TED ANKARA COLLEGE PRIVATE HIGH SCHOOL

Investigating the effect of the concentration of Fluoride on the reproduction of *S. Mutans*

Extended Essay (Biology)

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Abstract

The bacterium *S. Mutans* is known as the bacterium which is one of the reasons of the cavities in humans mouth. It basically combines with sugar and this makes the bacterium to adhere onto the teeth and after this the cavities begin to occur. To prevent the cavities, there is a substance called fluoride. Almost every toothpaste includes this substance and it fights against the *S. Mutans* by combining the inhibitor part to the bacterium. Some experiments have been done showing the effect of the concentration of the fluoride on *S. Mutans.* However there isn't enough experiment which shows the exact effect of the concentration of the fluoride on the reproduction of *S. Mutans.* As a result the research question of this study is; how does the amount of fluoride effect the reproduction of *S. Mutans* on the teeth surface?

Throughout the experiment it was found that when the concentration of the fluoride was increased the concentration of the bacteria was decreased. The greatest concentration of the bacteria was observed when there is no fluoride concentration in petri dishes, it was 0,527 od±0,005. In addition to that the lowest concentration of *S. Mutans* was observed when the fluoride concentration was the greatest (2000±0,5 ppm), the mean concentration of the bacteria was 0,173 od±0,005 which is the lowest concentration in the experiment. In addition to that a decrease was observed in the concentration of the bacteria when the fluoride concentration was increased, which shows the experiment is suitable with the hypothesis. On the other hand the results of the annova test also shows that the experiment is meaningfull with a p value which is smaller that 0.05. The result of the experiment shows that the fluoride has an effect on the reproduction of the bacteria and the regression in the decrease of the bacteria is directly proportional with the concentration of the fluoride.

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Introduction

Almost all people brush their teeth everyday and everybody uses a toothpaste to brush their teeth. However most of the people don't know what a toothpaste includes and don't know what substance kills and fight against bacteria in their mouth. After a little research I found out that it was fluoride[1] but I still don't know the exact effects of fluoride on our teeth. Does it kill bacteria or prevent bacteria to stick on our teeth?

After that I started to search the effect of the fluoride on teeth and I found that it prevents cavities and bacteria. After that I think about the fluoride concentrations in different tooothpastes, all of these toothpastes have fluoride concentrations about 1100-1500 ppm (%0,30-0,33). In addition to that in my research I also see that in USA there is something called fluoride water and it contains fluoride approximately 5000 ppm and this is being used especially for the ones who has cavities in their mouth.[2] After I read that I totally understood that this substance helps us prevent cavities in our mouth with fighting against bacteria and killing them. After this research I thought that I can do an experiment in which I can observe the effect different concentrations of fluoride in cavities.

I began searching on the bacteria which were located in oral cavity and I found out that the bacterium is *Streptococcus mutans*[3] which comes from *streptococcus viridans* as one of it's oral species. It was mostly found on tooth surfaces and intrestingly if one teeth has high concentration of this bacteria the teeth which is next to that will have a small concentration of this bacteria.[4] This bacterium has receptors which helps it to stick into teeth. It makes cavities by metabolizing sucrose to lactic acid and this acidic environment causes the highly mineralized tooth enamel to be vulnerable to decay.

An experiment of W.J. Loesche, R.J. Murray, J.R. Mellberg shows the effectt of fluoride and also effect of the amount of fluoride on the reproduction of *S. Mutans*. In the experiment different gels (which includes different fluoride concentrations) were given to the people who were between 14 – 16 years old. After

[1] http://www.animated-teeth.com/tooth_decay/t4_tooth_decay_fluoride.htm

[2] *http://www.fluoridealert.org)*

[3] tıp ve diş hekimliğinde genel ve özel mikrobiyoloji

[4] http://en.wikipedia.org/wiki/Streptococcus_mutans

that the effect of different fluoride concentrations were tried to observed. At the end of the experiment it was understood that when the concentration of fluoride incereases in the gel which was used, the concentration of *S. Mutans* decreases.[5]

As a result my experiment will specifically focus on the effect of fluoride on reproducing of *S. Mutans* on teeth surface. This paper will focus on the research question: how does the amount of fluoride effect the reproduction of *S. Mutans* on the teeth surface? And also show how this experiment done and appraising the results which I get from the experiment.

