IB BIOLOGY EXTENDED ESSAY

"INVESTIGATING THE CHANGE IN GRAM NEGATIVE AND POSITIVE BACTERIA NUMBER PRESENT ON TOP OF THE BEVERAGE CANS BEFORE AND AFTER CLEANING THE REGION AND COMPARING THE NUMBERS WITHIN THE CLEANING METHODS WHICH ARE CLEANING WITH TISSUE, WET WIPE, TAP WATER AND SOAPY WATER"

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ABSTRACT

Beverage cans encounter with external contacts which causes microorganisms including pathogenic bacteria dispersed. Found on top region of the cans, these bacteria increase the contamination risk of diseases to human beings. The purpose of this study is to compare the bacteria amount (CFU/ml) on top region of the beverage cans before and after cleaning the area with four methods (cleaning with tissue, wet wipe, tap water and soapy water). Additional aim is to investigate which cleaning method is most effective in reducing the bacteria amount (CFU/ml).

The research question of this study is: ‘‘Is there a significant difference on the amount of bacterial colonies of gram negative and positive bacteria found at the surface of the steel beverage cans before and after cleaning the top surface region with different methods as cleaning with tissue, wet wipes, distilled water and water with soap?’

Bacteria count from the samples is done by taking cultures from the surface, diluting and leading them to stay 24 hours at 37°C in petri dishes containing sheep blood agar in incubation. Then colony number count is done for five groups and used in data analysis.

Mean values are 280, 29, 8.8, 21.2 and 3.2 CFU/ml for the controlled and the cleaned groups (with tissue, wet wipe, tap and soapy water) respectively. ANOVA test supports the hypothesis that there is difference between the bacteria amount before and after cleaning the top region of the cans ($P=5.3825 \times 10^{-6}$). The second ANOVA test is done to see the difference between the cleaning methods. It shows the most effective method is cleaning with soapy water ($P=1.36 \times 10^{-4}$). The conclusion is that after cleaning the top region of the can there is a decrease in bacteria number and the most decrease is seen when it is cleaned with soapy water.

Word Count: 299

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INTRODUCTION

The first time I came across with the topic of this extended essay was when I heard on the news that a woman in Switzerland who drank directly from the beverage can while having a picnic near Genfer Lake lost her life. The autopsy revealed that the woman died of Leptospirosis Fulgurante which is a disease caused by an infection transmitted to humans by allowing water that has been contaminated by animal urine. After a short while, it was also heard that number of kids got seriously poisoned by Coca Cola cans in Belgium. The poisonous cans were recalled and import of Coke from Belgium was banned in France, Holland and Luxembourg. The producers of Coca Cola in Belgium declared that the events took place due to the contact of cans with a chemical used in wood and tree protection. Even though, these events were not proved and then called as rumours, it is an undeniable fact that the surface of the drinking cans are not clean and they are open for bacteria nests. So, adhesion of microorganisms to food processing equipment surfaces has become one of the serious concerns in food industry. Profesor Wolfgang Graninger from Vienna Clinic of Infections and Tropical Diseases expresses that the spread of Diarrhea and Hepatitis A from contaminated beverage cans is highly probable, especially in Southern countries.[1]

It was also found out by Centers for Disease Control and Prevention in the USA that 76 million people get sick, 300.000 are hospitalized and 5.000 die each year due to foodborne illnesses including the illnesses caused by the contact with the microorganisms at the surface of the cans.[2]

[1] http://www.mypac.de/fakten.php?cl=2#le

[2] http://www.cdc.gov/foodborneburden/index.html

Even though, the beverage cans have advantages such as providing a longer shelf life, keeping products tasting fresh and preventing external factor and organisms to affect the products and despite the fact that the technology is developing in this field, there are still some concerns and unavoidable cases that can lead several health and hygiene problems about these cans. During the processes of manufacturing, storage, shipping and putting up for sale, it occurs many unhygienic and unhealthy conditions such as contamination of hazardous bacteria and then diseases. When the free floating bacteria attach to a surface they form a biofilm by sticking together. Within this biofilm it becomes easier for them to reproduce and spread, which means it decreases the probability of the bacteria to swept away leading an increase in the risk of contamination of infectious diseases.

