TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Comparing the efficiencies of different commercial cleaning products in reducing the growth of bacterial flora within the school environment.

Biology Extended Essay

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Abstract

The aims of this extended essay are to identify what types of bacteria are present at school environment and what type of cleaning product is the most effective at decreasing their growth when the surfaces are cleaned manually. In other words, bactericidal properties of various cleaning agents are compared within the scope of this study.

My research question is: “Is there a statistically significant mean difference between the selected cleaning products, which are named as soft soap, multi-purpose cleaner 1 and 2, alkaline disinfectant and alkaline surface cleaner, in terms of their bactericidal effects on bacterial flora found in school environment?”

It was hypothesized that; there would be a significant mean difference in terms of bactericidal effect in between the cleaning agents used. Alkaline disinfectant would be the most effective, followed by the other alkaline products (alkaline surface cleaner and soft soap), and then the neutral products (multi-purpose cleaners).

In order to test the hypothesis, bacterial samples were collected from each student’s desk before and after wiping with each product. According to the variety and amount of bacteria that grow after overnight incubation, types of bacteria present, and bactericidal actions of the products were determined. Amount of bacteria was reported in colony forming units per milliliter, and the units were counted by microbiologists.

Resultantly, alkaline surface cleaner reduced the largest number of colony forming units, followed by alkaline disinfectant, soft soap and multi-purpose cleaners respectively. Cheaper multi-purpose cleaner reduced more units than the expensive one, so it was concluded that the expensive was not worth its price. ANOVA results revealed that there was a significant mean difference between the agents in terms of their bactericidal effects, with the alkaline surface cleaner being the most effective, followed by alkaline disinfectant, soft soap and multi-purpose cleaners.

Word Count: 294

Abbreviations

SS: Soft Soap (ABC®)

MPC-1: Multi- purpose Cleaner 1 (Ajax Fabuloso®)

MPC-2: Multi- purpose Cleaner 2 (Amway L.O.C. ®)

AD: Alkaline Disinfectant (Surfanios ®)

ASC: Alkaline Surface Cleaner (Kiehl Dipex®)

MHB: Mueller Hinton Broth

EMB: Eosin Methylene Blue

CFU: Colony forming unit

cfu / mL: Colony forming units per milliliter

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I. Introduction

Increasing contamination in schools worldwide is an undeniable fact of our century. There are several reasons for this inconvenience. One of the main reasons is the increasing number of students. As cities grow, number of children that attend schools increase, thus number of student per classroom augment and result in contamination of the classrooms. Another factor is the quality of public education. As it hasn't improved over time as much as it had to, behavioral changes among public arose resulting in poor personal hygiene such as poor hand-washing. Besides, public began to give less importance to infections, and elimination of microbes, so contamination increased not only in schools but also in other public places (Lederberg, Shope, Oaks, 1992, p. 14). Another important reason for contamination is the worsening physical and hygienic conditions that the schools provide. As global economic crisis influences the whole world, schools are forced to decrease their expenses, resulting in dismissal of janitors and obtainment of low quality cleaning products. In other words, as low quality products are used and classrooms are not cleaned daily, contamination increases.

Contamination in schools plays a major role in transmission of infections and hence causes children to catch various diseases including gastrointestinal and skin diseases. They are mainly due to increasing number of possible human pathogens as a result of contamination. Therefore, schools need to prefer cleaning products that are effective at elimination of potential pathogens.

Cleaning agents, or cleaning products, are classified as acidic, alkaline, neutral and degreasers in general. Acidic products contain strong mineral acids or chelants, while alkalines contain strong bases as active ingredients. On the other hand, neutral products contain anionic surfactants, whereas degreasers contain solvent-containing surfactants. (1)

There are lots of ongoing debates over what kind of cleaning products should be used at schools. According to Bog Standard Campaign (a campaign to promote better toilets for pupils in United Kingdom) Guidelines, general environmental surfaces need regular manual cleaning with general purpose detergent and hot water. Besides, disinfectants or sodium hypochlorite may be used in case of an infection risk and must be rinsed after use. It is also noted that disinfectants will not be effective on dirty surfaces. (2)

1. "Cleaning agent." Wikipedia, The Free Encyclopedia. Retrieved on 13 October 2010 at 07:59. <http://en.wikipedia.org/wiki/Cleaning_agent>
2. "Bog Standard Campaign. " Campaign Guidelines. <http://www.bog-standard.org/guidance_whole_school.PDF>

Moreover, sodium hypochlorite, which contains hypochlorous acid, or similar acidic cleaning products are not recommended to use at schools or other public places by Hacettepe University Public Health Unit, Ankara as they may be harmful for public health and cause serious skin diseases or allergic reactions in pupils. Besides, "Sustainable Procurement Guidelines for Cleaning Products and Services" Background Report submitted by United Nations Environmental Program (UNEP) states that chlorine based disinfectants, such as sodium hypochlorite, have been found to have many health and environmental impacts. It is clearly stated that the main problem about bleach is the accidental mixing of it with other cleaners. If bleach reacts with particular ingredients of these chemicals, it may give off toxic gases. If these gases are inhaled, they might cause serious health problems [(3)]. Based on all this information, chlorine-based disinfectants, or acidic products in general- are not recommended for use at public places. To sum up, there are many opinions about how schools should be cleaned, but no worldwide standard regarding these procedures.

