The Antimicrobial Effects of Metals Copper, Barium and Silver on the Growth of Bacteria.

Research Question:

To what extent do the antimicrobial properties of metals copper, barium and silver aid in inhibiting the growth of bacteria *Staphylococcus albus* and *Micrococcus luteus*?

Subject: Biology

Referencing style: Harvard

Word count: 3860

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Research Question

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Introduction

In this essay I would like to explore an alternative method to antibiotics at inhibiting the growth of bacteria. This idea stemmed from my interest in the problem of serious bacterial infections caused by resistant bacteria such as Methicillin-resistant *Staphylococcus aureus* (MRSA) that pose a threat to many hospital patients in the UK today. After the discovery of Penicillin in 1928, its success has led to the discovery of a plethora of antibiotics such as Methicillin. However the widespread use of Methicillin to treat *S.aureus* bacterial infections has resulted in resistance to this antibiotic. This is due to variation amongst bacterial populations whereby random genetic mutations make one bacterium resistant to the antibiotic, so survives. The transfer of genes for antibiotic resistance by conjugation[1] or asexually by binary fission[2] is what allows the uncontrollable growth of these superbugs.

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[1] Rogers and Kadner (2017). *bacteria - Exchange of genetic information* p.7

[2] Micro.cornell.edu. (Anon n.d.). Binary Fission and other Forms of Reproduction in Bacteria | Department of Microbiology

This problem enthused me to investigate other suggested methods of inhibiting bacterial growth. In doing so, I came across the oligodynamic effect which refers to the ability of metals, such as silver and copper, to have toxic effects on bacterial cells[3]. To test the ability of metal ions at inhibiting bacterial growth, I planned an experiment to investigate the mechanisms of action and test the antimicrobial properties of salt solutions barium chloride, silver nitrate, copper sulphate and copper chloride. I investigated the metals silver and copper due to their association with the oligodynamic effect and the alkaline earth metal barium to investigate the antimicrobial properties of a more reactive metal. The cost of solid metals for use in a school lab limited my experiment to using salt solutions containing the metal ions I wished to study.

I tested the antimicrobial properties of these three metals against the gram-positive bacterium *Staphylococcus albu*s and gram-negative bacterium *Micrococcus luteus.* I chose to investigate these two non-pathogenic bacteria as model organisms for their pathogenic relatives of gram-positive *Staphylococcus aureus* and other pathogenic gram-negative bacteria such as *Escherichia coli* due to the limitations of using pathogenic bacteria in a school laboratory. By investigating one gram-positive and one gram-negative bacteria species, my results could suggest the potential impact of metal ions on a wider range of bacteria.

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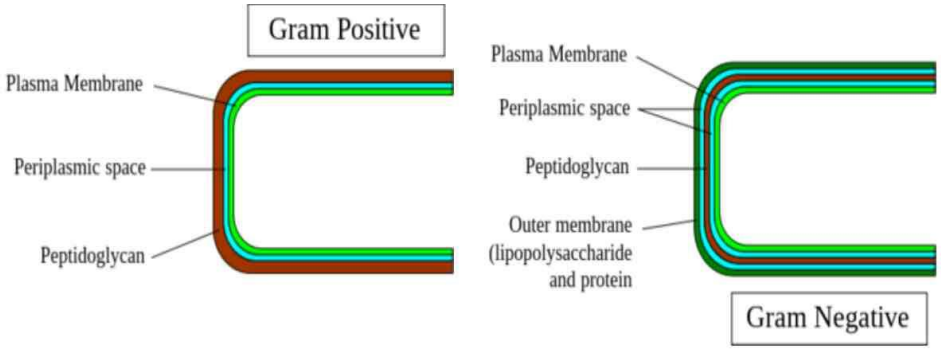
[3] Shrestha et al (2009). Oligodynamic Action of Silver, Copper and Brass on Enteric Bacteria Isolated from Water of Kathmandu Valley

Background Information:

The Structural Differences of Gram-positive and Gram-negative Bacteria

Knowing the structural differences of gram-positive and gram-negative bacteria is crucial in predicting the impact of metal ions on their growth. Their structure may determine whether one type of bacteria is more resistant to the antimicrobial properties of a metal than the other. As seen in **Figure 1**, a gram-positive bacterium has a thicker peptidoglycan cell wall and its plasma membrane is located on the interior of this cell wall. A gram-negative bacterium has a thinner peptidoglycan cell wall in-between two membranes; the outer lipopolysaccharide membrane and the interior phospholipid bilayer.

Figure 1- Diagrams showing the structure of gram-positive and gram-negative bacteria[4].



[4] T, K. Sehgal, P and Jasuja, N. (n.d.). Gram-positive vs Gram-negative Bacteria - Difference and Comparison | Diffen

Mechanisms of Action

The metals silver, barium and copper use a range of mechanisms to inhibit the growth of bacteria including oxidative stress, antioxidant depletion and the blockage of pore proteins caused by high concentrations of metallic ions. Their individual mechanisms of action could help determine the most effective metal at inhibiting bacterial growth of *S.albus and M.luteus*.

Barium Ions Blocking the Transport of Essential Ions

Barium is an alkali earth metal more reactive than both copper and silver. The build-up of barium ions intracellularly is toxic to a cell. These ions, non-essential to the functioning of bacterial cells, competitively block proteins that transport less reactive essential ions, such as calcium, through the cell membrane[5]. The competitive blocking of these ion channels allows an intracellular accumulation of metal ions, overpowering the ability of the cell's efflux proteins to maintain metal ion homeostasis[6]. Gram-negative bacteria are less vulnerable to this accumulation due to their double membrane (see **Figure 1**) which reduces the uptake of metal ions as they have to pass through transport proteins within both the outer and inner membrane[7].