[5] http://content.karger.com/ProdukteDB/produkte.asp?doi=10.1159/000259852

Hypothesis

It is known that fluoride prevents our teeth from cavities by killing and decreasing the numbers of sticking of bacteria to our teeth. "*That said, fluoride mouth rinses have many advantages. Fluoride re-mineralizes teeth with weakened enamel. Fluoride doesn't make teeth harder, but it does make them stronger and more impervious to plaque, tooth decay and cavities. Children who have grown up with fluoridated water generally have better overall oral hygiene. They typically have fewer cavities and less tooth decay."*[6]

The average value of fluoride in a toothpaste is about 1100-1500 ppm (%0,30-0,33). On the other hand fluoride water which includes fluoride about 5000 ppm is mostly using for the ones who has teeth decays and cavities in their mouth. As it can be understood that when the concentration of fluoride increases the effect of the toothpaste or fluoride water is also increasing.

Therefore the hypothesis can be; <u>as the amount of fluoride increases, the amount of bacteria will decrease.</u>

[6] http://www.associatedcontent.com/article/5768052/benefits_of_fluoride_mouth_rinse_in.html?cat=69

Method Development and Planning

In order to prove or reject the proposed hypothesis and answer the given research question, a convenient method should be used in the experiment, however to expose a proper method you should have pass over some problems. One of them is the reproducing of bacteria, where will they reproduce and how? After a little research I found that microorganisms can reproduce in mediums[7](agars) and the media is used by planting bacteria onto it. The base medium that I used in the experiment is a standart medium and the base of the medium is sheep blood and it includes the neccesary things needed for the reproduction of *S. Mutans.* The reproduction of the bacteria is an important point. The reason for this is; the bacteria population reach the maximum number of individuals about 24 hours.

A further problem is that where will I find *S. Mutans.* -This bacterium is using in the experiment because it is one of the bacteria which was located in mouth cavity and it is one of the reasons of the cavities.- You can find the bacteria from the medicals, however I do my experiment in Başkent University and from my previous researchs I know that *S. Mutans* is not a dangerous bacterium so I thought that I can find the bacterium in the university laboratoy. As I guess I found it in laboratory and the biggest obstacle I faced is now passed. The universtiy bought the bacteria from ATCC. “ATCC is a company which provides culture items for the science researchers throughout Europe and India.” [8]

In order to prepare different solutions of medium – fluoride, there must be fluoride in the form of aqueous or solid. However it is not easy to find fluoride in the faculty of medicine so I ask it to the faculty of dentistry and it is said that they have solid fluoride. It is usefull for me because I can make different solutions with mixing them in different ratios. Inspite of having bacteria and fluoride I don’t know the concentrations which I can prepare to see the effect of fluoride exactly. From my previous researchs I know that a standart toothpaste include fluoride about 1100 – 1500 ppm and fluoride water includes about 5000 ppm so I make my concentrations around these values. My fluoride values in different concentrations are 0, 500, 1000, 1250, 1500 and 2000 ppm. After that to create different concentration of fluoride – medium mix the medium with the fluoride and different concentration of fluoride –

[7] http://www.disknet.com/indiana_biolab/b029.htm

[8] http://www.atcc.org/

medium was occured. For example to make 1500 ppm fluoride – medium; 5cc medium was mixed with 15 cc %0.6 fluoride solution. On the other hand the formula (0,X gr fluoride + 100 mL water = Xx1000 ppm) is used to prepare different fluoride concentrations and 3 different ppm values for fluoride were prepared. For an instance to prepare 4000 ppm fluoride; 0,4 gr fluoride is added to the 100 mL water. In addition to that the formula formula (%X solution = Xx1000 ppm x 0.0001) is used to find the concentrations of the solutions. For example the %0.4 fluoride concentration is the concentraion of 4000 ppm.

My dependent variable is the amount of fluoride and my independent variable is the number of bacteria (the bacteria that will be used should be reproduced for 24 hours because at that time it has maximum number of individual) but some other factors must stay constant during the experiment. Temperature is the most important factors which must stay constant. It is easy to hedge the temperature because there isn't any window so the temperature remains constant during the experiment. However after planting bacteria, a suitable temperature must be used so the media are put into incubator and after 20 – 24 hours the bacteria were reproduced in the medium. In addition to that in this time interval the bacteria population has it's crowded population because after this period the bacteria begin to die. To see the exact effect of the fluoride concentration on the reproducing of the bacteria I should wait 20 – 24 hours because as I said before the number of the bacteria that was produced was maximum in that time. Secondly I should keep the acidity of the medium constant. I have prepared all the media from same materials so there isn't anything which can change the acidity of the medium. However when there is reproduction some reactions occur and this can effect the pH of the media. As the reproduction will not be same in all media depending on our estimations, the pH may also differs in media. Actually it is not easy to avoid this problem but the pH change will be really small so it's affect won't be so much on the results of the experiment. Another problem is that I don't want the bacteria to gather at one point so the medium should blend during reproducing of the bacteria before puting fluoride solution and with fluoride solution in the incubator and the apparatus (shaker) which blends the petri dishes is present in the laboratory.