Based on this fact, the main focus of this study will be trying to find out what type of cleaning products should be used in schools, as there is no current study concerning this issue. Therefore, the topic of this research is *"Comparing the efficiencies of different commercial cleaning products in reducing the growth of bacterial flora within the school environment".* The fact that the study will focus on daily manual cleaning in classrooms should be taken into consideration while reading this paper. In order to test the efficiency of each cleaner when the surfaces are cleaned manually, samples will be collected from each student's desk before and after the desk is wiped with each product. According to the variety and amount of bacteria that grow after overnight incubation, bactericidal actions of the products will be determined. Therefore, no bacteria from a stock culture will be used during this experiment. In other words, this investigation attempts to examine real life situations, not the conditions in laboratory setting.

The term "bacterial flora"- aforementioned above- includes both pathogenic and non-pathogenic species of school environment. According to a research -by microbiologists from Amravati University in 2009- , the most infectious bacteria species that can be found in school environment are *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteusmirabilis*, *Citrobacter freundii* and *Enterobacter aerogenes* (Tambekar, Shirsat, Kakde, Ambekar, 2009).

3. "Sustainable Procurement Guidelines for Cleaning Products and Services Report" (submitted by UNEP) <www.pnuma.org/.../UNEP%20Purchasing%20criteria%20-%20cleaning%20-%20Background%20Doc.pdf>

Moreover, according to another research -by Illinois Department of Health-, in recent years number of infections caused by Methicillin-resistant *Staphylococcus aureus*, which is known as a non-pathogenic type of bacteria, has increased [(4)]. Therefore, it is expected that some of those bacterial species will grow from the samples collected from school during the investigation.

Please note that while carrying out the experiment, collected bacterial samples will be cultivated in laboratory. The purposes of bacterial cultivation are to grow and isolate all bacteria present, and to obtain sufficient growth of relevant bacteria to allow identification and characterization. After the bacteria are cultivated, I expect to see the formation of macroscopically visible growths of microorganisms on petri dishes. These formations are called “colonies” (Pelczar, Chan, Krieg, 1986, p. 883). Moreover, aggregate of cells which gives rise to a single colony in the plate is called “colony forming unit” (Pelczar, Chan, Krieg, 1986, p. 883). Besides, each type of bacteria has its own colony characteristics when it is cultivated in the appropriate medium in terms of size, margin, surface texture, elevation, and colour. In other words, different species of bacteria growing on the same medium may appear quite differently. So, these cultural characteristics are useful for identification of bacterial species. (Pelczar, Chan, Krieg, 1986, Chapter 8)

Last but not least, other than testing the antibacterial abilities of selected products, this study will try to find a type of cleaner that is also affordable. That’s why I am going to include both expensive and cheap cleaning agents in my study. However, it is better to bear in mind that main aim is still to find the type of product that provides the healthiest environment; being affordable is the secondary focus of this study.

4. IDPH Guidance for Schools: Students and Community Associated Staphylococcus Aureus (CA-MRSA) Infections, January 2009, <www.ipdh.state.il.us>

II. Hypothesis

The cleaning products whose effects will be observed throughout the study are,

- Amway L.O.C.®(Legacy of Clean) Multi-purpose cleaner
- Surfanios ® Alkaline disinfectant
- ABC® Soft soap
- Ajax Fabuloso® Multi-purpose cleaner
- Kiehl Dipex® Alkaline surface cleaner

As stated before, cleaning products can be classified as acidic, alkaline, neutral or degreasers. Among the products above, multi-purpose cleaners that many people prefer to use at their home are pH-neutral, thus can be described as neutral cleaning products. They contain anionic surfactants that are highly antibacterial and also, various acids or alkalis in order to maintain a pH around 7.

On the other hand, the three other products above are referred as alkaline products. Alkaline disinfectant, soft soap and alkaline surface cleaner are being marketed with a pH range of 8-12. They mainly contain alkalis as their active ingredients. In addition, they play an important role in cleaning and disinfection of various surfaces, together with acidic products [(5)]. As acidic products have some negative health impacts as stated earlier, alkaline cleaners are preferred by a majority of people nowadays. Based on this information, alkaline products are expected to be more efficient in inhibiting bacterial growth than neutral multi-purpose cleaners.

