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Survey of Microbial Contamination on The Floor of Computer Rooms in A University

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ABSTRACT

Infectious diseases are those in which pathogens such as viruses and bacteria invade the human body and multiply, causing various adverse effects on the body. To prevent infectious diseases, it is important to sterilize and remove the causative microorganisms, as well as prevent the introduction and movement of microorganisms from the external environment. In our laboratory, we investigated the status of microbial contamination of shoes and floors shared within the university and confirmed that the degree of microbial contamination varied depending on the location. In this study, we investigated general live bacteria, fungi, *Staphylococcus aureus*, *Escherichia coli*, and coliform bacteria to determine the status of microbial contamination on the floors of two computer rooms. The number of colonies of general live bacteria and *S. aureus* tended to increase with the number of users, and vice versa; this trend was similar for fungi. No characteristic tendency was observed for *E. coli* and coliform bacteria.

INTRODUCTION

Infectious diseases are those in which pathogens such as viruses and bacteria invade the human body and multiply, causing various adverse effects on the body. The transmission routes of infectious diseases include contact, droplets, and air, and the novel coronavirus infection (COVID-19), which is currently infecting an increasing number of people rapidly, is considered to be transmitted mainly by droplets and contact[1)]. To prevent infectious diseases, it is important to sterilize and remove the causative microorganisms, as well as prevent the introduction and movement of microorganisms from the external environment. In previous studies, multiple microorganisms were detected input buttons of elevators in hospitals used by an unspecified number of people, computer mice, and smartphones[2)], and it was shown that microorganisms moved *via* fingers, *etc*.[3-5)]. An epidemiological study of the rate of Trichophyton spread in people in their teens and twenties indicated that this genus may spread into the environment not only from the feet but also from socks[6-7)].

In our laboratory, we conducted surveys on the status of microbial contamination on shared footwear and floors in the university, and confirmed that the degree of microbial contamination differed depending on the location[8-9)]. In this study, we investigated general live bacteria, fungi, *Staphylococcus aureus*, *Escherichia coli*, and coliform bacteria to determine the status of microbial contamination on the floors of two computer rooms.

MATERIALS AND METHODS

Overview of measurement locations

The measurement locations were the floor of the information practice room at the University of Kochi Ike campus headquarters building (referred to as **I**) and the floor of the information practice room in the common building (referred to as **II**)(at the university, the computer room is called the information practice room). A total of 12 aisles and seating positions, which were considered to have different degrees of contamination, were selected as measurement points (I: **1**-**12**, II: **A**-**L**).In each room, the measurement points were denoted as**12** and in front of the rear entrance, **1**, **3**, **5**, **6**, **8**, **10**, **11**, **A**, **C**, **E**, **F**, **H**, **J**, and **K** the floor under the desk where the student computer was installed, and **2**, **4**, **7**, **9**, **B**, **D**, **G**, and **I** in the passage. The points at the front were denoted as **1**-**5** and **A**-**E**, those on the window side were denoted as **1**, **6**, and **11**, and **F**-**K**, and those on the corridor side were denoted as **5**, **10**, **12**, and-**E**. The sampling area for each floor was approximately 50 × 50 cm^2. The floors of both rooms are

carpeted, and they are supposed to be used by changing from outside shoes to slippers; however, many users do not use them because they are not obliged to wear them. In addition, as a countermeasure against COVID-19, windows and doors are opened for ventilation during use.

The features of the room **I** was one door in the front and one in the rear, six windows on the side, and an outdoor toilet near the rear door. Only the rear door is used as the doorway, and the front is opened only during use, for air circulation. With regard to the usage of shoeboxes installed near the rear door, there is a tendency to distinguish between upper and lower storage for slippers and outer shoes, respectively, but not all users comply with it. In contrast, there is one door in the front and two in the rear, and two windows in the rear in **II**. Only the rear door is used as a doorway. As for the usage of shoe boxes, slippers and outer shoes are mixed, and there is a tendency for many people to take off their outer shoes in the corridor in front of the doorway and move with socks or bare feet.

August 15 to September 13 is the summer vacation period, and from September 14 to September 30, there is almost no classroom in use. The number of users was obtained from the usage books installed in **I** and **II** (Table 1). This usage book does not include the number of people using the class. However, to prevent the spread of COVID-19 during this survey period, the number of face-to-face lessons was extremely low; hence, the actual number of users was determined from the number of people listed in the usage book.