At the end of the experiment a method should be used in order to understand the concentration of bacteria in the solutions. Two methods can be used to see the difference first one is photographing. As I make different media I put slides in these

media and at the end of the experiment I paint the slides and look at them under a microscope. By attaching a special photograph machine to the top of the microscope, the *S. Mutans* chains can be easily photographed. Second method I used is basically occurs by painting the bacteria. After putting the solutions into small cavities which are on the same tablet I paint the cavities with four different colours after washing it. If more bacteria is present in the cavity then the concentration of the colour will be greater, because the bacteria are attaching to the dye. The name of the dye which was used is crystal viole and the reason why I have chosen this dye is that; it can easily pass from the cell wall and it paints the cell walls, so it is a proper dye to paint *S. Mutans*[9]. After painting, when the tablet is put into a machine which reads the dye concentration the different concentrations of bacteria can be easily seen. In addition to that the wavelength of the light of the promotograph is 540 nm and the results were get at this wavelenth.

For the accuracy of the experiment I made 4 prelimary work before doing the experiment and in this works I mainly try the techniques that I will use in my experiment. In addition to that I found the exact concentrations of fluoride that I will use in the experiment.

[9] http://www.bio.net/mm/cellbiol/1995-May/002236.html

Materials

- *S. Mutans* which is taken from ATCC (American Type Culture Collection)
- 10 gr sodium fluoride
- Solid medium which is suitable for *S. Mutans*
- Aqueous medium which is suitable for *S. Mutans*
- Incubator
- Apparatus to shake the petri dishes in the refrigator (shaker)
- Heater
- Sterile stick which has got cotton at the tip x 6
- Petri dish x 6
- The purple dye which is used to paint the *S. Mutans*
- Promotograph (the machine which reads the concentration of the dye)
- A pot which includes small 2 mL pits and suitable for the promotograph
- Droppers
- Distilled water (500mL)
- Goggles
- Glove

Method

1. After taking the *S. Mutans* from the ATTC put the solid medium to the incubator (the heat of the incubator should be 37°C) and left them for one night.
2. In the morning take the sterile stick which has got cotton at the tip and expel the stick to the solid medium with this process you have got the bacteria on the stick (you must do this process near to a heater because of the danger of the bacteria).
3. Put the bacteria in your stick to the aqueous medium by this way you have the bacteria in aqueous form. (this process must be done near a heater becasue there can be contamination easily)
4. After preparing the aqueous medium with *S. Mutans* start to prepare the different concentrations of fluoride. (Look to the method development part while preparing different fluoride concentrations)
5. After preparing the bacteria and the fluoride concentration, the medium which includes different fluoride concentration should be made. To prepare the medium add different fluoride concentrations to the media;

- For the 500 ppm fluoride medium; add 2.5 cc %0.4 fluoride to the 17.5 cc medium.
- For the 1000 ppm fluoride medium; add 5 cc %0.4 fluoride to the 15 cc medium.
- For the 1250 ppm fluoride medium; add 5 cc %0.6 fluoride to the 15 cc medium.
- For the 1500 ppm fluoride medium; add 15 cc %0.6 fluoride to the 5 cc medium.
- For the 2000 ppm fluoride medium; add 10 cc %4.6 fluoride to the 10 cc medium.
- Put only 20 cc medium for the 0 ppm fluoride medium.

6. Left the mediums in the incubator (the temperature should be 25°C) for one day and put the apparatus (shaker) which will shake the petri dishes in the incubator.
7. After 24 hours time take out the media and put 2 mL media to the pits of the pot. Repeat this action two more times for each media. You have to have 18 pots filled with 6 different media.
8. After waiting 15 minutes spill the solutions in all 18 pots of media but you must be fast while turning the pot, then put 2 mL distilled water to the pots and spill the distilled water after 5 minutes (do this process one more time (only the water part)). The reason for the washing of pots are to spill the bacteria which have not adhere to the bottom of the pots.

9. After that add the purple dye (bacteria are sticking to the purple dye) to the pots and after waiting 15 minutes spill this too.

10. Put the pot the promotograph and it will give values. The biggest value is where the concentration of the dye is the greatest which means the greatest number of bacteria. From these results the pots which includes more bacteria or less bacteria can be easily seen and as it is known which pot is belong to the which medium the result can be easily processed. (If the value is shwon as OUT it means the concentration is bigger than 3,000 od)

Data Collection and Processing

After the reproduction of the bacteria in different concentrations of fluoride, the petri dishes were put into the promotograph. The difference between the concentrations of the bacteria (dye) in different solutions was seen in the promotograph.

Table-1: Different concentration of the dye (bacteria) for different amount of fluoride concentrations.