Types of bacteria that I expect to grow within the school environment, can grow optimally between pH range 6.7 and 7.5 .[(6)] Therefore, it can be said that the alkaline products used in this study are expected to provide a better inhibitory action against bacteria, since they provide a higher pH than the upper limit of the optimum range for bacteria.

In light of the information above, the hypothesis of this experiment is;" There will be a significant mean difference in terms of bactericidal effect in between the cleaning agents used". The alkaline disinfectant is expected to be the most effective, followed by other alkaline products and then, neutral multi-purpose cleaners.

5. "Cleaning agent." Wikipedia, The Free Encyclopedia. Retrieved on 13 October 2010 at 07:59. <http://en.wikipedia.org/wiki/Cleaning_agent>
6. "Bacteria". < http://science.jrank.org/pages/714/Bacteria.html>

III. Method Development and Planning

The primary aim of this study is to find out what type of cleaning products should be used in schools in order to provide students with the healthiest environment. During the time students spend at schools, they interact with many surfaces. In this investigation, it was decided to collect samples from students' desks as they are the surfaces that students interact mostly. The reason why only one type of surface was chosen was that by this way, we would be able to limit the extent of bacteria species as similar types of bacteria would proliferate in similar surfaces. I have learned that in many schools, desks are cleaned manually by janitors at the end of every school day. Therefore, I had to examine how well products work when the surfaces are cleaned manually. Hence, a method that is fully performed in laboratory conditions wouldn't be suitable since it wouldn't mimic real life situations. So, I decided to develop a method that was capable of both testing manual cleaning and comparing the efficiencies of different cleaning products.

As a school environment, I selected TED Ankara College High School since it is the school that I am attending to. I learned that Kiehl Dipex® Alkaline Surface Cleaner was used to wipe student desks. Therefore, Dipex was determined to be one of the test products for this investigation.

I wanted to compare different types of cleaners since my aim was to determine a type of cleaner that every school could use. However, after talking to Hacettepe University Public Health Unit, I learned that acidic products were not recommended to use in schools. I also found out that UNEP state that acidic products would cause serious diseases in pupils. Therefore, I decided not to include an acidic product among selected products for this research.

I learned that in many hospitals in Ankara, an alkaline disinfectant called Surfanios® was preferred for surface cleaning due to its microbiological qualities. The only problem about it is the fact that it is not easy to access and is rather expensive. However, this makes it convenient for this study since the secondary aim of the study was to find out whether the products were worth their price or not. Therefore, I decided to use this disinfectant as the second material.

I wanted to include another alkaline product in the experiment so that I would be able to compare the alkalis among themselves. I think that one of the traditional cleaning materials in Turkey, called "soft soap" could be suitable for this purpose since it contains potassium hydroxide –a strong base- in its composition. In addition, it was cheaper. Therefore, I selected it as my third material.

In modern houses, commercial multi-purpose cleaners are preferred for surface cleaning as they are cheaper and more accessible. They are marketed as neutral, so they don't cause health problems. Therefore, I added two different multi-purpose cleaners to the materials from different price ranges, so I could also find out whether they are worth the price or not. An expensive one- Amway L.O.C.® and an inexpensive one- Ajax Fabuloso® were chosen.

After all cleaning products were selected; I had to develop a method that could compare their bactericidal abilities. I decided first to collect samples from a classroom at the end of a school day, before and after cleaning the desks with each solution that is diluted according to its manufacturer's instructions. Then, with the help of my mother, I would transport the samples to laboratory.

After overnight incubation in the laboratory, number of colony forming units (CFU) in each sample would be determined. Since bacteria tend to aggregate, they can't be counted individually. That's why the growth of bacteria would be reported in cfu/mL (Pelczar, Chan, Krieg, 1986, p. 127). After the number of CFUs that grow from the samples is reported, the reduction in CFUs after the desk is wiped with a cleaning agent would be determined by writing all data in their base 10 logarithm values and subtracting the final value (after wipe) from the initial (before wipe). It is accepted that a fine disinfectant should be capable of at least 5-$\log_{10}$ reduction of bacteria (Mazzola, Penna, Martins, 2003). Obtained results would be evaluated accordingly.

Even though this method carries the risk of technical defects, it was seen as the best way to mimic real life situations since the main aim of my study is to find out which product is best to use at schools where manual cleaning is being applied. Therefore, rather than being efficient in laboratory settings, it is important for a cleaning product to be efficient in real life situations within the scope of this study.