Instruments and equipment

To prepare sterilized water for use in experiments, the pure water production equipment, Auto still WS200 (Yamato Scientific Co., Ltd., Tokyo, Japan), the ultrapure water production equipment, RFU414BA (Advantech Toyo Co., Ltd., Tokyo, Japan), and an automatic high-pressure steam sterilizer, Lab Autoclave MLS-3020(Sanyo Electric Biomedica Co., Ltd., Tokyo, Japan), were used. A sterile cotton swab (code 06526, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) was used to collect microorganisms. The DG-1 ultrasonic cleaner with an oscillation frequency of 43 kHz (Iuchi Seieido Co., Ltd., Osaka, Japan) was used for ultrasonic processing.

The MIR-154 incubator (Sanyo Electric Co., Ltd., Tokyo, Japan) and the ADS161SHUG clean bench (Yamato Scientific Co., Ltd.) were used for microbial culture. The temp/RH TM-413 air velocity meter (Tenmers, Taipei, Taiwan) was used for temperature and humidity

measurements. Temperature and humidity were measured in the center of the room during non-air-conditioned and air-conditioned hours (results not shown).

Medium and water

The Compact Dry "Nissui" simple medium (Nissui Pharmaceutical Co., Ltd.) was used for general live cell, yeast/mold (rapid type), *S. aureus*, and *E. coli*/coliform bacteria measurements. After confirmation with the manufacturer, a kit for yeast/mold measurement was used for fungi.

The pure water obtained from the pure water production equipment was processed into ultrapure water with a specific resistance value of ≥18 MΩ·cm using ultrapure water production equipment, and sterilized at 121°C for 15 min in a high-pressure steam sterilizer to prepare sterilized water. Then, 6 ml of sterilized water was dispensed into a sterilized 15 ml centrifuge tube on a clean bench by aseptic operation with a micropipette, and used for measurement.

Operation

On the mornings of July 27, August 24, September 27, and October 26, 2020, the floors were wiped in the order of **I** and **II**, and samples were taken before the class started. A sterile cotton swab was moistened with sterile water in a centrifuge tube, and the measurement location was wiped off thrice. The swab was added to the centrifuge tube containing the remaining sterile water, being careful not to touch it with hands (assuming that the original amount of sterile water remained). The centrifuge tube containing the sterilized cotton swab after wiping was ultrasonically treated for 5 min and stirred with a touch mixer on a clean bench; immediately after this, 1 ml of each microbial suspension was inoculated in four types of media and cultured. The culture conditions, following the instruction manual, were as follows: general live cell measurement was performed at 35°C for 2 days, fungus measurement was performed at 25°C for 3 days, and *S. aureus* and *E. coli*/ coliform bacteria measurements were performed at 35°C for 4 days. The number of bacteria was visually measured as the number of colonies; two people measured each medium to increase the accuracy, and the average value was converted and used as the result for the floor surface. According to the instruction manual, the colonies to be measured are red colonies for general live bacteria, all colonies for fungi, light blue to blue colonies for *S. aureus*, blue to bluish purple colonies for *E. coli*, and pink to magenta colonies for coliform bacteria.

RESULTS AND DISCUSSION

Comparisons between and within rooms

Microbial species other than fungi were more likely to be detected near the doorway and in the back in both **I** and **II**, but fungi were more likely to be detected near windows and in the front. General live bacteria, fungi, *S. aureus*, *E. coli*, and coliform bacteria were all affected by the increase or decrease in the number of users. It was speculated that the fungi that invaded through the windows might have affected the number of microorganisms detected. As shown in Figs. 1, 3, 4, and 5, a large number of general live bacteria, *S. aureus*, *E. coli*, and coliform bacteria were detected near and behind the exit in both **I** and **II**. When using the information practice room, users must pass from behind because they can only enter and exit through the rear door. In addition, when using the information practice room outside of class, the seats are not fixed, and it is thought that this may be because many people prefer to use the rear seats rather than the front seats.

As shown in Fig. 2, the number of fungi detected in **1** and **6** in **I** and **L** in **II** tended to be large; however, the proximity of the windows might have affected these measurement points. Fungi are larger than bacteria[10),11)]; hence, it is possible that the fungi that invaded through the window immediately fell and adhered to the vicinity of the bottom of the window. According to previous reports, many fungi have been detected in unused sandals[12)], and environmental conditions that are prone to fungi include long periods of unuse of places, air retention, and dust[13)]. Therefore, many fungi were detected in the anterior direction because they proliferate in rarely used places but are scarce in places that are used frequently.

