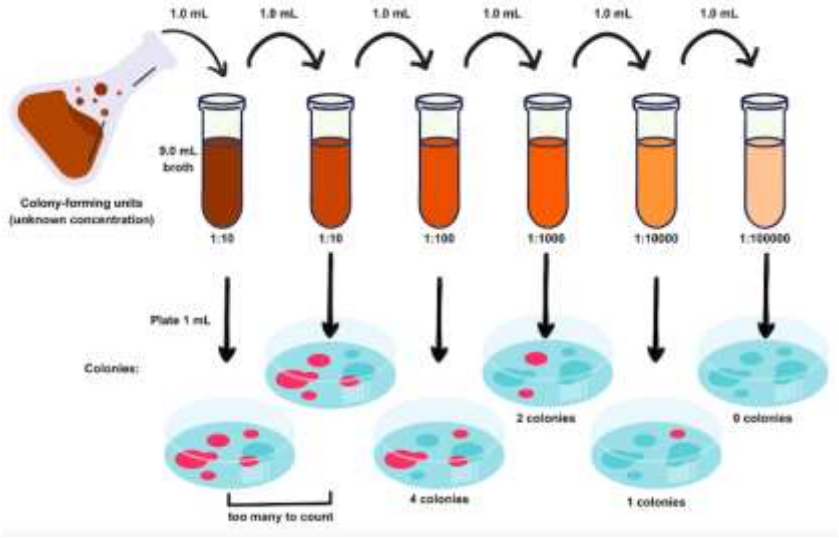

Figure 1: Serial dilution technique for the viable count of microorganisms.

Characteristics of Viable Microorganisms

The characteristics of the viable microorganisms grown onto the NA plate were observed after 24 hours incubation from different swabbing areas.

Students' Palm

Figure 2 shows the grown NA plate divided into gender category which is male and female subjected personnel. Three best NA plate were chosen from each category. It was obviously observed that the palm area of the male and female students is not clean and consists of different types of bacteria colonies. **Table 1** describe the characteristics of the viable colonies on each plate. Overall, there were two to three of different colonies were obtained from male and female NA plate. The colonies exhibited round and irregular in shape, different colour of white, yellow, pale white, yellowish white and pale white. The colonies isolated from the students' palm exhibited texture like opaque and translucent.

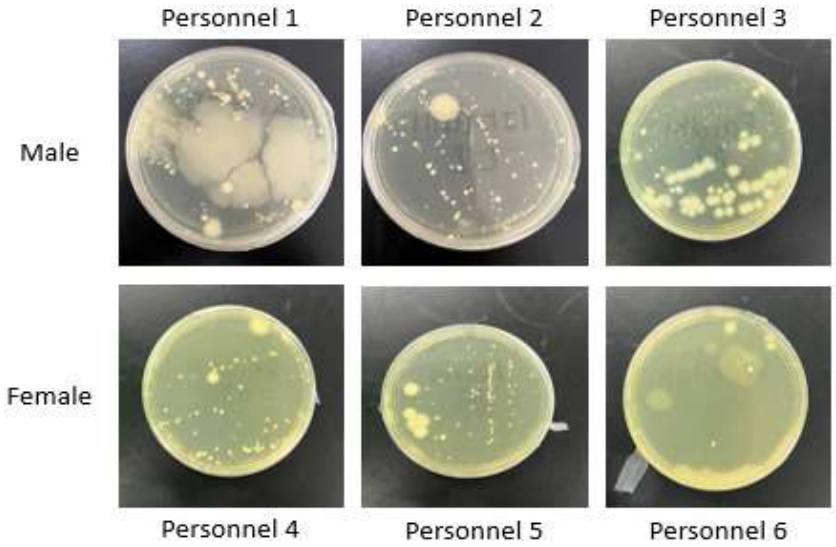

Figure 2: Selected NA plates of microorganisms' swabbing from student's palm after 24 hours incubation.

Table 1: Characteristics of bacteria colonies observed after 24 hours incubation on NA plate medium from student's palm.