Concentration of fluoride (ppm±0,5)	Concentration of the dye (bacteria) (od±0,005)			Temperature (°C±0,5)
	Trial 1	Trial 2	Trial 3	
0,0	0,351	0,890	0,340	22,1
500,0	0,202	0,187	0,253	22,1
1000,0	0,153	0,197	0,243	22,1
1250,0	0,265	0,233	0,283	22,1
1500,0	0,191	0,197	0,240	22,1
2000,0	0,173	0,129	0,209	22,1

As it seen from the table-1 the greatest concentration of the dye -so the bacteria- is seen where there isn't fluoride. On the other hand the smallest concentration of the bacteria is seen where there is 2000,0 ppm fluoride.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	1,581	0,527	0,098857
Row 2	3	0,642	0,214	0,001197
Row 3	3	0,593	0,197667	0,002025
Row 4	3	0,781	0,260333	0,000641
Row 5	3	0,628	0,209333	0,000714
Row 6	3	0,511	0,170333	0,001605

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,263519	5	0,052704	3,010491	0,054662	3,105875
Within Groups	0,210081	12	0,017507			
Total	0,4736	17				

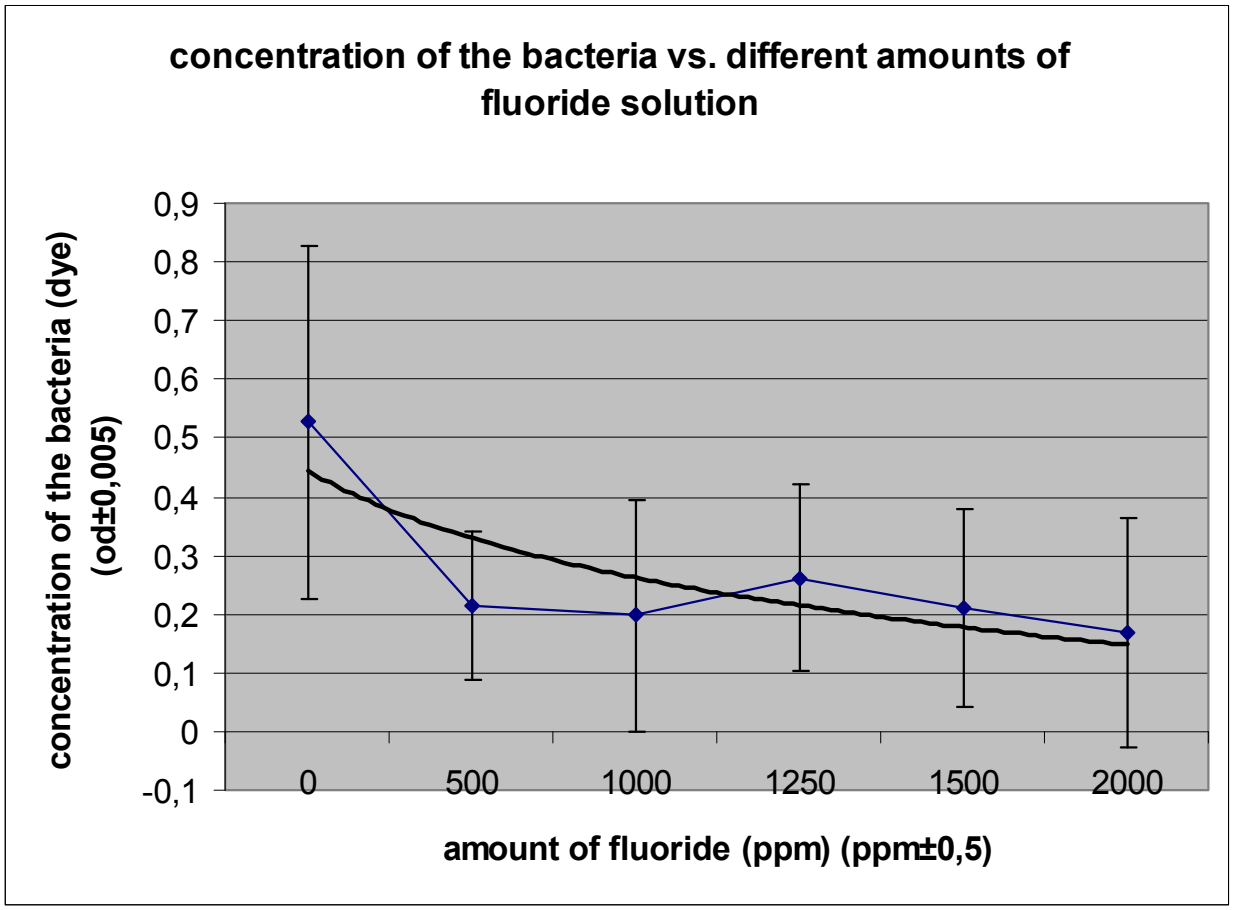
Descriptive Statistics

Mean, median, standart deviation, standart error, T value and %95CI are found for each group.