As shown in Fig. 3, *S. aureus* was frequently detected near and behind the exit in both **I** and **II** but was detected at low levels in front of **I**. It is thought that this is because *S. aureus* is a part of resident skin microbiota that exist in the nasal cavity, skin, intestinal tract, *etc*. of the human body[14)], and is greatly affected by differences in users and their working conditions.

As shown in Fig. 4, *E. coli* were more abundant near the exit of **I** than in **II**. Toilets are installed around **me**, and *E. coli* is abundant in feces[15)]. It is possible that *E. coli* was brought in by a person who used the toilet near the doorway behind **I** by using it as it was.

As shown in Fig. 5, coliform bacteria were abundant near the exit of **II**. Coli forms are widely distributed not only in human and animal feces, but also in soil, water, and air[15)]. Even in **I**,

coliforms may have been detected near the doorway because they fall on the floor when shoes are placed on the shoe box and adhere when changing from shoes to slippers. In **II**, the slippers placed in the shoebox were often placed in the position of outer shoes. It is possible that coliform bacteria derived from environments such as soil were transferred to the slippers from the outer shoes and moved from the slippers to the floor surface. In **II**, there is a tendency for many people to take off their outer shoes in the corridor in front of the doorway and move with socks or bare feet inside, which is one of the reasons why many coliform bacteria were detected near the doorway. In this survey, it was considered that the number of coliforms detected on the floor, due to the mixture of slippers and outer shoes, and the coliforms brought in by walking in the corridor with socks or bare feet, increased. It is thought that coliforms are widely distributed not only in feces but also in the air. However, in this study, many coliforms were detected at the exit and rear; therefore, soil- and fecal-derived coliforms were more affected. In **I** as well, coliforms were detected near the doorway, but their number was low, and it was considered that the number of coliforms detected differed depending on the condition of the shoebox and the individual's foot.

In the second measurement of **6**, high numbers of *E. coli* and coliform bacteria were detected, and this may have been because the hair was mixed in one tube at the time of sampling. Ikeda *et al.*have shown that a large number of bacteria get attached to the hair and that *S. aureus* is killed by various shampoos. However, it has been reported that some *P. aeruginosa* and *E. coli* continue to adhere alive even after shampooing[16]; hence, it is possible that hair affected the amount of *E. coli* and coliforms detected.

Comparison based on sampling date

For general live bacteria and *S. aureus*, the number of microorganisms detected increased with the number of users, and it was speculated that the number of users might affect the microbial load. For fungi, the number of microorganisms detected decreased as the number of users increased, and vice versa. Since there were many places where *E. coli* and coliform bacteria did not change depending on the sampling date, it was not possible to compare the ratios unconditionally.

In **I**, general live bacteria decreased in number from the first to the second and from the second to the third sampling dates and increased in number from the third to the fourth sampling dates. Since it has been reported that the number of general live bacteria tends to

reflect the frequency of use[17]), it was considered that the number of users affected the microbial load in the rooms. In **II**, there was almost no difference in the ratio of the decreased part to the increased part from the second to the third sampling dates. Compared with that, the higher room temperature in **II** may have had an effect from the second to the third sampling dates (room temperature and humidity data are not shown). Although temperature and humidity were both measured in this survey, it was considered that a single measurement was insufficient to determine the relationship between temperature and the number of detected microorganisms. It is necessary to consider this in the future.

In **I**, the change in the number of microorganisms depending on the sampling date of fungi increased from the first to the second and from the second to the third sampling dates, and decreased from the third to the fourth sampling dates. The first to third sampling days were summer holidays, and it is thought that the decrease in the number of people using the information practice room may have been the cause of the increase. In contrast, for **II** results were different from those for **I** was obtained. The cause may have been related to different conditions for each user, such as the temperature of an individual's foot, humidity due to sweating, and type of socks.

In **I**, the change in the number of *S. aureus* depending on the sampling date decreased from the first to the second and from the second to the third sampling dates and increased from the third to the fourth sampling dates. The level of *S. aureus* detected decreased as the number of users decreased, and vice versa, suggesting that the number of users affected the *S. aureus* load. For **II**, some results that were different from those for **I** were obtained. One of the causes was that *S. aureus* is a part of resident skin microbiota; hence, it is considered that a large difference may have occurred depending on the number of microorganisms possessed by an individual.

Regarding the change in the number of *E. coli* and coliform bacteria detected depending on the sampling date, there were many places where *E. coli* and coliform bacteria were not detected in either information practice room. Therefore, it was judged that it is not possible to compare the rate of increase/decrease unconditionally depending on the sampling date.