While researching this issue, I came across with several studies on profiles of the bacteria that are found on the surface of the beverage cans of the drinks. These researches focused especially on the kind of the bacteria and the colony numbers found at the top of the beverage cans. So, it is a fact that there are bacteria nesting and living on the surface of the cans such as E.coli and S.aerius, where our mouth is contacting with it. These bacteria can be carried to these surfaces by air of the production, transportation and marketing mediums, by various organisms such as vectors and animals or even by human.

The reason I chose this topic, the bacteria and dirt on the surfaces of the beverage cans, was that the issue is so popular and it is important to be cared because these cans are widely used all around the world and are part of our lives. So, we are contacting with these bacteria almost every day taking the risk of contamination of contagious diseases. Even though, this paper will focus on the amount of the bacteria found on the steel cans after

detecting the bacteria profile, it will also mention the precautions that can be taken with their effectiveness are also useful and easy to decrease the contamination risks of the bacteria.

I aimed to give and have a wider, detailed and scientific view and approach about this actual issue and to determine and find out the profile and especially the amount of the bacteria found on the top of the beverage cans. I also aimed to give several advice and suggestions in order to decrease the risk of the possible hygiene related diseases like applying some simple but significant methods on the surface of the beverage cans like cleaning with tissue, wet wipes, distilled water and water with soap. Therefore, this research will be dealing with the microorganisms found at the surface of the beverage cans and the significance and the effect of the cleaning methods with the light of the research question 'Is there a significant difference on the amount of bacterial colonies of gram negative and positive bacteria found at the surface of the steel beverage cans before and after cleaning the top surface region with different methods as cleaning with tissue, wet wipes, distilled water and water with soap?' This essay will also focus on the preparation process of the experiment, results gathered at the end of it and their validity and outcomes.

HYPOTHESIS

There is evidence that there exists a number of microorganisms on the surface of the beverage cans used in the daily life. It is also known that these bacteria are causing illnesses such as diarhea and hepatits A, and rarely death. However, researches and clinical tests till today are only profiling the number and type of these microorganisms, especially focusing on the bacteria, but not giving any solutions to overcome the problem or to state a difference between cleaning the top region of the cans or not. There is a fact that disinfectants and chemicals such as detergents used in houseworks and other areas have a decreasing effect on the amount of the bacteria and other microorganisms. Nevertheless, because it is hard to find hygiene agents like these every time in outdoors and the chemical effects of these agents are hazardous and toxic to human body, some other healthy, easy and cheap methods may be used. Cleaning the surface of the cans with tissue, wet wipe, water and water with soap can have a probable effect on decreasing the number of the bacteria colonies present in the top surfaces of these cans. Therefore, it can be hypothesized that there is a significant difference in the number of the colonies of bacteria present in the top region of the beverage can of coke before and after this region is cleaned with the 4 methods mentioned above. In addition to this hypothesis, the most effective method in reducing the amount of bacteria was thought to be the method where the top region was cleaned by water with soap.

METHOD DEVELOPMENT AND PLANNING

Designing an available method in order to support or reject the suggested hypothesis and answer the research question has aroused several problems. One of the problems was, because investigating the behaviour of the bacteria, such as their reproduction and dispersion, is hard to observe in school laboratory conditions, it was needed a hospital laboratory condition where the profiling of the bacteria can be done easily and conditions for inoculation and observation of the rate of reproduction and dispersion of the bacteria are more available.

In order to investigate the behaviour of the bacteria, the inoculation of the examples gathered from the top region of the beverage cans should have inoculated. So, another significant problem aroused from the determination of the technique that was going to be used because there are many types of inoculation changing from the bacteria type that is under investigation. With the light of the previous researches, it was found that the most common isolated microorganisms were Bacillus spp. (53.5%), Diphtheroid bacilli (41.7%), coagulase negative Staphylococcus (CNS) (35.4%), B.subtilis (30%), methicilline sensitive Staphylococcus aureus (MSSA) (21.2%), Mould spp. (16.5%), and Escherichia coli (7.%8)[3] which are gram positive and negative bacteria. After some research, it was found that inoculation to the sheep blood agar medium was the best way to investigate the functions of the sample of the gram negative and positive bacteria.