Materials Used in the Experiment:

- Amway L.O.C.®(Legacy of Clean) MPC (2)
- Surfanios ® AD
- ABC® SS
- Ajax Fabuloso® MPC (1)
- Kiehl Dipex® ASC
- 6 X 10 test tube containing MHB
- 6 X 10 sterile cotton tip swab
- Parafilm
- Herous Incubator
- 6 X 10 Petri dishes with 5 % Sheep Blood Agar (Oxoid,UK)
- 6 X 10 Petri dishes with EMB Agar (Oxoid, UK)
- Bacteriologic Loop
- Gram stain
- Light microscope (Olympus, Japan)
- Micro- pipette(100 µL)
- Bunsen burner
- Gloves
- Lab coat
- Goggles
- A piece of cloth
- 10 student desks
- Ruler

IV. Method

Mask and gloves must be worn during the procedure at all times to minimize the effect of external contamination to the samples and agar plates.

Procedure:

A. Sample Collection

1) Solutions of the cleaning agents are diluted according to manufacturer's instructions.
2) Basal samples from ten desks are collected by sterile cotton-tip swabs before cleaning. Collected samples are put into tubes that contain 10 mL of Mueller Hinton Broth (MHB) to transfer them safely to the laboratory. Mouths of the tubes are covered with parafilm to minimize the effect of external contamination.
3) All desks are divided into five equal sections. Each section of a desk is wiped with ASC, AD, MPC-2, MPC-1 and SS respectively.
4) After 1 minute of drying period, samples are collected from each section by cotton-tip swabs and put into MHB containing tubes that are numerated accordingly. Each tube is again covered with parafilm. I paid attention to collect the samples homogeneously from each section.
5) All tubes are transferred to the laboratory.

B. Cultivation and Incubation of Samples

1) All tubes are left for overnight incubation in the incubator at37 °C.
2) 10 µL of incubated MHB, are taken from each tube by using micro-pipette. This inoculum is spreaded over agar surfaces by using the bacteriological loop. Each time the loop is used, it is flamed for sterilization before streaking. (For details, see Appendix 2.) This step is repeated for both 5 % sheep blood agar and eosin methylene blue agar (EMB).
3) All agars are again left for overnight incubation in the incubator at 37 °C.

C. Isolation of Microorganisms

After overnight incubation, colonies on each agar plate are counted under supervision of an experienced microbiologist and multiplied by 100. The colonies on blood agars are reported as colony forming unit per mL (cfu/mL). It was noted that there was no growth on EMB agars, so no further examination involving those agars was made.

D. Identification of Microorganisms

1) For identification of the grown bacteria; typical colony appearance is important. Also Gram staining, catalase and coagulase tests are performed. Few 4-5 colonies with typical appearance were taken, gram-stained (see Appendix 3) and examined under light microscope. They are identified as either gram-positive cocci or gram-positive bacilli.
2) The typical colonies (unpigmented, smooth and slightly raised) that were gram-positive cocci, catalase positive and coagulase negative were identified as *coagulase negative Staphlylococcus* (CNS).
3) The typical colonies (unpigmented, smooth but larger than CNS) that were gram-positive cocci, catalase positive and coagulase negative were identified as Micrococcus spp.
4) The typical colonies (irregular, flat) that were gram-positive bacilli, catalase positive and coagulase negative were identified as Bacillus spp.

Please note that tests done for identification of microorganisms were only applied on the colonies obtained from basal samples during the investigation.

I. Results

Results obtained from the experiment are displayed in Tables 1 and 2 below.

Table 1: Raw results obtained from the experiment, showing the number of colony forming units for each desk before and after wipe with five different cleaning agents named as SS, MPC-1, MPC-2, AD and ASC.

Desk Number	Colony Forming Units (cfu/mL)					
	Before Wipe	After Wipe				
		SS	MPC-1	MPC-2	AD	ASC
1	10^8	10^4	10^4	10^3	10^3	10^3
2	10^8	10^5	10^7	10^6	10^3	10^3
3	10^{10}	10^7	(-)	10^5	10^3	10^3
4	10^8	(-)	10^{10}	10^7	10^4	10^4
5	10^{10}	10^4	10^3	10^2	10^3	(-)
6	10^{10}	10^4	10^8	10^6	10^5	(-)
7	10^{10}	10^8	10^4	10^7	(-)	(-)
8	10^{10}	10^8	10^6	10^6	10^3	10^4
9	10^7	10^7	10^7	10^7	10^4	10^4
10	10^7	10^5	10^5	10^5	10^4	10^4

Table 2: Types of bacteria that have grown from the samples taken from each desk

Desk Number	Types of Bacteria
1	Bacillus, CNS
2	CNS
3	Bacillus, CNS
4	Bacillus, CNS
5	CNS
6	CNS
7	CNS