CONCLUSION

In this study, we investigated the microbial contamination status on the floor of an information practice room at a university. Comparisons between and within rooms indicated

that general live bacteria and *S. aureus* was present at the back of each information practice room in large numbers, fungi were near the windows of each information practice room, coliforms were near the exit of information practice room **I**, and coliform bacteria tended to be detected near the exit of information practice room **II**.

Based on the sampling date, the number of microorganisms detected for general live bacteria and *S. aureus* decreased as the number of users decreased, and vice versa. This tendency was different from that of other microorganisms. *Escherichia coli* and coliform bacteria were not detected in many places, and it was not possible to compare the rate of increase/decrease unconditionally depending on the sampling date.

In this survey, there was no clear relationship between the increase/decrease in the number of microorganisms due to the increase in indoor temperature and humidity. However, the temperature and humidity were measured only a few times. In the future, it will be important to consider increasing the measurement date and time. In this study, we investigated the presence or absence of air conditioning, window ventilation, and floors carpeted with the same material, and obtained similar or different results between and within rooms. In the future, it will be necessary to investigate the pollution status of toilet floors and further consider it as a comparison factor when the floor material is investigated under the same conditions.

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Table 1 Sampling date and number of users in each information practice room

Sample number	Sampling date	Survey period	Number of users	
			I	II
1	July 27	June 28-July 26	405	560
2	August 24	July 27-August 23	234	323
3	September 27	August 24-September 26	183	73
4	October 26	September 27-October 25	258	388

Sampling was done in 2020.

The number of users was obtained from the usage book.

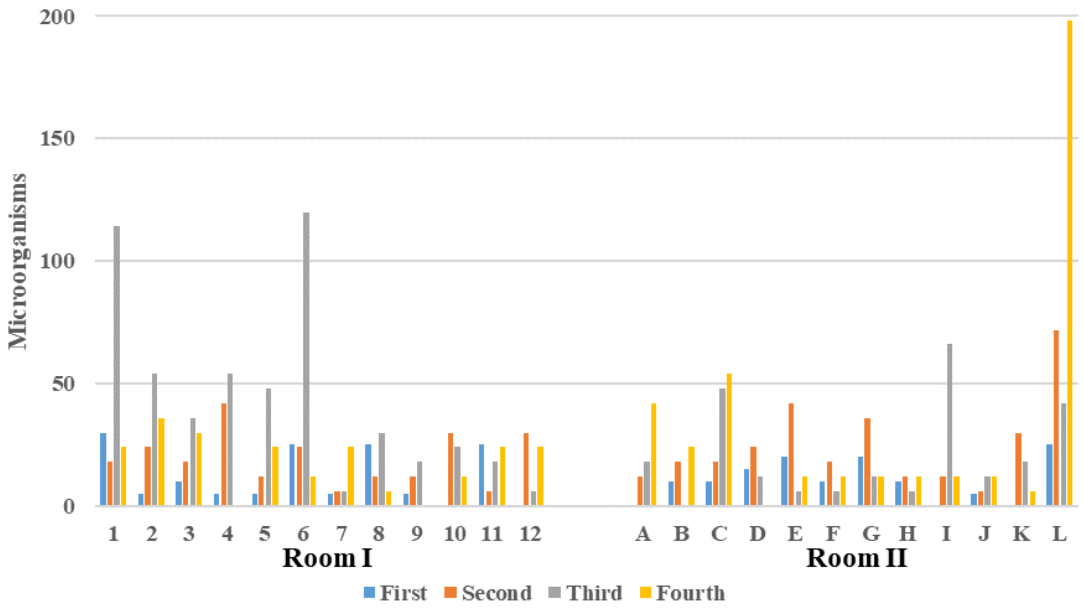

Fig. 1 Number of general live bacteria detected on each floor

Floor positions in the rooms: Forward, **1**-**5**/**A**-**E**; Rear, **6**-**12**/**F**-**K**; Window side, **1**,**6**, and **11**/**F**-**K**; Corridor side, **5**,**11**, and **12**/**A**-**E**; Near the door, **12**/**F**,**J**; Under the desk, **1**,**3**,**5**,**6**,**8**,**10**, and **11**/**A**,**C**,**E**,**F**,**H**,**J**, and **K**; Aisle, **2**,**4**,**7**, and **9**/**B**,**D**,**G**, and **I**.