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[5] Lemire et al (2013). Antimicrobial activity of metals: mechanisms, molecular targets and applications p.374

[6] Begg et al (2015). *Dysregulation of transition metal ion homeostasis is the molecular basis for cadmium toxicity in Streptococcus pneumoniae*

[7] Lemire et al (2013). Antimicrobial activity of metals: mechanisms, molecular targets and applications p.377

Silver Ions in Depleting the Antioxidant Reserves of Bacterial Cells

Silver is a metal whose ions are non-essential to the functioning of bacterial cells. As tested on the gram-negative bacteria *E.coli*, silver ions are responsible for the intracellular depletion of antioxidant reserves by oxidising cellular thiols such as glutathione [8]. This mechanism leaves proteins within the bacteria more vulnerable to attack by reactive oxygen species (ROS) that contribute to the lethal oxidative stress on the cell. Once inside both gram-positive and gram-negative bacteria, silver ions can bind to thiol groups of amino acids, denaturing cellular enzymes vital to cell function[9].

Oxidative Stress and Membrane Damage by Copper ions

Unlike silver and barium, copper ions are essential to the functioning of microbial cells. Copper ions act as a redox co-factor which means they assist biochemical pathways[10] such as electron transport, oxidative respiration and denitrification[11]. At higher concentrations, copper ions catalyse the production of reactive oxygen species (ROS) in a cell such as the production of hydroxyl radicals[12]. The resulting oxidative stress overwhelms the cell's

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[8] Harrison JJ, e. (2009). Chromosomal antioxidant genes have metal ion-specific roles as determinants of bacterial metal tolerance. - PubMed – NCBI

[9] Gordon, O et al (2010). Silver Coordination Polymers for Prevention of Implant Infection: Thiol Interaction, Impact on Respiratory Chain Enzymes, and Hydroxyl Radical Induction

[10] Mishra, A. and Prasad Mishra, K. (2015). Bacterial response as Determinant of oxidative stress by Heavy metals and antibiotics

[11] Argüello et al (2013). *Mechanisms of copper homeostasis in bacteria*.

[12] Lemire et al (2013). Antimicrobial activity of metals: mechanisms, molecular targets and applications p.376

antioxidant protection, permanently impairing cell function. Copper is also associated with lipid peroxidation resulting in a loss of cell membrane rigidity in the gram-negative bacteria *E.coli*[13]. The impairment of membrane function results in the leakage of cellular components, inhibiting any processes necessary for cell growth.

Hypothesis:

I predict that copper will be the most effective metal at inhibiting bacterial growth of *Staphylococcus albu*s and *Micrococcus luteus* due to its involvement in catalysing the oxidative stress on bacterial cells. I predict that *M.luteus* will be more susceptible to denaturation by copper and *S.albus* by silver and barium due to the difference in their structures.

[13] Hong et al (2012). Membrane Lipid Peroxidation in Copper Alloy-Mediated Contact Killing of Escherichia coli.

Method

Independent variables

- The salt solutions used: barium chloride, copper chloride, copper sulphate and silver nitrate.
- The concentrations of each salt solution (0.5M and 1M for copper chloride, copper sulphate and barium chloride with 0.025 and 0.0125 for silver nitrate).
- The type of bacteria, either *M.luteus* or *S.albus.*

Dependent variables

- The zone of inhibition (mm^2) ±4mm^2 measured using graph paper with 2x2mm squares.

Controlled variables

- The volume and type of agar medium.
- The volume of salt solution added to each filter paper disc.
- The size of the filter paper discs must remain constant.
- The number of repeats per salt solution concentration is 30.
- The temperature at which the inoculated plates were stored (in the same incubator).
- Sterile conditions should be maintained throughout by using sterile apparatus, a sterile work space and a flaming Bunsen to maintain a zone of sterility around the experiment.

Method Development

In the experiment I aimed to test and compare the effectiveness of silver, copper and barium metals at inhibiting bacterial growth. These metals are worth testing due to their known mechanisms of action in inhibiting the growth of bacteria.

In a preliminary investigation I deduced that growing the bacteria *M.luteus* and *S.albus* in an agar medium was the best method to test the antimicrobial properties of metal ions. This is because the filter paper discs, dipped in selected salts of barium chloride, copper sulphate, copper chloride and silver nitrate, produced a clear ring of clarity after 48 hours in an incubator (See Appendix D) allowing me to measure their subsequence areas of bacterial inhibition using graph paper. On each agar plate I consistently used $2cm^3$ of either cultured *M.luteus* or cultured *S.albus* to maintain consistent growth.

Initially I planned to test the antimicrobial properties of a range of solid discs of elemental metals although, their cost and availability was limiting. As a result of this I chose to use salt solutions containing metal ions of barium, copper and silver as a cheaper and more accessible alternative for solid metal pieces. Using salts containing metal ions of barium, silver and copper in combination with various non-metallic anions in the preliminary experiment, I deduced that the anions had no impact on the zone of inhibition. This is because with the same metal but a different anion, the areas of inhibition were very similar. Although, changing the metal and not the anion had a dramatic impact on its subsequent ring of clarity. In finding this, I chose to test the salts of barium chloride, silver nitrate, copper chloride and copper sulphate for their antimicrobial properties. With the assumption

that the anions have no antimicrobial properties, I would expect the mean zone of inhibition for copper sulphate and copper chloride to be the same.