It was important to gather the samples of the bacteria from the top of the beverage cans from an equal region with same amounts. In order to carry out this, the top region of the

[3] Ayçiçek H,Küçükkaraarslan A., Microbial Profile of External Surfaces of Beverage Cans, *YYÜ. Vet. Fak. Derg. 2003, 14 (1):118-123*

beverage cans was contacted to sterilised nylon bag which contained 200 ml 0.9% Saline which is a sterile solution of sodium chloride that has a dilutive effect. After rubbing the top of the cans with the nylon bag, the samples of the bacteria were collected to nylon bag. With this method a homogeneous dispersion was formed. The following incubation processes were done from this nylon bag.

Conserving the controlling groups for 5 cans, after cleaning the top region of the rest 20 beverage cans with tissue, wet wipe, water and water with soap, the inoculation of the bacteria to the petri dishes from the nylon bag with the samples of the bacteria and saline took place. Then the kind of bacteria that reproduced was detected. It was found that the bacteria present on top of the cans were gram negative and positive bacteria. The time needed to dishes to stay in incubation was found to be 24 hours[4]. After leaving the petri dishes to incubation in laboratory for 24 hours, the data gathering process started. It was hard to count the number of the bacteria in petri dishes one by one. So, instead of the numbers of the bacteria individually, the colony number was detected according to the formula "Number/ml = CFU (colony forming unit) = (Colony number X Dilution Factor) / volume that was transferred from dilution tube to petri dish (ml)" for each petri dish.

Now it became important to make sure that all variables were being controlled. First, the 20 beverage cans were picked up randomly from one market. The size of the top region of the cans was the same of 23 cm^2. Then the optimum temperature for aerobic gram negative and positive bacteria to perform their behaviour without damaging the organisms was needed. After some research, the optimum temperature for gram negative and positive bacteria was

[4] "Counting Methods in Microbiology, Prof. Dr. Velittin Gürgün, Assoc. Prof. Dr. Kadir Halkman, The Association of Food Technology, vol.2, Ankara, 1990

determined to be as 37ºC[5] so the temperature should have been stabilized in this value. The thermometer in the incubation region was scaled to 37°C. Because they affect the behaviour of the bacteria, pH value, oxygen and carbon dioxide amounts and light intensity were kept constant in the incubation place by measuring them with appropriate probes in constant time intervals. Moreover, the type, amount and density of the Saline in nylon bags of 200 ml of 0.9% and, the petri dishes' area of 25 cm^2 containing sheep blood agar were also kept constant. The best time that should be given to bacteria for them to reproduce and spread and take the dishes out the incubation process was determined as 48 hours for each of the petri dish. The surface area of the beverage cans' tops were also the same of 23 cm^2 in order to keep the dispersion areas of the bacteria the same. The cleaning processes were also kept constant. The tissues that were used in the first stage of cleaning were from the same brand and with the same surface areas of 10cmx10cm were contacted to the top of the cans and pressured by the same person for 2 minutes. The same method was done by the same kind of wet wipes of the same sizes as 10cmx10cm that were changed after each cleaning. For the cleaning with water, for each can 100 ml of tap water was poured from 5 cm away from the top of the can and for the last cleaning type, that was water with soap, 100 ml of water that contained 25 g of same liquid soap was poured from the same distance to the top regions of each can. Each pouring process was done for 10 seconds.