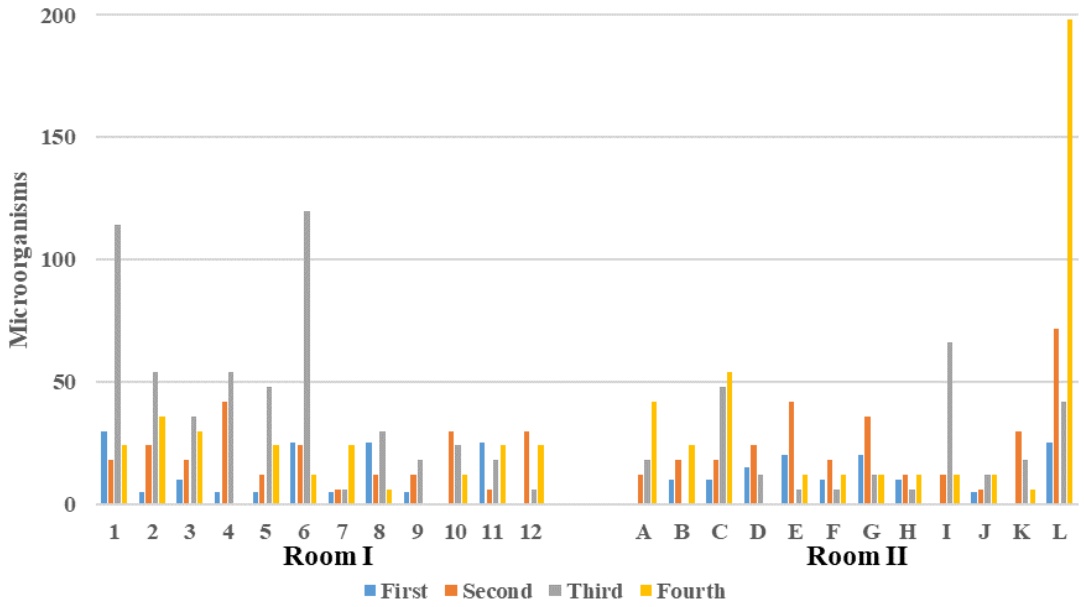

Fig. 2 Number of fungi detected on each floor

The positions of the floor in the rooms were the same as those in Fig. 1.

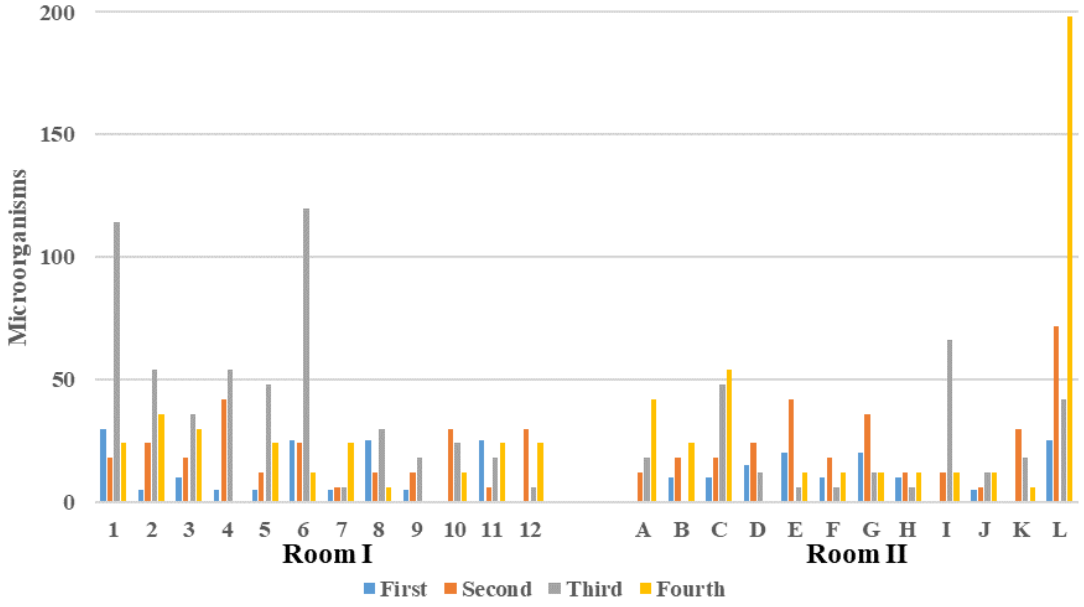

Fig. 3 Number of *S. aureus* detected on each floor

The positions of the floor in the rooms were the same as those in Fig. 1.