8	Bacillus, CNS
9	Bacillus, CNS, Micrococci
10	Bacillus, CNS, Micrococci

CNS: Coagulase (-) staphylococci

II. Data Analysis

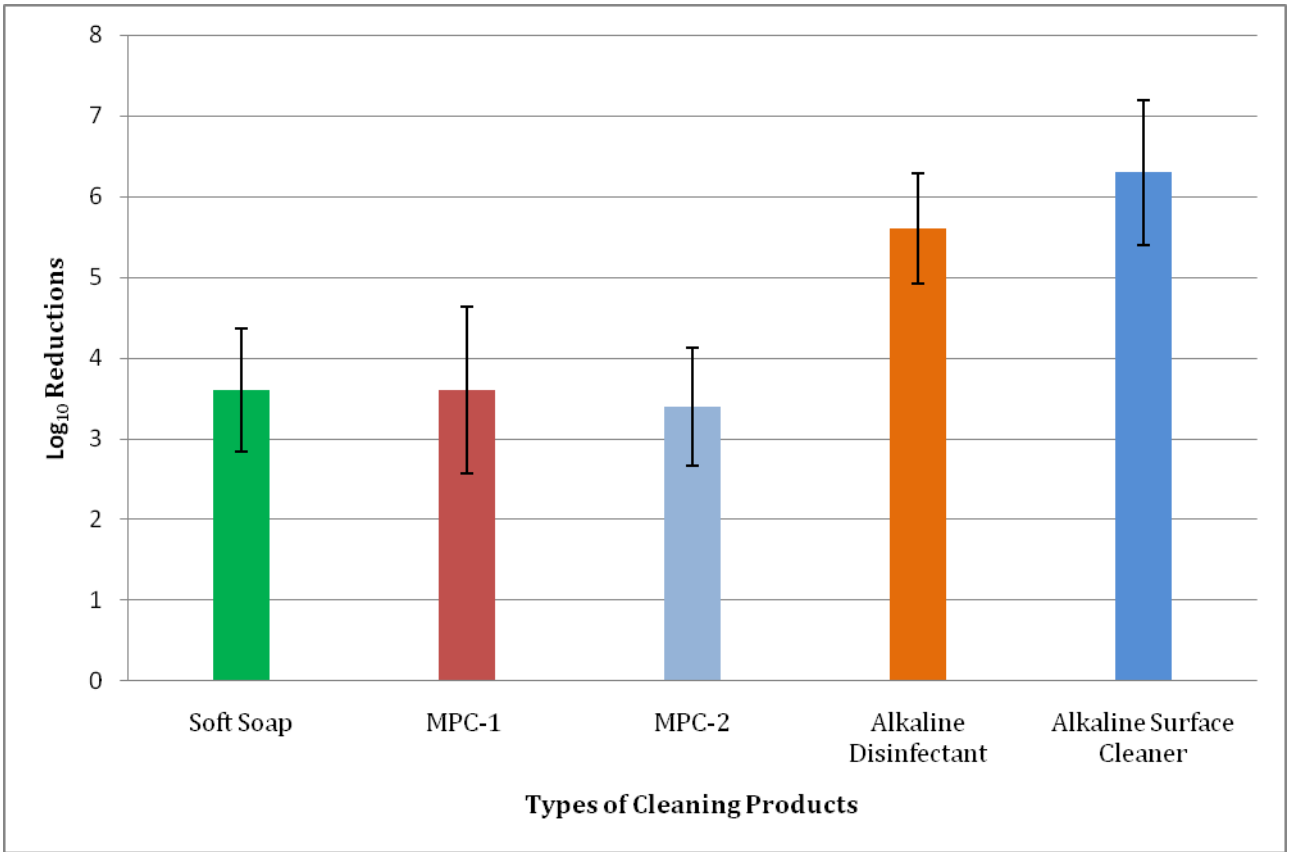
Analysis of data displayed in Table 1 is shown in Tables 3-4-5-6 below. In order to analyze the data easily, all values were written as base 10 logarithm values and further calculations were done accordingly.

Table 3: Each product's $\log_{10}$ reductions of identified colony forming units deployed on each desk. Log_{10} reduction means the subtraction of final colony forming unit numbers (written as $\log_{10}$) from their initial values (also written as $\log_{10}$).

Agent / Desk Number	Log_{10} Reductions				
	SS	**MPC-1**	**MPC-2**	**AD**	**ASC**
1	4	4	5	5	5
2	3	1	2	5	5
3	3	10	5	7	7
4	8	0	1	4	4
5	6	7	8	7	10
6	6	2	4	5	10
7	2	6	3	10	10
8	2	4	4	7	6
9	0	0	0	3	3
10	2	2	2	3	3

Table 4: Relevant Descriptive Statistics (Average values, Standard error, Standard deviation and Confidence interval) for log_{10} reductions of each cleaning product

	Soft Soap	MPC - 1	MPC-2	Alkaline Disinfectant	Alkaline Surface Cleaner
Mean	3.600	3.600	3.400	5.600	6.300
Standard Error	0.763	1.035	0.733	0.686	0.895
Standard Deviation	2.413	3.273	2.319	2.171	2.830
Confidence Interval (95.0 %)	1.726	2.341	1.659	1.553	2.025



Graph 1: The comparison of bactericidal effects of products against environmental bacteria in terms of log_{10} reduction. The higher the log_{10} reduction, the more effective is the product. Error bars were drawn according to standard errors for each product displayed in Table 4.