Table-2: Shows the descriptive statistics of each group.

Groups (different amounts of ppm)	mean	median	Standart deviation	Standart error	T value	%95 CI
0	0,527	0,351	0,31441533	0,095	3,182449	0,302
500	0,214	0,202	0,034597688	0,045	3,182449	0,127
1000	0,197667	0,197	0,045003704	0,062	3,182449	0,197
1250	0,260333	0,265	0,02532456	0,05	3,182449	0,159
1500	0,209333	0,197	0,026727015	0,053	3,182449	0,168
2000	0,168	0,173	0,040066611	0,061	3,182449	0,194

Graph-1: Graph of the experimental data, showing the average concentrations of the bacteria (dye) as the concentration of the fluoride was varied.



Conclusion and Evaluation

In this experiment my aim was to show that; when the fluoride concentration increases, there will be a regress on the reproduction of the *S. Mutans*. In order to observe the exact effect of the fluoride concentration I have prepared 5 different concentrations which are close to an avreage toothpaste's fluoride concentration. The main reason for this is that I want the experiment to be actual. On the other hand *S. Mutans* has been used during the experiment because this bacterium is located in mouth cavity and it is not dangerous so I can use it easily.

In the experiment I have made different fluoride solutions and the liquid media were mixed with these different fluoride solutions so different concentrations of fluoride were obtained. The petri dishes which includes fluoride and the bacteria were left for 24 hours for reproduction, after 24 hours time the samples from petri dishes were put into the promotograph and the results of the promotograph shows the different concentrations of the bacteria so the difference in the reproduction of the bacteria was observed from the results.

The results which I have got at the end of the experiment is supporting my hypothesis, as I expect the concentration of the bacteria is the greatest when the bacteria is left without fluoride, in addition to that the concentration of the bacteria is lowest when it was put into the 2000 ppm fluoride concentration (the greatest fluoride concentration in the experiment). The success of the experiment can be also seen when the descriptive statistics were compared. The mean and the median of the groups are close the each other (the mean and the median for the 500 ppm group is 0.214 and 0.202 respectively and these are same for the other groups; for 1000 ppm the values are 0.197667 and 0.197, for the 1250 ppm they are 0.260333 and 0.265 respectively and the values are 0.168 and 0.173 respetively for the 2000 ppm group) and this results with low standart errors and standart deviations, (0.095, 0.045, 0.062, 0.05, 0.053, 0.061) are standart error values for the groups and (0.31, 0.03, 0.04, 0.02, 0.02, 0.04) are values for standart deviation. These low standart errors and deviations are the indicators of the success of the experiment.

Anova single factor is also applied to the data to understand if the experiment is meaningful. The p value is found out as 0.044662, regarded to anova single factor if the p value is <0.05 it means the experiment is meaningful, so my experiment is significant according to anova single factor. As a result my hypothesis: "as the

amount of fluoride increases, the amount of bacteria is going to decrease" is true for the experiment.

To conclude, it can be understood that the fluoride is preventing the reproduction of *S. Mutans* and the differentation of the concentration of the bacteria can be seen in graph-1 and the huge gap between the "0 fluoride" and the "500 fluoride" can be seen in the graph-1 which means low concentrations of fluoride is also effective on the reproduction of the bacteria. The concentration of the bacteria for the other fluoride values can be also seen from the graph-1, the concentrations of the bacteria are 0.527 od, 0.214 od, 0.197667 od, 0.260333 od, 0.209333 od and 0.168 od respectively for the 0 ppm, 500 ppm, 1000 ppm, 1250 ppm, 1500 ppm and 2000 ppm of fluoride concentrations. As it seen the concentration of the bacteria is decreasing while the concentration of the fluoride is increasing and this is exactly what I expect from the experiment. The best fit line also confirm the regress in the concentraion of the bacteria.

The result of the experiment is suitable with the literature. My hypothesis for the experiment is; "As the amount of fluoride increases, the amount of bacteria is going to decrease". The literatures that I have serached confirm the results of the experiment, the research of W.J. Loesche, R.J. Murray, J.R. Mellberg is parallel to my research, they basicaly use a gel which includes fluoride in it and expel this gel to the children who are between 14 – 16. After that they begin to look the effect of the gel so the fluoride periodicly and after 6 days %75 reduction was observed on the concentration of *S. Mutans*. This experiment mainly shows the effect of the fluoide on the reproduction of *S. Mutans*[10]. On the other hand the research of A. Brunelle and J.P. Carlos shows the effect of the amount of the fluoride on the mouth disease. They observed that the people who drink fluoridated drinking waters has less cavities and less mouth diseases than the ones who haven't used the fluoridated drinking waters as much as the others. This experiment shows the effect of the amount of fluoride and this results confirm the results of my experiment. Same with my experiment when the amount of fluoride increase the amount of the bacteria in the mouth cavity decreases.[11]

The results of the experiment is suitable with the hypothesis, however there are some weaknesses and limitations in the experiment. To improve these

[10] (yaershttp://content.karger.com/ProdukteDB/produkte.asp?doi=10.1159/000259852).