In my final method, the salts of 1M and 0.5M copper sulphate, 1M and 0.5M copper chloride, 1M and 0.5M barium chloride and 0.025M and 0.0125M of silver nitrate were used as these concentrations produced a big enough zone of inhibition to measure (see Appendix C). The concentrations of silver nitrate used are limited by the cost of this salt, restricting the experiment to lower concentrations. I chose to test two concentrations of each solution to examine the impact of increasing metal ion concentration on the growth of bacteria. This has relevance when using the metals in a medical environment to treat bacterial infection whereby, if effective, lower concentrations of metals could be used at a lessened cost.

Maintaining an aseptic environment to prevent any contamination by microbes in the laboratory is essential. To do this, proper aseptic technique was needed throughout the experiment. A sterile environment was set up by wiping down the laboratory bench with alcohol wipes, sterilising all equipment using Virkon solution, wearing gloves and a laboratory coat. Flaming the neck of the bacteria cultures and salt solutions as they were opened and before they were closed helped prevent the entry of unwanted microorganisms. Maintaining an aseptic environment is achieved by working within the 30cm zone of sterility of a Bunsen burner (for risk assessment see Appendix E).

My final method involved placing three filter paper discs that had been dipped in a chosen solution onto each agar plate. I repeated this for the same solution ten times which meant

that overall I could collect 30 areas of inhibition for each chosen salt, at each concentration. By repeating each solution 30 times, I could identify any anomalous areas of inhibition, calculate a reliable mean and conduct a statistical test on this data.

Results

In general, I noticed that salt solutions of a higher concentration created a larger ring of clarity although there were a few exceptions to this. Copper solutions also produced the largest rings of clarity for both bacteria.

Raw Data

All the measured areas of inhibition for my experiment were recorded in a raw data table (See Appendix F) and used to create Table 1 demonstrating the average zone of inhibition formed by each salt solution on the two tested bacteria.

Mean Zone of Inhibition Table 1:

A table demonstrating the average zone of inhibition formed by salt solutions on the bacteria *S.albus* and *M.luteus*

Bacteria	Salt solution and concentration (M)	Average zone of inhibition (mm^2) ± standard deviation	% error of standard deviation
S.albus	Barium Chloride 1M	210 ±52	25%
	Barium Chloride 0.5M	213 ±42	20%
	Copper Chloride 1M	518 ±52	10%
	Copper Chloride 0.5M	252 ±97	38%
	Copper Sulphate 1M	425 ±54	13%
	Copper Sulphate 0.5M	264 ±54	20%
	Silver Nitrate 0.025M	95 ±20	21%
	Silver Nitrate 0.0125M	80 ±11	14%
M.luteus	Barium Chloride 1M	192 ±43	22%
	Barium Chloride 0.5M	86 ±29	34%
	Copper Chloride 1M	536 ±83	15%
	Copper Chloride 0.5M	579 ±57	10%
	Copper Sulphate 1M	544 ±76	14%
	Copper Sulphate 0.5M	404 ±76	19%
	Silver Nitrate 0.025M	76 ±15	20%
	Silver Nitrate 0.0125M	62.1 ±9.4	15%

Calculations

Calculating the Mean:

I used the raw data (See Appendix F) to calculate the mean zone of inhibition created by a particular salt solution at a particular concentration.

$$\textbf{\textit{Average area of inhibition}} \pm \textbf{Std Dev.} = \frac{\textbf{\textit{The sum of}}\ \mathbf{30}\ \textbf{\textit{areas of inhibtion}}}{\mathbf{30}}$$

Error Calculations:

The error bars for the mean area of inhibition seen on Graph 1 were calculated using standard deviation above and below the mean. The values for standard deviation seen in Table 1 are quoted to two significant figures and the means are subsequently quoted to the same number of decimal places. The following calculation was used in excel to determine standard deviation:

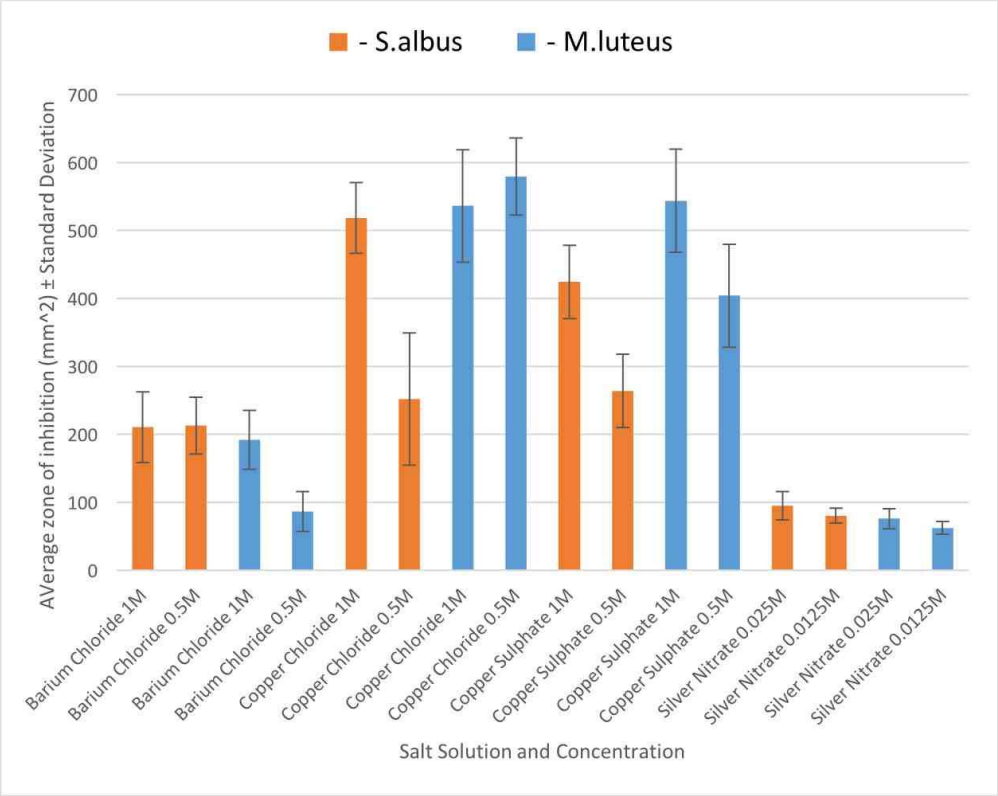
$$\boldsymbol{SD} = \sqrt{\frac{\sum(\boldsymbol{x} - \bar{\boldsymbol{x}})^2}{\boldsymbol{n} - \mathbf{1}}}$$