[5] Fotadar U, Zaveloff P, Terracio L, "Growth of Escherichia coli at elevated temperatures". *J. Basic Microbiol.* **45** (5): 403–4, 2005

PHOTOGRAPHS

Photograph 1: One of the top of the beverage cans where the culture of bacteria were taken

Photograph 2: Example of a bacteria colony present in controlled groups in petri dish

Photograph 3: Example of a bacteria colony in petri dish after cleaning the top of the can with tissue

Photograph 4: Example of a bacteria colony present in petri dish after cleaning the top of the can with wet wipe

Photograph 5: Example of a bacteria colony present in petri dish after cleaning the top of the can with tap water

Photograph 6: Example of a bacteria colony present in petri dish after cleaning the top of the can with soapy water

METHOD

Materials and Apparatus

1. 25 identical beverage cans of coke
2. 6 petri dishes with sheep blooded agar Oxoid-CM0854
3. 25 graded pipes
4. Incubator arranged to 37°C
5. 25 sterilized nylon bags
6. 100 ml of 0.9% salin per bag
7. Binocular microscope (olympus CX21)
8. 5 tissues of 10cmx10cm
9. 5 wet wipes of 10cmx10cm (with pH of 5.5)
10. 1000 ml tap water with pH of 6.5
11. 125 ml liquid soap with pH of 5.5
12. pH meter
13. thermometer
14. stopwatch

15. ruler (30 cm)

16. etiquettes

17. graded beakers

18. stirring sticks

19. graded tube

25 beverage cans of coke were lined. The petri dishes were divided into 5 parts labelled by etiquettes. Starting from the controlled group, the beverage cans' tops were put in and rubbed for 2 minutes to the nylon bags containing 0.9%, 100 ml of Saline, which is a classic microbiological method. With the help of the graded pipe 1 ml from the nylon bag's content (saline and bacteria sample) was soaked and put in a graded tube. 9 ml of Saline was added to this tube and by this the dilution of the sample was gained (dilution is needed to fit in the samples of bacteria to the petri dishes). The diluted solution then was inoculated to the petri dish's first division containing sheep blood agar. The same method was repeated for the rest 4 cans of the controlled group inoculated to the following division parts. Taking the second 5 beverage can group a tissue with size 10x10 was contacted and pressured by the same person for 2 minutes. The third group was cleaned by identical 10x10cm wet wipes in the way that the tissue was used. The fourth group was exposed to cleaning by tap water whose pH was measured as 6.5 with pH meter. With the help of the ruler 5 cm was measured from the top of the can and 100 ml of tap water (measured by graded beakers) was poured to the top of the beverage cans. The last group was cleaned by water with soap. First, for each 100 ml of water 25 g of liquid soap was

mixed and stirred. The pH value was measured 7.5. This mixture was again poured from 5 cm away from the top of the cans. After the cleaning procedures the rubbing and inoculation methods were applied to these four groups. When taking samples process is finished the petri dishes were placed to the incubator arranged to 37°C. This temperature value was controlled during the experiment process. After waiting for 48 hours the petri dishes were taken out from the incubator and with the help of the binocular microscope the colony investigation and counting took place. The data were taken and noted down.