[11] http://www.fluoridealert.org/health/teeth/caries/nidr-dmfs.html

weaknesses and limitations some developments should be applied to set up and to the experiment. First of all as it can be seen from the graph the results of the “1250 ppm” group are not the results that we predict. According to our predictions so the hypothesis, the concentration of the bacteria should decrease when the concentration of the fluoride increases however the concentration of the bacteria in “1000 ppm” group is lower than “1250 ppm” group. - The results of the other groups are the results that we foresee - Actually the reverse of this situation must be occured for the “1250 ppm” group. The main reason for this error can be the contamination (which means some other kinds of bacteria were reproduced in the petri dishes). While I was putting the bacteria from the solid medium to the liquid medium or from the liquid medium to the petri dishes I have been working near a heater to prevent contamination however inspite of this a contamination was occured. In order to prevent the contamination the preson who is changing the place of the bacteria must be fast (I wasn’t fast enough because this was my first time that I have worked near a heater and with bacteria). In addition to that two heaters can be also used to prevent the contamination becasue two heater means higher temperature and the bacteria will die in higher temperatures easily.

Some other experiments can be done towards my experiment and some other previous experiments that have been done. For an instance an experiment can be designed which has greater fluoride concentrations than I have like; 6000 ppm, 8000 ppm. With this experiment the effect of fluoide waters that I have mentioned in introduction part will be observed. In addition to that the effect of greater fluoride concentrations will be observed too. On the other hand it is known that great amount of fluoride concentrations threatens the health of the teeth so an experiment can be designed which will process the danger of fluoride. In this experiment the fluoride concentrations can be

same with the above experiment that I have mentioned, however the difference is that; real teeth will be used in this experiment so the effect of great amount of fluoride concentration on the teeth surface will be observed by putting the teeth to different fluoride concentrations. Beside this experiment another experiment which will investigate the adhesion of the *S. Mutans* in different fluoride concentrations can be designed. In this experiment a slide will be put into the petri dishes which also

includes fluoride and the bacteria. After that the petri dishes will be left for one day for the reproduction and the adhesion of *S. Mutans,* after one day the lames will be painted with a dye (same in my experiment) and the effect of the fluoride on the adhesion will be observed under a microscope.

Appendices

Appendix 1

The medium which was used durin the experiment includes;