The standard deviation represents the average value that each data set differs from the mean of that set and can be used to analyse the spread and reliability of data in relation to the mean.

Processed Data

Mean Zone of Inhibition Graph 1:

A graph showing the average area for the zone of inhibition of *M.luteus* and *S.albus* by different salt solutions.



Analysis and Discussion

This graph demonstrates how named salts of different concentrations have impacted the area of inhibition produced on the bacteria *S.albus* and *M.luteus*. The error bars demonstrate the standard deviation above and below the mean value for those areas enabling further analysis on the range of data presented and therefore its reliability.

For both bacteria, the most effective salt solutions at inhibiting bacterial growth were copper chloride and copper sulphate as they produced the largest mean area of inhibition. 1M concentrations of copper sulphate and copper chloride produced similar zones of inhibition on both bacteria as each solution had the same concentration of copper ions. Unexpectedly, with the same concentration of copper ions in 0.5M copper chloride and copper sulphate the zone of inhibition produced on each bacterium varied drastically. The mean zone of inhibition produced by 0.5M copper chloride and 0.5 copper sulphate on *M.luteus* were 579mm^2 and 404mm^2 respectively whereas for *S.albus* they were 252mm^2 and 264mm^2. This indicates that the gram-negative bacteria *M.luteus* is more vulnerable to the antimicrobial properties of copper ions as it produced a larger mean zone of inhibition than *S.albus*. This can be explained by the presence of a thin peptidoglycan cell wall and two lipid membranes in the gram-negative bacteria *M.luteus* which are left more vulnerable to lipid peroxidation by copper ions. In contrast, *S.albus* has a thicker peptidoglycan cell wall and a singular phospholipid membrane, protecting it from the mechanism of lipid peroxidation.

Barium chloride of 1M and 0.5M was less effective at inhibiting bacterial growth than the copper solutions. Both concentrations of barium chloride produced similar zones of inhibition on the bacteria *S.albus*. In contrast, 1M barium chloride produced a similar zone of inhibition on *M.luteus* as for *S.albus* although 0.5M of barium chloride produced a zone of inhibition on M.luteus less than half that of the one produced on S.albus. This suggests that barium is less effective at inhibiting the growth of M.luteus than it is for S.albus. A similar result is seen for the zones of inhibition produced by silver nitrate whereby the zone of inhibition produced by 0.025M and 0.0125M silver nitrate on *S.albus* were 95mm^2 and 80mm^2 respectively whereas they were only 76mm^2 and 62mm^2 for *M.luteus*. This is explained by the double membrane in the structure of the gram-negative bacteria *M.luteus* that restricts the uptake of metal ions. This is because metal ions have to bypass pore proteins in both the inner and outer membrane of *M.luteus* instead of the single plasma membrane of *S.albus*.

As quoted in Table 1, the area of inhibition on *S.albus* by the 0.5M copper chloride solution has a standard deviation value that is 38% of its mean. This implies an unreliably large spread of data. This needs consideration when trying to extract its effectiveness at inhibiting the growth of *S.albus* compared to other solutions. The standard deviation values of 25% for 1M barium chloride and 20% for 0.5M barium chloride on the mean zone of inhibition for *S.albus* suggests that there could be a greater difference in their total means than what is seen in Graph . Further repeats would be needed to extract the effect of changing their concentration on the growth of *S.albus*.

For both S.albus and M.luteus I noticed that varying the silver nitrate concentration had little impact on the area of inhibition produced. Due to cost limitations in a school lab I could only access concentrations of 0.025M and 0.0125M of silver nitrate, limiting its comparison to other solutions and making the zones of inhibition produced very small. I used $4mm^2$ graph paper squares ($\pm 4mm^2$) to measure the area of inhibition but for such small areas, the percentage uncertainty on these values is large. For example, the percentage uncertainty for the mean area inhibited by 0.0125M silver nitrate on M.luteus is ±6% $\left(\frac{\pm 4mm^2}{62.1} \times 100 = 6\%\right)$ implying the recorded value of 62.1mm^2 is inaccurate.

Statistics

I chose a T-test to assess whether each individual metal is more effective at inhibiting the growth of one bacteria than the other. With 30 repeats I had a large enough sample size to conduct a 2 tailed unpaired T-test to deduce whether there is a statistically significant difference between the sets of data I obtained for *M.luteus* and *S.albus* for each salt, at each concentration.