Table 5: Single Factor Analysis of Variance (ANOVA) Test Results for all Groups

Source of Variance	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	72.8	4	18.20	2.6275	0.046763	2.578739
Within Groups	311.7	45	6.927			
Total	384.5	49				

Table 6: p-values (two tail) obtained from independent t-test comparing the difference between two observed means (statistically significant results / $p < \alpha = 0.05$ are written bold)

	MPC- 1	**MPC-2**	**Alkaline Disinfectant**	**Alkaline Surface Cleaner**
Alkaline Disinfectant				0.257
MPC-2			**0.012**	**0.002**
MPC-1		0.775	**0.021**	**0.015**
Soft Soap	1.000	0.823	0.088	**0.022**

III. Evaluation

The research question of this investigation was; “Is there a statistically significant mean difference between selected cleaning products in terms of their bactericidal effects on bacterial flora found in school environment?” Based on this research question, aims of the study were to identify what types of bacteria were present at school environment and what type of cleaning product would be the most effective at decreasing their growth. It was hypothesized that there would be a significant mean difference between the cleaning products in terms of their bactericidal activities. Among the selected products; AD was expected to be the most effective, pursued by other alkaline products and finally, neutral products.

During the study, instead of the types of bacteria species that were mentioned earlier; *coagulase negative Staphylococcus*, Bacillus and Micrococcus species were identified. These species are also abundant in school environment and although they are not pathogenic, they may cause serious diseases in easily-irritable pupils. The most probable reasons why we haven’t spotted one of the most infectious species in this study might be the facts that the classroom from which the samples were taken is cleaned daily and students obey the common hygiene rules.

As it can be seen in Graph-1, ASC was found to have the greatest bactericidal effect by reducing bacteria with an average value of $\log_{10}$ 6.3. AD was the second effective; it reduced the bacteria by $\log_{10}$ 5.6 in average. Accordingly, MPC-1 and SS showed equal bactericidal effect in average, they both reduced the bacteria by $\log_{10}$ 3.6. Finally, MPC-2 was found to have the least bactericidal effect, as it reduced the bacteria only by $\log_{10}$ 3.4 in average.

Looking at Table 4, it can be said that standard errors are relatively small when compared to mean values. The biggest error was made while testing MPC-1 (1.035). Despite these small errors, standard deviations are large when compared to mean values. Again, the largest deviation is in MPC-1’s results. These large values indicate that the data is distributed heterogeneously for each experimental group, even though it should have been distributed

homogeneously. 95 % confidence interval values are also large. In brief, all these descriptive statistics show that the data obtained from the investigation is not 100 % reliable.

In order to understand whether the results are statistically significant, a number of statistical analyses were done. The first one was ANOVA-Single Factor test. The null hypothesis was that there would be no significant mean difference between the selected cleaning products in terms of their $\log_{10}$ reductions of identified colony forming units. After ANOVA Test was done between groups, p-value was found to be 0.046763 which is a smaller value than α(=0.05). Therefore, the null hypothesis was rejected, and alternative hypothesis suggesting there would be a significant mean difference between groups was supported. In addition, the groups were also analyzed by independent t-tests comparing the differences between two observed means. Those tests revealed that there was a significant mean difference between some of the groups as their p-values were smaller than α, whereas there was no significant mean difference between other groups. Significant results were shown in bold numbers in Table 6.

In light of these analyses, the main hypothesis was partially supported. Even though there was a significant mean difference between the cleaning agents in terms of their bactericidal effects, a different ranking among them was expected. It was expected that AD would be the most effective, followed by other alkaline products and then, neutral products. However, this expectation was rejected since ASC was the most effective followed by other alkaline products and the neutral products.

Also stated above, AD was expected to show a more effective bactericidal action. However, ASC, whose abilities were obviously underestimated, showed the best action. This can result from many reasons, but the most logical explanation would be the possibility that ASC is more effective against non-pathogenic bacteria that mostly exists on surfaces, whereas AD might be effective against pathogenic bacteria, as it is a disinfectant that is not highly recommended for use in schools except for emergency. Another point that was not expected before the method was conducted is SS's relatively low bactericidal action when compared to other alkaline products. Since it contains potassium hydroxide- a very strong base- it was expected to be more efficient, but it showed almost the same action with neutral products. Depending on this result, it can be concluded that traditional methods may not be as good as modern technology's methods are.