OBSERVATIONS

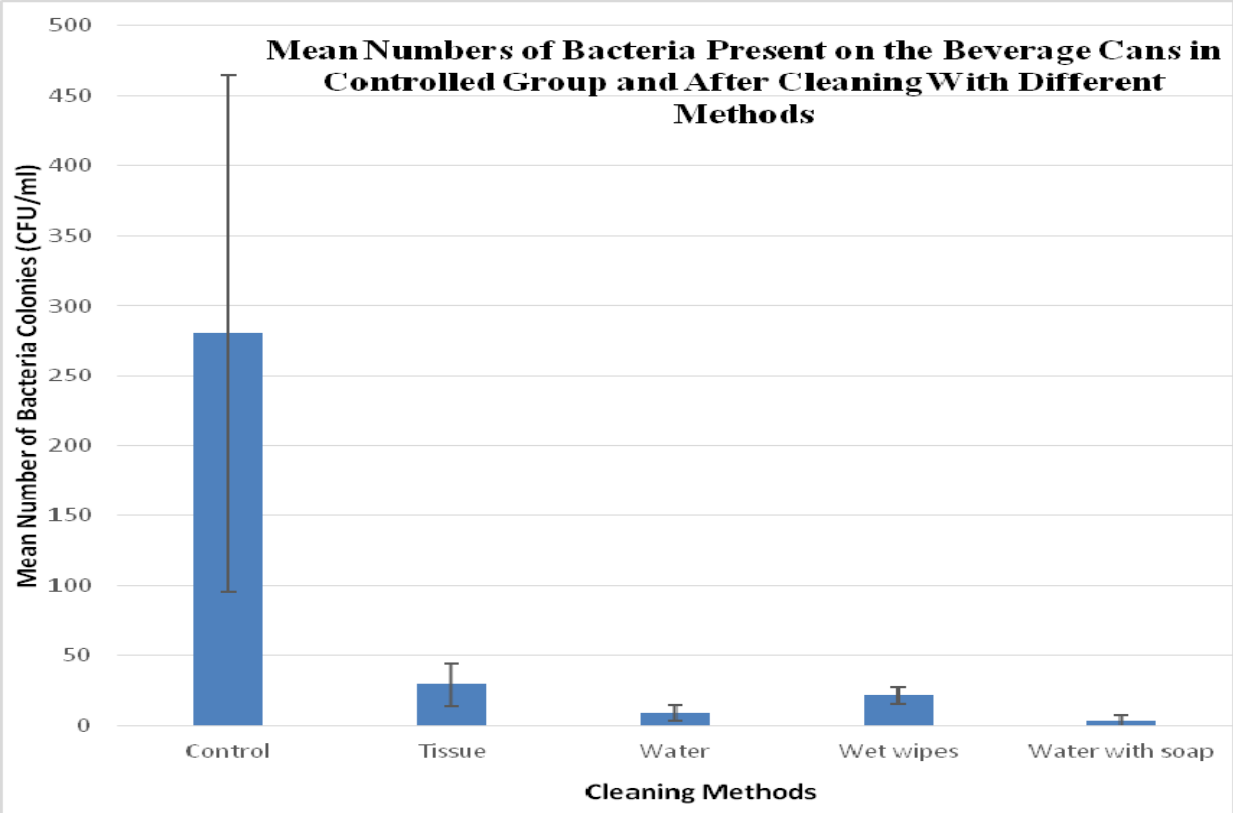
Cleaning Method	Trials	Amount of gram negative and positive bacteria (CFU/ml)	Temperature of the incubator (±0.5°C)	Size of the top region of the can (±0.1cm^2)	Amount of saline (±0.5ml)	Concentration of saline (%)	Amount of water used (±0.5ml)	Amount of soap used (±0.001g)
controlled group	1	300	37.0	23.0	200.0	9	0.0	0.000
	2	200	37.0	23.0	200.0	9	0.0	0.000
	3	300	37.0	23.0	200.0	9	0.0	0.000
	4	100	37.0	23.0	200.0	9	0.0	0.000
	5	500	37.0	23.0	200.0	9	0.0	0.000
cleaned with tissue	1	20	37.0	23.0	200.0	9	0.0	0.000
	2	25	37.0	23.0	200.0	9	0.0	0.000
	3	30	37.0	23.0	200.0	9	0.0	0.000
	4	20	37.0	23.0	200.0	9	0.0	0.000
	5	50	37.0	23.0	200.0	9	0.0	0.000
cleaned with wet wipe with pH of 5.5	1	16	37.0	23.0	200.0	9	0.0	0.000
	2	18	37.0	23.0	200.0	9	0.0	0.000
	3	25	37.0	23.0	200.0	9	0.0	0.000
	4	20	37.0	23.0	200.0	9	0.0	0.000
	5	27	37.0	23.0	200.0	9	0.0	0.000
cleaned with tap water with pH of 6.5	1	3	37.0	23.0	200.0	9	100.0	0.000
	2	10	37.0	23.0	200.0	9	100.0	0.000
	3	15	37.0	23.0	200.0	9	100.0	0.000
	4	9	37.0	23.0	200.0	9	100.0	0.000
	5	7	37.0	23.0	200.0	9	100.0	0.000
cleaned with water with soap with pH of 7.5	1	0	37.0	23.0	200.0	9	100.0	25.000
	2	0	37.0	23.0	200.0	9	100.0	25.000
	3	5	37.0	23.0	200.0	9	100.0	25.000
	4	8	37.0	23.0	200.0	9	100.0	25.000
	5	3	37.0	23.0	200.0	9	100.0	25.000