Personnel	Number of colonies	Colony	Characteristics		
			Shape	Colour	Texture
Male					
1	3	1	Round	White	Opaque
		2	Round	Yellow	Opaque
		3	Irregular	White	Translucent
2	3	1	Round	Yellow	Opaque
		2	Round	White	Opaque
		3	Irregular	Pale white	Translucent
3	3	1	Round	White	Opaque
		2	Irregular	Pale white	Translucent
Female					
4	2	1	Round	Yellow	Opaque
		2	Irregular	Yellowish white	Translucent
5	3	1	Round	White	Opaque
		2	Round	Yellow	Opaque
		3	Irregular	Pale white with some yellow	Translucent
6	3	1	Round	Yellow	Opaque
		2	Round	Yellow	Translucent
		3	Irregular	Yellowish white	Translucent

Student's attire

Student's attire is one of the surface environments that can encourage the growth of pathogens where the pathogens able to remain viable on the attire surface. Swabbing of student's attire like collar shirts (boy) and scarf (girl) are necessary to demonstrate to the student's cleanliness of their school attire. **Figure 3** demonstrated the NA plate grown bacteria colonies from student's attire. It was clearly observed that NA plate for collar shirt exhibited more bacterial colonies as compared to the girl's scarf.

The characteristics of the isolated bacterial colonies were described in **Table 2**. The colonies exhibited round and irregular in shape, different colour of white, yellow, pale white, yellowish white and pale white. The colonies isolated from the students' attire exhibited texture like opaque and translucent. Previous study had also demonstrated the screening of bacteria on the attire surface like coats, curtains and ties and results obtained demonstrated a significant number of bacterial contamination where it possible to expose to other person through direct contact (Catano et al., 2012).

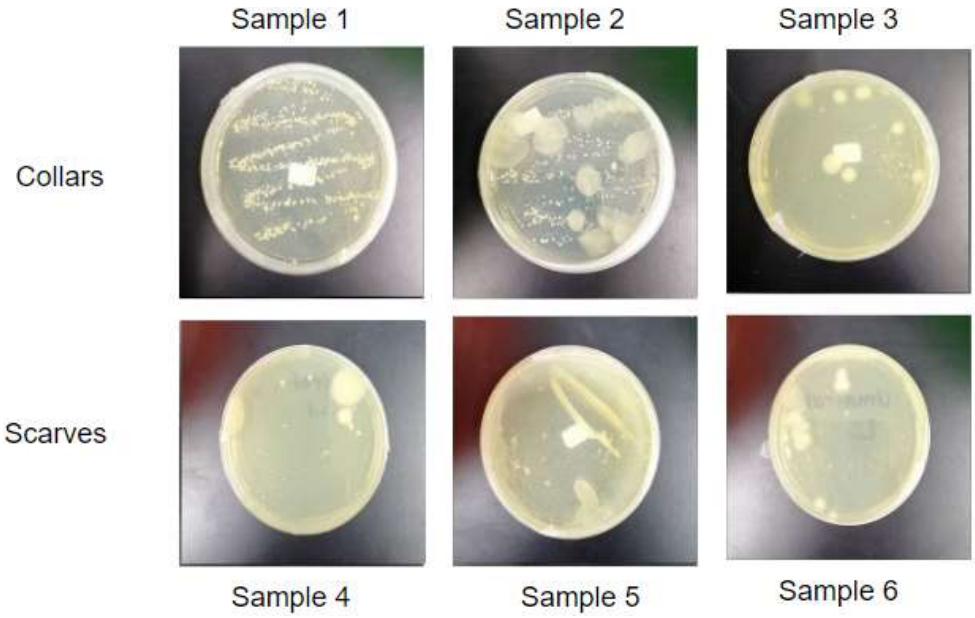

Figure 3: Selected microorganisms swabbing students' attires; from collars shirt (boys) and scarves (girls) grown onto NA plates after 24 hours incubation.

Table 2: Characteristics of bacteria colonies observed after 24 hours incubation on NA plate medium from student's attire.