- 5% sheep blood
- 95% amino acid (peptan), glucose

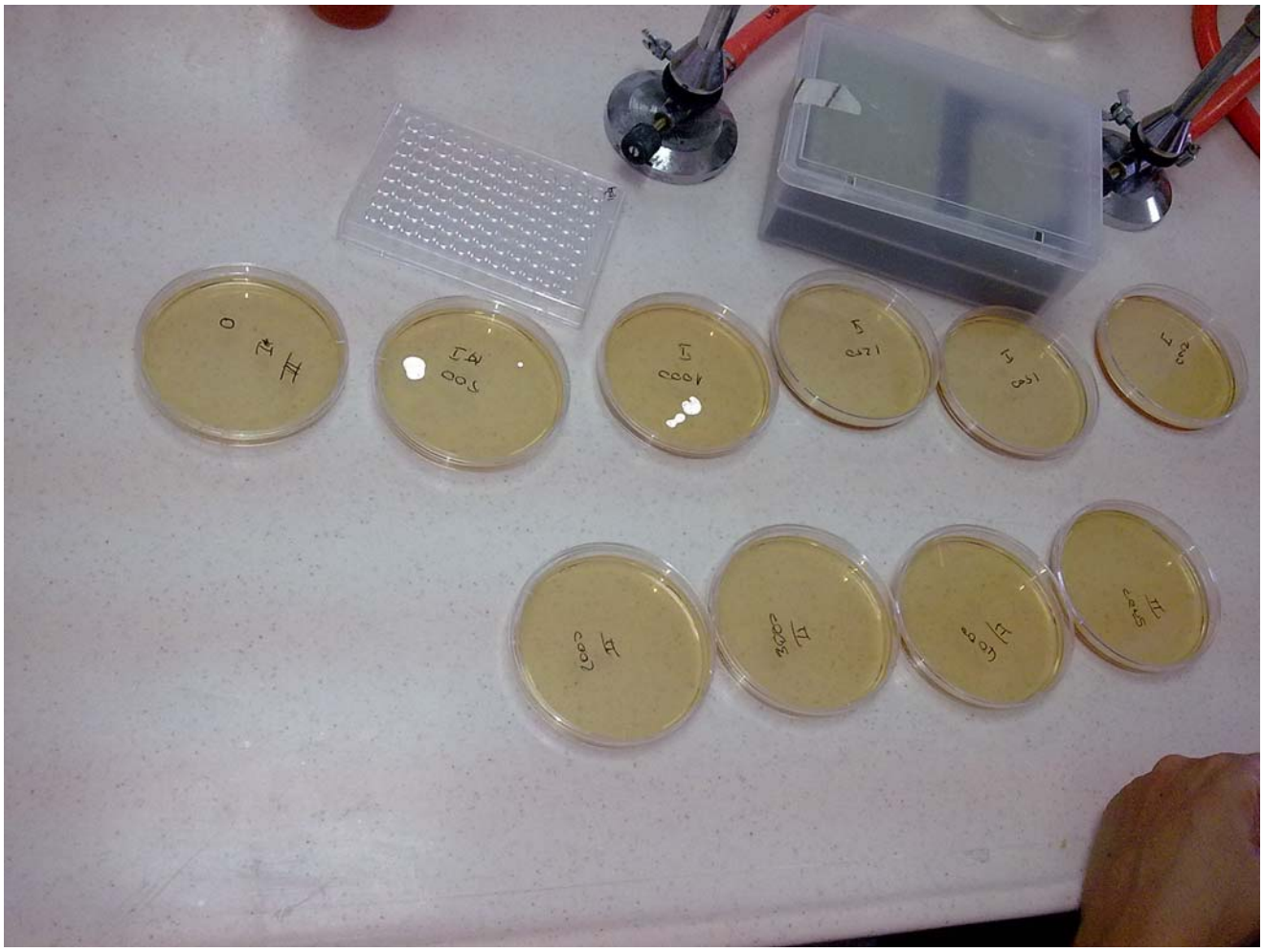


Photo 1: differet mediums which were prepared with different fluoride concentrations.

Appendix 2

In the planting process the stick which has circle on the top was drawn to the solid medium which includes *S. Mutans,* by this process the bacteria was taken from the solid medium. After that the stick was put into the liquid medium so the bacteria was planted to the liquid medium.

Appendix 3

The photos of *S. Mutans* in different fluoride concentrations which is taken from the microscope.

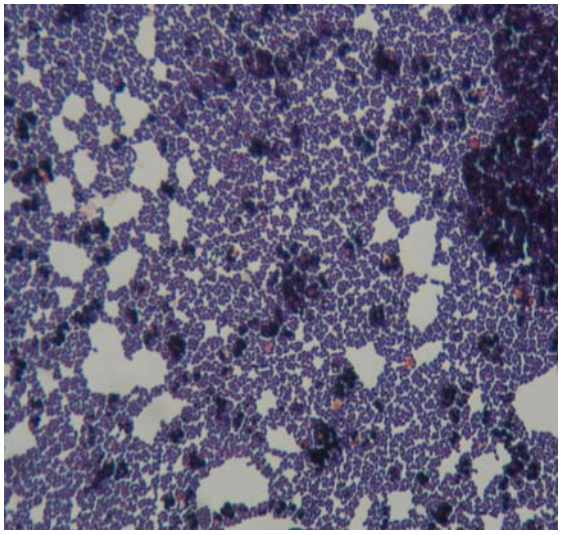


Photo2: the seem of *S. Mutans* when there is no fluoride

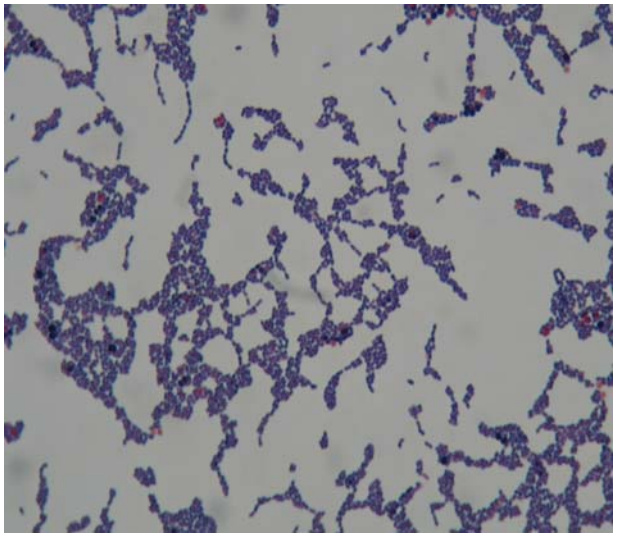


Photo 3: the seem of *S. Mutans* when the concentration of fluoride is “1000 ppm”