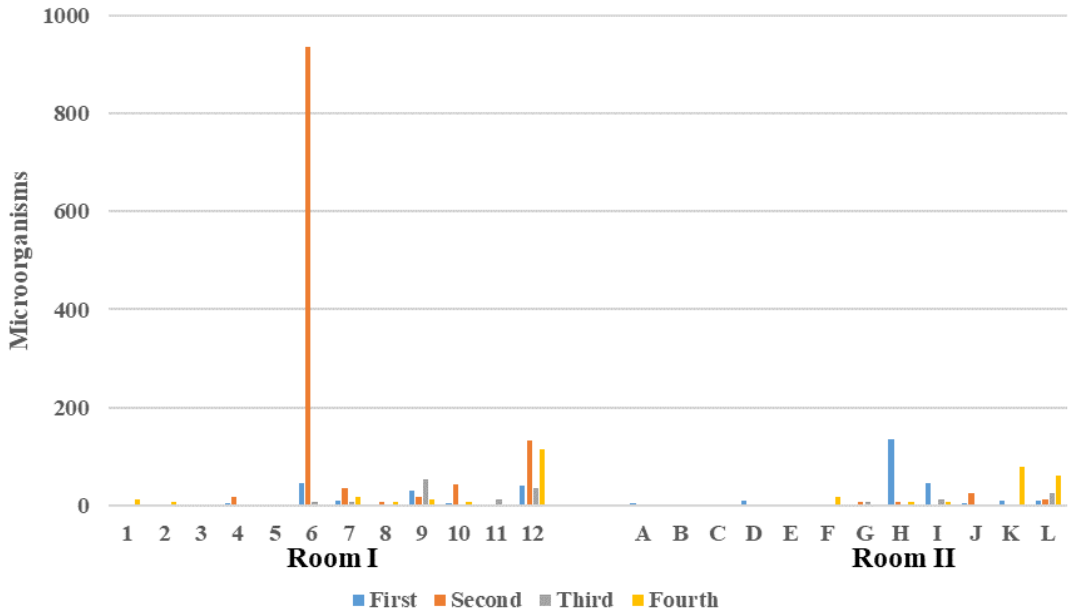

Fig. 4 Number of *E. coli* detected on each floor

The positions of the floor in the rooms were the same as those in Fig. 1.

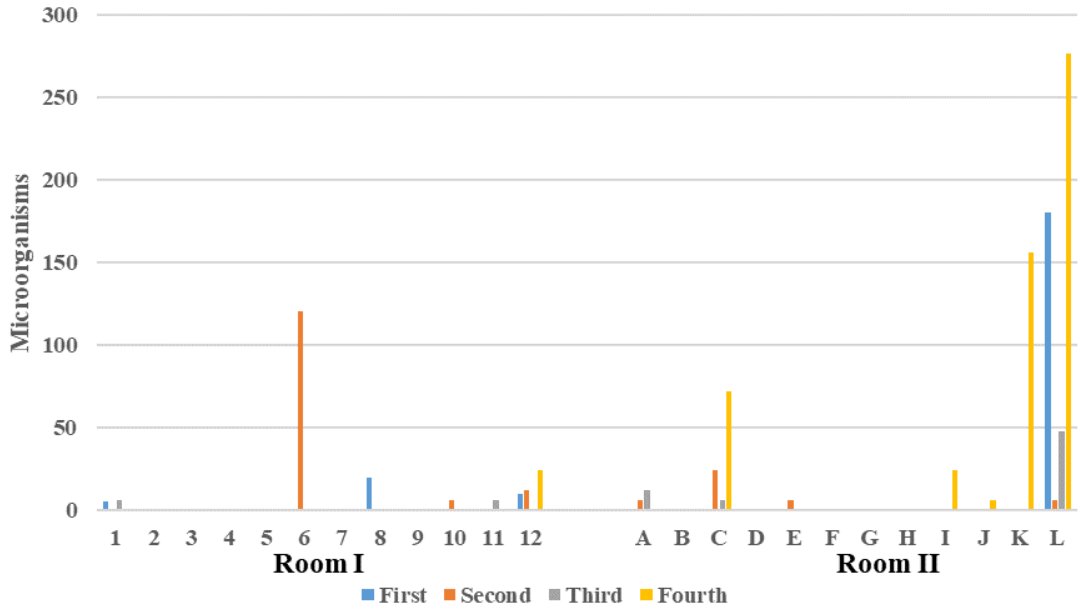

Fig. 5 Number of coliforms detected on each floor

The positions of the floor in the rooms were the same as those in Fig. 1.