The null hypothesis: There is no significant difference in the area of inhibition between *M.luteus* and *S.albus* for each salt, at each concentration.
Alternate hypothesis: There is a significant difference in the area of inhibition between *M.luteus* and *S.albus* for each salt, at each concentration.

In this test, if t is greater than the critical value (when p=0.05), then the null hypothesis will be rejected (if $|\boldsymbol{t}| > \boldsymbol{t\ crit}$) and the alternate accepted.
This is the equation used to find the t-values:

$$\boldsymbol{t} = \frac{|\boldsymbol{x_1} - \boldsymbol{x_2}|}{\sqrt{\frac{(\boldsymbol{S_1})^2}{\boldsymbol{n_1}} + \frac{(\boldsymbol{S_2})^2}{\boldsymbol{n_2}}}}$$

The degrees of freedom for this T-test are:

$$(n_1 + n_2) - 2 = (30 + 30) - 2 = 58\ degrees\ of\ freedom$$

From the table of critical values (See Appendix H) with 58 degrees of freedom when p=0.05, the t-critical value is 2.02.

Results of the T-test Table 2:

Salt solution and Concentration (M)	T-values	T-critical value	Result
Barium Chloride 1M	1.51	2.02	Accept null
Barium Chloride 0.5M	13.7	2.02	Reject null
Copper Chloride 1M	0.99	2.02	Accept null
Copper Chloride 0.5M	16.02	2.02	Reject null
Copper Sulphate 1M	7.02	2.02	Reject null
Copper Sulphate 0.5M	8.22	2.02	Reject null
Silver Nitrate 0.025M	4.15	2.02	Reject null
Silver Nitrate 0.0125M	6.63	2.02	Reject null

From this T-test we can accept the null hypothesis for 1M barium chloride and 1M copper chloride solutions that there is no significant difference in their produced area of inhibition on *M.luteus* and *S.albus*. This suggests that, at 1M concentration, they are equally effective at inhibiting the growth of either bacterium.

For the remaining solutions, we accept the alternate hypothesis that there is a statistically significant difference in the area of inhibition produced on *M.luteus* and *S.albus.* This suggests that the gram-positive and gram-negative bacteria do interact differently with the metallic cation in each solution. For all silver nitrate and copper sulphate solutions there is a significant difference in the zones of inhibition produced. This indicates that silver nitrate is indeed better at inhibiting the growth of *S.albus* than *M.luteus* and copper sulphate better at inhibiting the growth of *M.luteus* than *S.albus.*

However, for barium chloride and copper chloride the results are less clear. There is no significant difference in the 1M barium chloride at inhibiting either bacteria although for the 0.5M concentration there is a significant drop in the inhibition of *M.luteus* and not *S.albus.* This suggests that at lower concentrations, barium chloride is effective at inhibiting *S.Albus* but is less effective for *M.luteus.* Similarly, the higher concentration of copper sulphate inhibits both types of bacteria with no significant difference. Although, with a T-value of 8.22 much greater than the critical value (2.02), *M.luteus* is deemed significantly more sensitive to lower concentrations of copper sulphate than *S.albus*.

Evaluation

Method Evaluation

The method using agar plates was a success as a large number of repeats could be conducted in a short space of time, increasing the overall reliability of results. Although, there were cases where the line between the ring of clarity and the surrounding bacterial growth was unclear and so the area values recorded could be inaccurate. To eliminate this systematic error, I was consistent in what I counted as the ring of clarity for all repeats.

The fairly large squares on graph paper made measuring small areas, like those for silver nitrate, more inaccurate as they were quoted the nearest $4mm^2$. This could be improved by using graph paper with smaller squares and a backing light for clarity on the inhibited area.

Contamination from surrounding bacteria could form a smaller ring of clarity. This could explain any anomalously small zones of inhibition for example the $84mm^2$ area recorded for 0.5M barium chloride on *S.albus* (See Appendix F) in comparison to its mean of $213mm^2$ in Table 1. However, these anomalies were minimal in my experiment as I ensured that aseptic technique was maintained throughout.

A drip test was used to remove any excess solution from the filter paper discs. This is an inconsistent way of measuring the volume of salt solution therefore a fixed volume of salt solution would need to be added to each disc to improve the reliability of my results.

Evaluating Sample Size

In my experiment I was able to conduct 30 repeats for each solution at each concentration and this minimised the impact of anomalies on the mean. The sample size was also large enough to conduct a T-test for further analysis. In doing the statistical test, I can now deduce that each metal had a different impact on the growth of the two types of bacteria. However, a larger sample size would be beneficial to the statistical test as it would help support and validate these results.

The cost of silver nitrate solution was too large to access at higher concentrations therefore its antimicrobial effectiveness in comparison to the other salts is uncertain, limiting the conclusion of this experiment. At very low concentrations, silver nitrate had a reasonable impact on inhibiting bacterial growth although it is hard to predict how this will change at much higher concentrations. This means a further experiment involving a larger sample of silver nitrate solutions including 1M and 0.5M would better allow me to compare it to copper and barium solutions used.

Problems of Repeatability and Control Using Living Organisms

Even with sufficient measures in place to prevent the entry of bacteria form the surroundings into the experiment, there are still modes of contamination that are hard to avoid, increasing the possibility of anomalous data. The repeatability of my experiment is also limited to an identical source of bacteria to maintain consistency due to variation amongst bacterial populations.