Table 1: Observing the amount of gram negative and positive bacteria (CFU/ml) present on the top region of the identical beverage cans before and after cleaning the region with several methods as cleaning with tissue, wet wipes, tap water and water with soap at the same temperature

DATA ANALYSIS

	Controlled group	Cleaned with tissue	Cleaned with tap water with pH of 6.5	Cleaned with wet wipes with pH of 5.5	Cleaned with water with soap with pH of 5.5
MEAN of the number of the gram negative and positive bacteria (CFU/ml)	280	29	8,8	21,2	3,2
SE of the number of the gram negative and positive bacteria	66,33249581	5,567764363	1,959591794	2,083266666	1,529705854
SD of the number of the gram negative and positive bacteria	148,3239697	12,4498996	4,38178046	4,65832588	3,420526275
Count	5	5	5	5	5
Confidence level (95%) of the number of the gram negative and positive bacteria	184,1685333	15,45859211	5,440699045	5,784075537	4,247144331

Table 2: The descriptive statistics of the number of the gram negative and positive bacteria (CFU/ml) present on the top of the beverage cans varying due to the cleaning method change



Graph 1: The graph showing the changing mean values of the number of bacteria (CFU/ml) before (in controlled group) and after cleaning the top region of the beverage cans with four different methods

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	5	1400	280	22000
Tissue	5	145	29	155
Water	5	44	8,8	19,2
Wet wipes	5	106	21,2	21,7
Water with soap	5	16	3,2	11,7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	281789,76	4	70447,44	15,86111061	**5,38252E-06**	2,866081
Within Groups	88830,4	20	4441,52			
Total	370620,16	24				

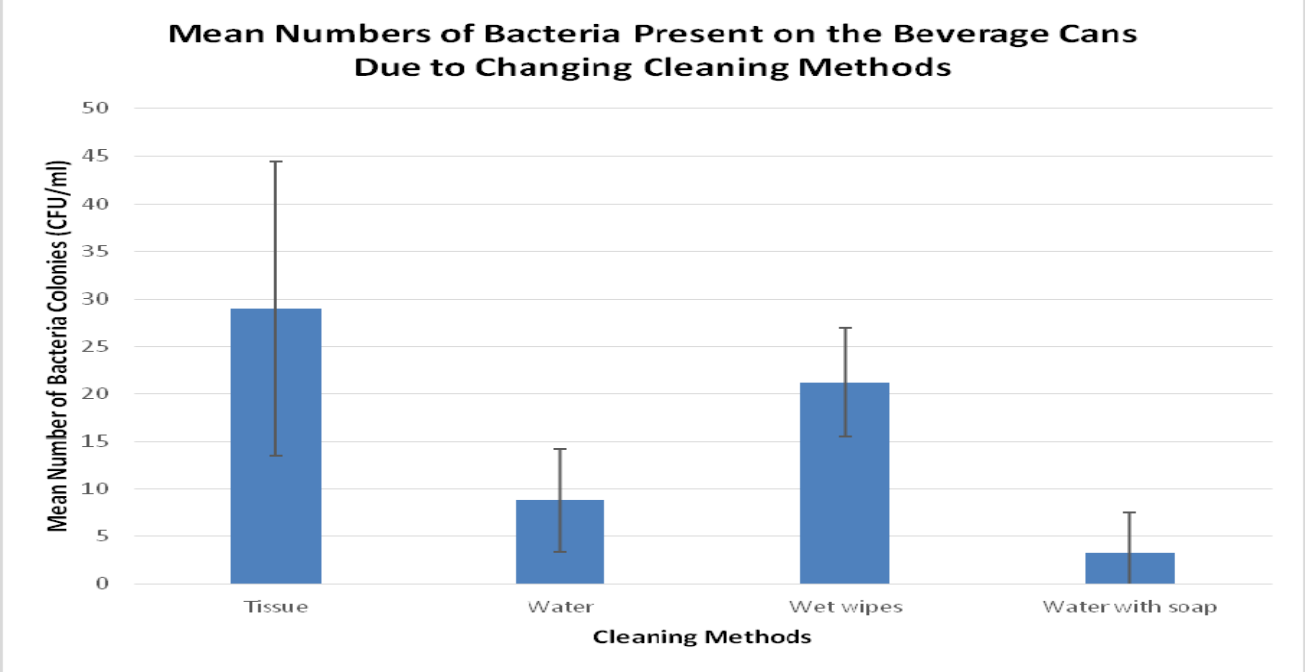
Table 3: Anova single factor test of the collected data from the controlled group and cleaning methods