Sample	Number of colonies	Colony	Characteristics		
			Shape	Colour	Texture
Collars					
1	2	1	Round	Yellow	Opaque
		2	Round	White	Opaque
2	3	1	Round	Yellow	Opaque
		2	Round	White	Opaque
		3	Irregular	Pale white	Translucent
3	3	1	Round	White	Opaque
		2	Round	White	Translucent
		3	Irregular	Yellow	Translucent
Scarves					
4	3	1	Round	Yellow	Opaque
		2	Irregular	White	Opaque
		3	Irregular	Yellow	Translucent
5	2	1	Irregular	Yellow	Translucent
		2	Irregular	Pale white	Translucent
6	2	1	Irregular	Yellow	Translucent
		2	Irregular	Pale white	Translucent

Contaminated surfaces

Contaminated surfaces are area or surfaces which frequently touched by the students such as desks, chairs as well as door handles. Microorganisms can grow on surfaces like desks, chairs or school areas through a process called biofilm formation. Biofilms are communities of microorganisms,

such as bacteria, fungi, and algae that adhere to surfaces and form a protective matrix of extracellular substances.

The process of biofilm formation typically occurs in several phase such as attachment, growth and colonization, maturation, and dispersion. In the attachment phase, microbes in the surrounding environment attach to the surface. This initial attachment can be facilitated by factors such as surface roughness, presence of organic material or electrostatic forces. Once attached, the microbes start to multiply and form colonies on the surface during growth and colonization phase. The microorganisms secrete a sticky, slimy substances known as extracellular polymeric substances that helps hold the biofilm together and protect the microorganisms. As the biofilm continues to grow, it becomes more structured, organized, and reached the maturation phase. In some cases, biofilms can release free-floating microorganisms allowing them to disperse and colonized other surfaces. In this phase, if the students touch a surface that has been overgrown with microorganisms, the microorganisms will stick to the surface of the hands, and if the students do not practice personal hygiene frequently such as washing hands, there is high possibility that the students can be infected by the microorganisms or spread them to other friends. (Catano et al., 2012)

In this study, there were 3 possible contaminated surfaces has been swabbed and streaked onto the NA plate. After 24 hours incubation, it was observed that only surfaces of classroom doorknob demonstrated most bacterial grown colonies. This might be due to the frequent touching by the students to go in and out of the class which allow the attachment of microorganisms on the surface of the doorknob. The other two surfaces of office and toilet doorknob shown less grown bacterial colonies as shown **Figure 4**. The characteristics of the viable microorganism's colonies were described in **Table 3**. Classroom doorknob demonstrated 2 different bacterial colonies with the characteristics of irregular shape, yellow and white colour, and translucent texture. Office doorknob also shown two different colonies with the characteristics of round and irregular shape, pale white colour with the texture of opaque and translucent. Toilet doorknob revealed the cleanest swabbed surface area as it only demonstrated one single colony with irregular shape, white in colour and translucent chapter.

It was suggested that office doorknobs are less contaminated due to the infrequent use of office doors by the students while toilet knobs show the fewest colonies due to the surface of the toilet knob being smooth (not rough) and the toilet is often cleaned by cleaning workers, so there is no adhesion and breeding microbes occurs on the surface of the knobs.

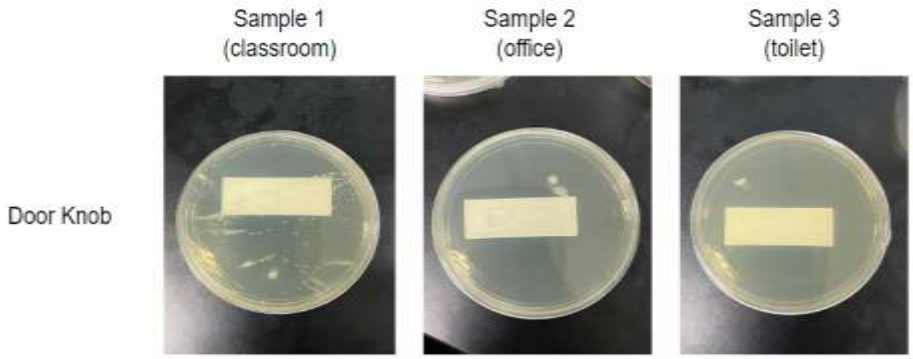

Figure 4: Selected microorganisms swabbing from doorknobs, grown onto NA plates after 24 hours incubation.