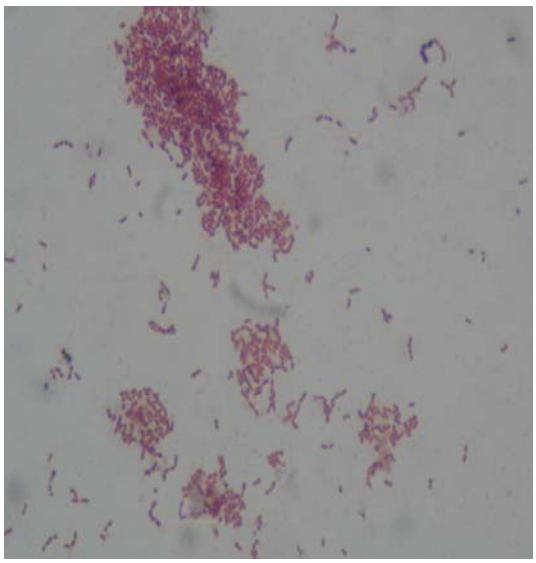


Photo 4: the seem of S. Mutans when the concentration of fluoride is “1500 ppm”

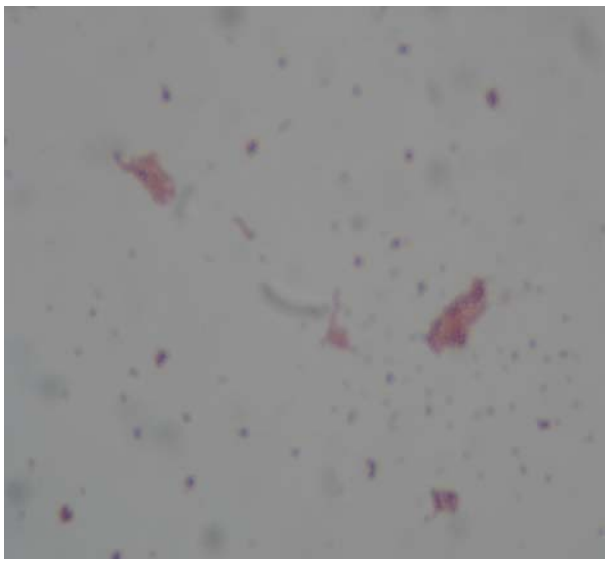
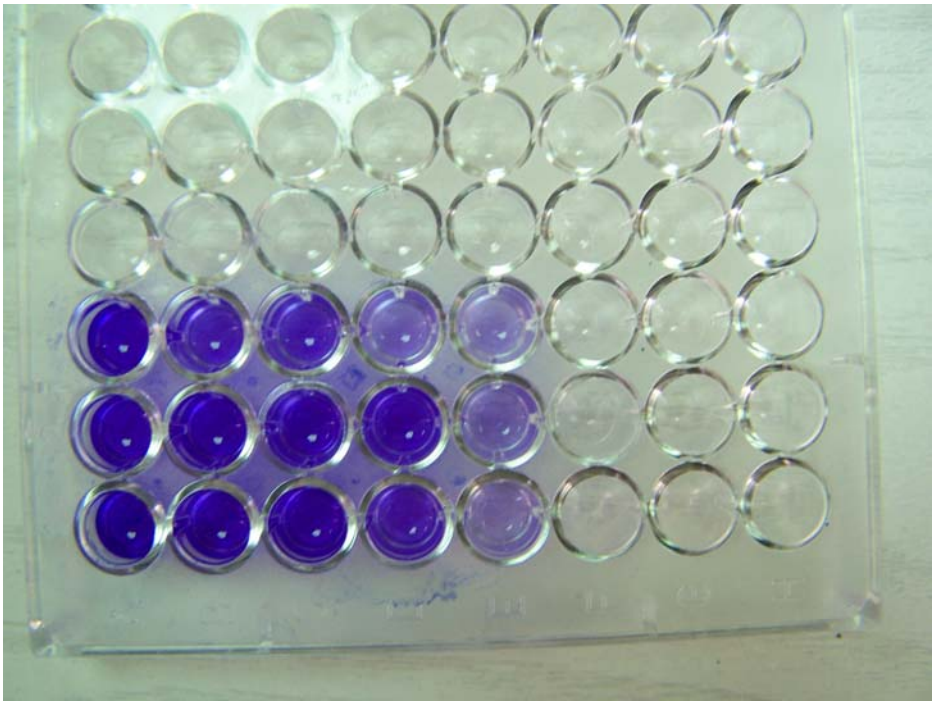


Photo 5: the seem of S. Mutans when the concentration of fluoride is “2000 ppm”

Appendix 4

The photos of the painting process. The dye which was used is crystal viole.



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  W.J. Loesche, R.J. Murray, J.R. Mellberg
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