P-value = 5.3825×10^{-6}

P-value < α

5.38252 < 0.05

Null hypothesis of there is no significant difference between the number of bacteria present on the beverage cans before and after cleaning the top region with 4 cleaning methods is rejected.



Graph 2: The graph showing the mean values of the number of gram negative and positive bacteria (CFU/ml) changing according to the different cleaning methods

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Tissue	5	145	29	155
Water	5	44	8,8	19,2
Wet wipes	5	106	21,2	21,7
Water with soap	5	16	3,2	11,7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2054,55	3	684,85	13,1955684	**0,000135672**	3,238872
Within Groups	830,4	16	51,9			
Total	2884,95	19				

Table 4: Anova single factor test for the collected data for only the cleaning methods applied on the top region of the beverage cans

P-value = 1.36×10^{-4}

P-value < α

$1.36 \times 10^{-4} < 0.05$

Null hypothesis of there is no significant difference between the cleaning methods applied to top regions of beverage cans is rejected.

EVALUATION

The aim of this study was to investigate the research question which was question 'Is there a significant difference on the amount of bacterial colonies of gram negative and positive bacteria found at the surface of the steel beverage cans before and after cleaning the top surface region with different methods as cleaning with tissue, wet wipes, distilled water and water with soap?' In consideration of this question, my hypothesis was that there will be a significant difference between the gram negative and positive bacteria amount before and after cleaning the top region with different methods. In addition to this hypothesis, I also hypothesized that the most effective method in reducing the bacteria amount was the method which the top region was cleaned with soapy water.

In order to analyze and see the mean comparisons before and cleaning processes experimental data is compared in Graph1. Cleaning methods are examples of qualitative variables, so that's why bar graph was preferred to line graph. The data from Graph1 indicates the difference between the amount of gram negative and positive bacteria. When the bacteria amount of the controlled group and of the cleaning methods are compared it is seen that the mean value of the bacteria amount in controlled group is 280 CFU/ml where this value is 29, 21.2, 8.8 and 3.2 CFU/ml when the top region is cleaned with tissue, wet wipes, tap water and soapy water respectively (See Table 2) With this amount of bacteria comparison, difference between the controlled group and the cleaning methods is done and observing the graph supports the first part of the hypothesis.

For a further support ANOVA test is held. The results supports this first part of the hypothesis that there is a significant difference between the amount of bacteria present on top region of the beverage cans before and after cleaning with 4 methods which were cleaning

with tissue, wet wipe, tap water and water with soap and the most effective method in decreasing the number of bacteria is cleaning with soapy water. In order to analyze the data collected from the top region of the beverage cans and prove the hypothesis, ANOVA test was advised by the supervisor and chosen. The ANOVA test which is performed with the data of the number of bacteria present on top of the beverage cans before and after cleaning this region with these methods has shown the P-value of 5.3825×10^{-6} (See Table 3) This value is lower than the α value which is 0.05 therefore, it can be assumed that there is a significant difference between the number of gram negative and positive bacteria present on top of the beverage cans before and after cleaning this area with four methods. So, the null hypothesis that there is no significant difference between the bacteria amount before and after cleaning methods is rejected.

For the second part of the hypothesis, comparison of the cleaning methods is done. At first, it was hypothesized that the most effective method in decreasing the amount of bacteria would be seen when the top region is cleaned with soapy water. Mean values of the amount of bacteria in cleaning methods are 29, 21.2, 8.8, 3.2 CFU/ml when cleaning with tissue, wet wipes, tap water and soapy water methods are applied to the beverage cans. This mean comparison is also seen in Graph 2.