Even though the hypothesis is partially supported and thus the method can be considered as a logical one, there are some remaining errors that need to be reduced. For instance; looking at Graph 1 error bars, it can be seen that the highest errors were made

while testing ASC and MPC-1. In order to reduce those errors, the method may be revised by following improvements:

- The samples were collected only from student desks in order to limit the extent of bacteria species. However, none of the most infectious bacteria species grew from our samples and hence we dealt with non-pathogenic bacteria that carry a low risk of infections. If pathogenic species had grown, we would have obtained a more valid result since they are the main cause of infections. In order to increase the possibility of pathogenic bacteria growth, samples might be collected from a different section of school such as the cafeteria, the computer lab or toilets where contamination level is expected to be the highest; if the method is repeated. Or, pathogenic bacteria from stock culture could be used; however that kind of an investigation wouldn't mimic real life situations.
- While collecting the samples, the sample that indicates the initial situation of the desk was taken first and then all desks were divided into five sections and further samples were collected after wiping each section with a different product. In this case, we assumed that bacteria were distributed homogeneously at all sides of the desk. This may have caused an error, since bacteria species would most probably exist in a denser way at the middle of the desk than they do at the sides. In order to eliminate this, desks can be divided into sections first and by increasing the number of swabs, initial samples may be taken from each section instead of taking only one initial sample from the whole desk. By this way, there will be an initial value for each trial with each product and the following calculations, such as $\log_{10}$ reductions, will be more precise.
- As stated above, each desk was divided into five sections and each section was cleaned with a different agent. Since the desks are not so wide, each agent cleaned a small surface; so the agents might have been more efficient than normal since surface area was less than the area that these agents normally clean. In order to eliminate this problem, the number of agents that are being tested can be fewer, or the experiment might be conducted on a wider surface than a student desk, such as a laboratory bench.

IV. Conclusion

As stated earlier, a disinfectant should be capable of at least 5-$\log_{10}$ reduction of bacteria, in order to be accepted as an effective one. In our study, only ASC and AD exceeded the criteria. So, it can be concluded that alkaline products are more suitable for use at surface cleaning in schools. If we are to specify which exact cleaning product that was tested in this experiment is the most suitable to be used in schools, we can say that ASC is the most convenient. Although AD showed a higher effect, it seems to me that it cannot be afforded by many schools since it is rather expensive when compared to ASC. As stated previously, the secondary aim of the investigation was to determine a cleaning product that could be affordable by many schools. Since alkaline surface cleaner is both effective against bacteria and affordable, it is the most convenient cleaning product for use in schools within the scope of this study.

To sum up, the reason why I chose this topic for my extended essay was to evaluate the hygienic conditions in schools and find what type of cleaning product provides the best cleaning for them. As my aim is fulfilled and my research question is answered, I consider the overall study successful. However, it seems to me that no matter how much research is done on this topic, the debates over what kind of cleaner should be used at schools or any other public place will last as long as technology renews itself and new products with better qualifications are produced and marketed.

V. Appendices

A. Appendix 1

The following is a brief explanation for laboratory cultivation (taken from Bailey and Scott's Diagnostic Microbiology, 11th Edition) and information on nutrient media used in the experiment (formulas are taken from "Oxoid" Website):

"Cultivation is the process of growing microorganisms in culture by taking bacteria from the infection site by some means of specimen collection and growing them in the artificial environment of the laboratory. The successful transfer of samples from the site that they are collected to the laboratory requires the nutritional and environmental growth needs of bacteria. Bacteria have several nutritional needs including different gases, water, various ions, nitrogen, sources for carbon and energy. In the laboratory, nutrients can be incorporated into an either solid or liquid media on or in which bacteria are grown. Media can be categorized according to their function and use as enrichment, supportive, selective and differential.

The solid media is called "agar", which contains agarose, nutrients and water. On the other hand, the broth in which nutrients are dissolved in water is the liquid media."

Mueller Hinton Broth:

It is a nutrient media that supports the growth of many microorganisms.

Typical Formula*	**gm/litre**
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5
pH 7.3 ± 0.1 @ 25°C	

* Adjusted as required to meet performance standards

Directions

Place 21.0 g in 1 litre of distilled water mix to dissolve completly. Sterilise by autoclaving at 121°C for 15 minutes. Chill and adjust cation levels if necessary.

Sheep Blood Agar (Mueller Hinton Agar):

It is the most commonly used supportive and rich medium for diagnostic bacteriology since it allows many organisms to grow. Its main purposes are the cultivation of fastidious microorganisms and determination of hemolytic reaction. (Some bacteria produce extracellular enzymes that cause hemolysis of red blood cells in the agar, and this hemolytic reaction is observed.)

Typical Formula*	**gm/litre**
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0
pH 7.3 ± 0.1 @ 25°C	

*** Adjusted as required to meet performance standards**

Directions

Add 38 g to 1 litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes.

Eosin Methylene Blue Agar, Levine:

It is a selective medium that is mainly used for the isolation of enteric bacilli found in stool according to their lactose fermenting abilities.