Table 3: Characteristics of bacteria colonies observed after 24 hours incubation on NA plate medium from school door knobs.

Sample	Number of colonies	Colony	Characteristics		
			Shape	Colour	Texture
Classroom					
1	2	1	Irregular	Yellow	Translucent
		2	Irregular	White	Translucent
Office					
2	2	1	Round	Pale white	Opaque
		2	Irregular	Pale white	Translucent
Toilet					
3	1	1	Irregular	Yellow	Opaque

Health effects and mitigation of hygienic classroom

The types of microorganisms found in classrooms can vary, but common ones include bacteria, viruses, fungi, and allergens. Some of the potential health risks associated with microbial contamination in classrooms include the spread of infectious diseases like colds, flu, and other respiratory infections. Additionally, certain molds and allergens can trigger respiratory issues and allergies, particularly in individuals with sensitivities. The comparison of hygienic environmental studies is shown in **Table 4** which describe the various microbial contaminants in our surrounding.

Table 4: Comparison of the current work on the hygienic classroom of high school students with other environmental hygienic studies.

Personnel involved	Area of contamination	Observed/Isolated microorganisms	References
High school students (age 13-17 years)	Students' attire (collar shirt, scarf), students' palm, door knobs	Bacterial colonies with different shape, texture and colour	Current work
Medical students	White coat	*Staphylococcus aureus*, *Bacillus* sp.	Muhadi et al., 2007
Childcare centers	Built environment (window, desk surface, door trim)	Human associated bacteria (Actinobacteria, Bacteroidetes, Cyanobacteria, Proteobacteria)	Beasley et al., 2022

To mitigate microbial contamination in the classroom environment, schools and educational institutions should implementing significant measures for hygienic practice in providing safe and hygiene school environments. **Table 5** suggested the mitigation measures and adequate activities for hygienic school classrooms environment. Regular monitoring and maintenance of the classroom environment can help identify potential issues and ensure a healthier and safer learning space for everyone.

Table 5: Mitigation and adequate activities for hygienic school environments.

Mitigation area	Mitigation activities	Suggested personnel involved
Classrooms	**Regular cleaning and disinfection:** Classrooms and frequently touched surfaces should be cleaned and disinfected regularly to reduce the presence of harmful microorganisms	Students
	Avoiding food consumption in classrooms: Encourage students to eat and drink only in designated areas to prevent food-related contamination.	Teachers
Classrooms/office/ lecture hall	**Proper ventilation:** Ensuring good airflow and ventilation in classrooms can help reduce the concentration of airborne contaminants	Students/school's technician
	Indoor plants: Some indoor plants can help improve air quality and reduce certain pollutants, contributing to a healthier classroom environment.	Students/teachers
Education	**Hygienic education:** Teaching students about proper handwashing and hygiene practices can limit the spread of germs.	Teachers
Person well-being	**Prompt response to illnesses:** Encourage staff and students to stay home when sick to prevent the spread of infections within the classroom	Principle/Teachers

To increase the awareness of high school students to implement hygienic practices, pocket posters have been distributed to the students. **Figure 5** shows the pocket poster about ways to maintain hygienic.

Figure 5: Ways to maintain hygiene pocket poster

4. Conclusion

This Environmental Microbiology Classroom is a great way to educate students about the state of microbial proliferation and contaminations on their learning environment. Students are encouraged to practice hygiene practises as part of daily living in agreement with the recommendation of the suggested guidelines on hygienic lifestyle. Hygienic classroom environment are maintained regularly to guarantee a healthier and safer learning environment for both students and teachers.

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