The second ANOVA test which was performed in order to have a stronger proof is done to see which cleaning method would be the most effective one. The test showed a P-value of 1.36×10^{-4} (See Table 4). So, the hypothesis that the most effective cleaning method to decrease the number of bacteria found on top region of the beverage cans is cleaning with water with soap is confirmed. At this point, the second null hypothesis that there is no

significant difference between the cleaning methods in reducing the number of bacteria is also turned down.

It is seen from the Graph 1 and Graph 2 the error bars for the controlled group is relatively high when compared with the bars for the cleaning methods. Even though this doesn't have an effect on the overall results of the experiment, to get more precise counts from the cans, the number of trials can be increased and repeated.

Even though the mean values and the results of the ANOVA test support the hypothesis, there occurred some limitations that prevented to get more accurate results. First limitation arouse from the pH difference between the wet wipes, tap water and soapy water. When these three cleaning methods were applied to the top region of the cans, pH difference wasn't taken into account. However, because the metabolism and the structure of the bacteria (cell wall, polypeptide chain) are dependent on the pH, pH differences should be considered. For further researches, wet wipes, tap water and soapy water which have the same pH value should be used in order to see more accurate and controlled results.

Another limitation was about the applied method when the top region was cleaned with tap and soapy water. Although the water was poured from same heights, the pouring process was done by a person. So, any shake or direction deviation can result a change in the results. In order to apply the water in the same direction in same time intervals, a device which is called perfusor can be used. By this way, with arranging the pressure and the time interval, more precise results can be obtained.

During this investigation, the samples were chosen from the same market from the same brand. The metal that is used on top of the beverage cans, can vary from company to company. As a result, properties of metal like adhesion properties can be different leading the results change due to changing brand. For the next studies, investigations can be held for the cans that are collected from different companies.

In this experiment, for the isolation of the bacteria, sheep blood agar was chosen. However, this medium is only useful when investigating the gram negative and positive bacteria. For further experiments, other agars such as Eosin Metilen Blue (EMB) and Sabouroud Dekstroz (SDA) Agar can be used to observe the other microorganisms such as yeasts and moulds. When the samples are inoculated in these different agars wider and more general results can be seen and compared. In addition, in this study the count was done for gram negative and bacteria without looking specific species. In order to get more specific results profiling the species like if they are pathogenic or not can be done.

CONCLUSION

The intention of this study was to investigate the research question ‘Is there a significant difference on the amount of bacterial colonies of gram negative and positive bacteria found at the surface of the steel beverage cans before and after cleaning the top surface region with different methods as cleaning with tissue, wet wipes, distilled water and water with soap? I hypothesised that there is a significant difference in the bacteria amount before and after cleaning the top region. Moreover, I also stated that the most decrease amount of bacteria is seen when the top region is cleaned with soapy water.

Because the top regions are always in contact with outside and open for any external factor such as dust or any touch, these areas become an optimum nest for bacteria. When looked to packaging, transportation and selling processes it is seen that there is not enough care or meticulousness. This results a possible risk to outcome in public health.

From the results obtained from this investigation, it is seen that whether the top regions are cleaned or not there are still bacteria present on the surface. However, it is also seen that with the methods discussed in this study, the amount of bacteria thus the disease caused by those bacteria can be decreased. Even though, cleaning with tap or soapy water is not always possible, carrying and using wet wipes can also help reducing the health risks.

With advancing and improving this study, general assumptions and more accurate results can be obtained and showed to public. New quality standards or technologies for the companies that produce the cans can be detected as well as marketing or transportation restrictions can be formed.

The reason why I chose this topic was that in our daily lives approximately each day we use beverage cans. So, we are facing the health risks every time we drink from cans

without cleaning the top region. With this study, I wanted to see that if there is a difference between the number of bacteria before and after cleaning the can. Furthermore, I wanted to see which cleaning method is the most effective one in reducing the disease factors. As a result, I saw that there is a significant difference in bacteria amount before and cleaning the cans, so I will try to clean the top region of the cans before drinking, if possible with soapy water in order to decrease the contamination risk of bacteria and other microorganisms.

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