Typical Formula*	**gm/litre**
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0
pH 6.8 ± 0.2	

* Adjusted as required to meet performance standards

Directions

Suspend 37.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 60°C and shake the medium in order to

oxidise the methylene blue (i.e. restore its blue colour) and to suspend the precipitate which is an essential part of the medium.

B. Appendix 2

The following is taken from Bailey and Scott's "Diagnostic Microbiology" Eleventh Edition (2002):

"The cultivation of bacteria from infections at various body sites is accomplished by inoculating processed specimens directly onto artificial media. To enhance the growth, isolation, and selection of etiologic agents, specimen inocula are usually spread over the surface of plates in a standard pattern (can be seen below):

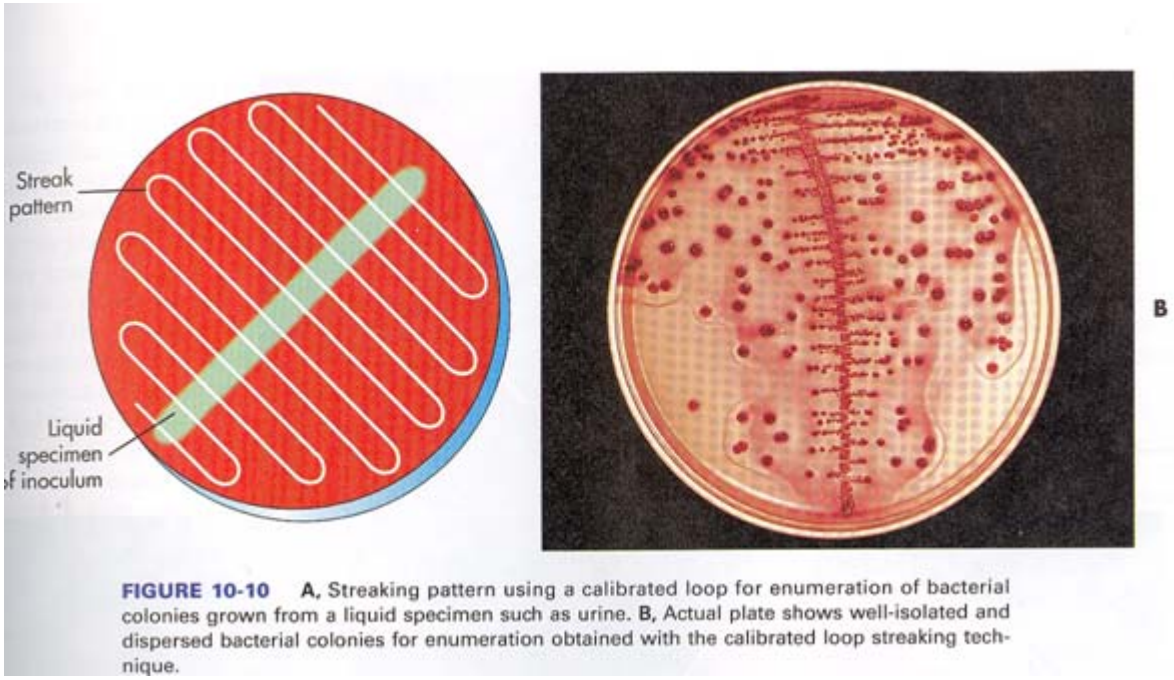


FIGURE 10-10 **A,** Streaking pattern using a calibrated loop for enumeration of bacterial colonies grown from a liquid specimen such as urine. **B,** Actual plate shows well-isolated and dispersed bacterial colonies for enumeration obtained with the calibrated loop streaking technique.

Streaking plates inoculated with a measured amount of specimen, such as when a calibrated loop is used to quantify colony forming units (CFUs) in urine cultures, is accomplished by spreading the inoculum evenly over the entire agar surface. This facilitates counting colonies by ensuring that individual bacterial cells will be well dispersed over the agar surface."

C. Appendix 3

The following is a brief explanation for use of Gram Staining taken from McGraw- Hill International Editions/ Microbiology Series.

"First devised by Hans Christian Gram, the purpose of Gram stain is to identify bacterial species under microscope. It divides the most bacterial species into gram- positive bacteria (those that take up crystal violet dye and stain purple) and gram- negative bacteria (those that allow the crystal violet dye to wash out and stain pink or red)

Procedure:

- Methanol Fixation: Slides are soaked into 95 % methanol in order to allow fixation of bacteria. Then, methanol is allowed to run off and the slides are air dried.
- Primary Stain with Crystal Violet
- Secondary Stain with Gram's Iodine
- Decolorization with Alcohol
- Counter- staining with safranin to stain the gram-negative bacteria pink or red.
- The slide is examined under microscope. Then, it is evaluated for colour, morphologies (cocci or bacilli) and the arrangements of the cells (chains, pairs etc.)."

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