The two bacteria used in the experiment; *Staphylococcus albus* and *Micrococcus luteus* are non-pathogenic and therefore non-disease causing. It needs to be considered that these bacteria were used purely as model organisms for pathogenic bacteria. Hence there is doubt whether the metallic ions of copper, barium and silver would be as effective at inhibiting the growth of pathogenic bacteria.

Evaluation of Online Sources

Information used in this essay comes from a range of scientific sources online. I focused on sources aimed at academics and sources published by companies credible and relevant to the research I was conducting. Many of the sources used in this essay were cited by a range of online publications. The publication date of sources in this essay varied significantly which impacted their currency. I therefore took care in extracting information from older sources or those without a date of publication.

Conclusion:

This experiment investigated the research question: To what extent do the antimicrobial properties of metals copper, barium and silver aid the inhibition of biofilm growth in hospitals?

In general, increasing the concentration of metal ions did increase the subsequent zone of inhibition except for the inhibition of *S.albus* by barium chloride and M.luteus by copper sulphate where there was little change. In a future investigation I would test a wider range of salt concentrations to get a better understanding of how their concentration may impact the growth of bacteria.

Based on the results of this experiment I deduced that copper was the most effective metal ion at inhibiting bacterial growth of *S.ablus* and *M.luteus*. This conclusion is supported by the increasing use of Antimicrobial Copper as a surface in hospitals to reduce bacterial growth of *MRSA, Staphylococcus aureus, Enterobacter aerogenes, Pseudomonas aeruginosa,* and *E. coli*[14]. The salt containing barium ions was the next most effective inhibitor of bacterial growth, followed by silver nitrate. Although, a T-test concluded that the effectiveness of these metals were significantly different for inhibiting the growth of gram-positive *S.albus* versus gram-negative *M.luteus*. The test revealed that silver and barium ions were generally better at inhibiting the growth of *S.albus* than *M.luteus* whereas copper ions were more effective at inhibiting the growth of *M.luteus* than *S.albus*.

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[14] Antimicrobialcopper.org. (Anon n.d.). *How it Works | Antimicrobial Copper Site*.

Further Questions

After completing this experiment, I explored the applications that metals silver, barium and copper could have in a medical environment to prevent bacterial infections. The antimicrobial properties of metals suggest that they can be used in hospitals to maintain clean surfaces although the extent that metal ions can diffuse from solid metal surface into the surroundings needs further investigation. Internal administration of metal ions to treat infections could cause equal damage to human cells as for the targeted bacteria, restricting their use. If proven safe, metals could provide a step ahead of resistant strains of bacteria in hospitals, potentially solving a widespread medical issue.

Resistant forms of bacteria such as MRSA can also harbour themselves within biofilms. “Biofilms are densely packed communities of microbial cells that grow on living or inert surfaces and surround themselves with secreted polymers” [15]. They therefore provide a reservoir for the growth of resistant bacteria impenetrable by antibiotics due to the surrounding polymers. Consequently I would question the role that metals play in dismantling these biofilms to treat chronic wounds and dental plaque[16].

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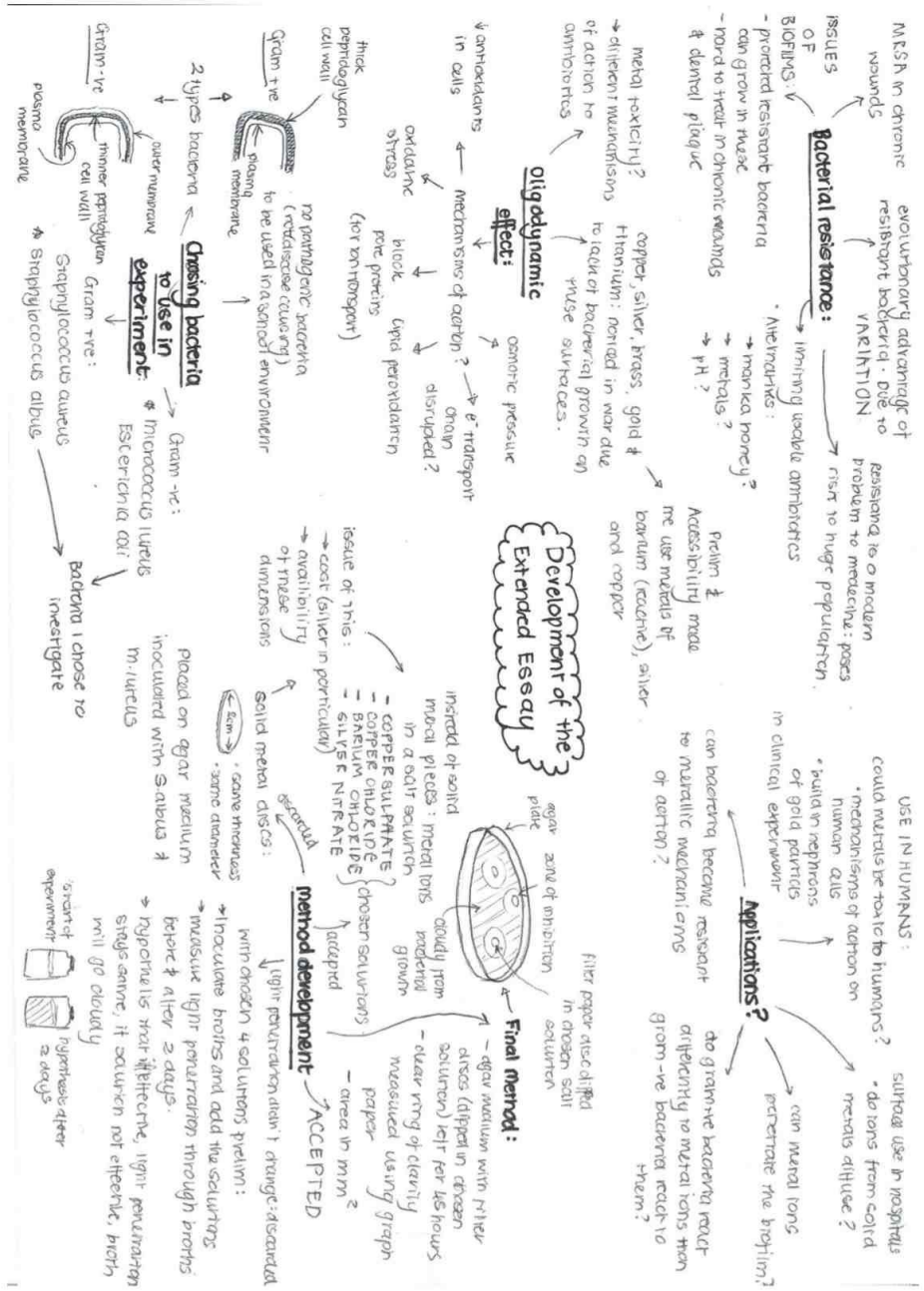
Appendices:

Appendix A:

Reflection Space:



Appendix B:

List of apparatus used in the experiment:

- 160 agar plates
- 2 x 10cm^3 sterile syringes
- 10 sterile plastic spreaders
- 480 x 6mm sterile filter paper discs
- 10 sterilised sharp end forceps
- A bunsen burner
- A heatproof mat
- Safety goggles
- A lab coat
- Gloves
- Alcohol wipes
- A biohazard bag

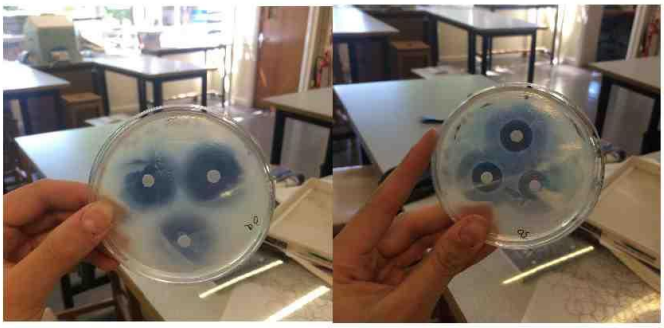
Appendix C:

A list of solutions used in the experiment:

- 350cm^3 nutrient broth inoculated with *M.luteus* in a conical flask with a sponge stopper.
- 350cm^3 nutrient broth inoculated with *S.albus* in a conical flask with a sponge stopper.
- 100ml of 1M and 100ml of 0.5M barium chloride in sealable glass jars.
- 100ml of 1M and 100ml of 0.5M copper chloride in sealable glass jars.
- 100ml of 1M and 100ml of 0.5M copper sulphate in sealable glass jars.
- 100ml of 0.025M and 100ml of 0.0125 silver nitrate in sealable glass jars.
- 250cm^3 beaker containing 200cm^3 of Virkon solution.

Appendix D:

Photos demonstrating the zones of inhibition produced by copper sulphate (1M) solutions:



Appendix E:

Risk assessment

Bacterial growth: there is a risk that possibly pathogenic bacteria from the surrounding of the experiment enters an agar plate. This could result in the growth of a harmful type of bacteria therefore gloves, goggles and a lab coat should be worn at all times and the work bench must remain sterile.

Burns: when working in such close proximity to a Bunsen burner to maintain a zone of sterility there is also a risk of burns. To prevent burns; allow any heated apparatus to cool before handling, use a heatproof mat and make sure that if the Bunsen is left unattended that it is turned off or left on a safety flame.

Virkon solution and alcohol wipes: These are both very flammable therefore must not be used near a lit Bunsen burner. To prevent a fire, ensure that the virkon solution and alcohol wipes are kept away from any flame and must not be used within short time before lighting a flame. For extra care in case of a fire, ensure that a fire extinguisher or fire blanket is at hand.

Appendix F:

Raw data tables for the zone of inhibition produced by each salt solution on *S.albus* and *M.luteus.*

S.albus

Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $BaCL_2$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $CuCl_2$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $CuSO_4$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $AgNO_3$ (M)
224	1	500	1	400	1	68	1
228	1	476	1	452	1	104	1
244	1	464	1	428	1	76	1
128	1	448	1	292	1	88	1
184	1	540	1	292	1	72	1
316	1	488	1	328	1	92	1
208	1	632	1	428	1	88	1
192	1	468	1	408	1	72	1
240	1	592	1	480	1	80	1
172	1	504	1	480	1	88	1
172	1	556	1	464	1	76	1
296	1	584	1	420	1	84	1
276	1	432	1	488	1	92	1
276	1	536	1	436	1	124	1
192	1	552	1	452	1	92	1
220	1	576	1	504	1	80	1
152	1	540	1	448	1	100	1
168	1	504	1	508	1	92	1
192	1	568	1	424	1	84	1
168	1	592	1	368	1	72	1
140	1	556	1	364	1	88	1
188	1	652	1	372	1	144	1
84	1	476	1	420	1	136	1
264	1	540	1	444	1	100	1
240	1	572	1	408	1	96	1
212	1	560	1	440	1	84	1
232	1	536	1	448	1	112	1

Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $BaCL_2$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $CuCl_2$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $CuSO_4$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $AgNO_3$ (M)
268	1	556	1	444	1	136	1
220	1	540	1	456	1	124	1
212	1	564	1	440	1	100	1
220	0.5	316	0.5	340	0.5	92	0.5
204	0.5	320	0.5	344	0.5	96	0.5
212	0.5	396	0.5	336	0.5	76	0.5
300	0.5	332	0.5	260	0.5	64	0.5
264	0.5	292	0.5	196	0.5	84	0.5
268	0.5	256	0.5	204	0.5	76	0.5
164	0.5	356	0.5	236	0.5	72	0.5
252	0.5	380	0.5	236	0.5	68	0.5
224	0.5	348	0.5	272	0.5	112	0.5
236	0.5	336	0.5	340	0.5	88	0.5
224	0.5	312	0.5	384	0.5	84	0.5
172	0.5	376	0.5	380	0.5	84	0.5
232	0.5	216	0.5	268	0.5	84	0.5
124	0.5	184	0.5	276	0.5	92	0.5
208	0.5	208	0.5	260	0.5	72	0.5
164	0.5	136	0.5	208	0.5	76	0.5
136	0.5	184	0.5	220	0.5	72	0.5
192	0.5	184	0.5	236	0.5	68	0.5
212	0.5	156	0.5	196	0.5	68	0.5
216	0.5	200	0.5	176	0.5	96	0.5
244	0.5	144	0.5	220	0.5	88	0.5
256	0.5	140	0.5	252	0.5	96	0.5
184	0.5	144	0.5	276	0.5	84	0.5
152	0.5	160	0.5	264	0.5	80	0.5
184	0.5	120	0.5	272	0.5	72	0.5
188	0.5	140	0.5	224	0.5	84	0.5
208	0.5	148	0.5	276	0.5	72	0.5
268	0.5	428	0.5	260	0.5	64	0.5
256	0.5	356	0.5	228	0.5	72	0.5
224	0.5	288	0.5	276	0.5	68	0.5

M.luteus

Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $BaCL_2$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $CuCl_2$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $CuSO_4$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $AgNO_3$ (M)
208	1	524	1	488	1	76	1
208	1	408	1	560	1	68	1
212	1	420	1	528	1	84	1
156	1	508	1	508	1	68	1
284	1	540	1	460	1	64	1
212	1	604	1	524	1	68	1
196	1	480	1	580	1	108	1
236	1	584	1	600	1	88	1
108	1	540	1	584	1	108	1
216	1	536	1	512	1	60	1
144	1	560	1	608	1	52	1
120	1	464	1	584	1	64	1
232	1	592	1	640	1	68	1
244	1	576	1	604	1	68	1
248	1	536	1	412	1	72	1
236	1	616	1	472	1	72	1
104	1	632	1	412	1	76	1
188	1	628	1	468	1	68	1
164	1	356	1	536	1	76	1
192	1	432	1	580	1	84	1
168	1	416	1	468	1	120	1
172	1	476	1	572	1	80	1
192	1	468	1	768	1	84	1
216	1	572	1	500	1	76	1
160	1	596	1	588	1	56	1
164	1	596	1	564	1	68	1
164	1	692	1	476	1	68	1
236	1	492	1	620	1	76	1
164	1	696	1	616	1	80	1
208	1	540	1	480	1	72	1
104	0.5	600	0.5	424	0.5	72	0.5
120	0.5	520	0.5	368	0.5	56	0.5
84	0.5	516	0.5	376	0.5	64	0.5

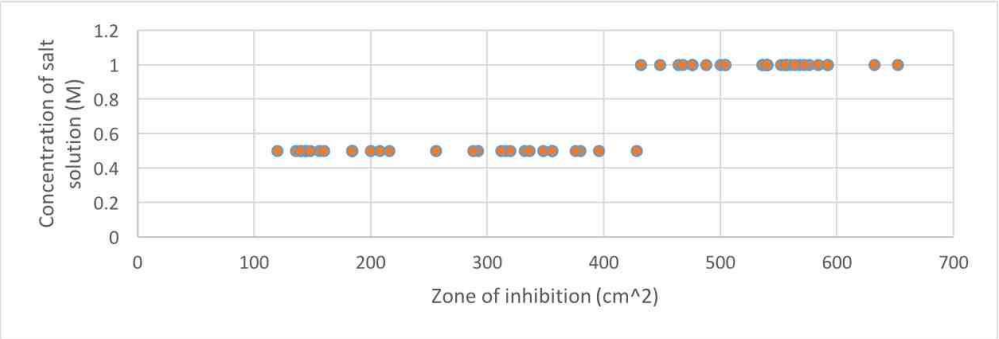
Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $BaCL_2$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $CuCl_2$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $CuSO_4$ (M)	Zone of inhibition $(mm^2) \pm 4mm^2$	Conc-entration $AgNO_3$ (M)
68	0.5	524	0.5	300	0.5	44	0.5
44	0.5	500	0.5	284	0.5	56	0.5
60	0.5	544	0.5	276	0.5	72	0.5
84	0.5	588	0.5	348	0.5	64	0.5
108	0.5	644	0.5	296	0.5	60	0.5
68	0.5	604	0.5	344	0.5	64	0.5
96	0.5	596	0.5	292	0.5	68	0.5
68	0.5	592	0.5	312	0.5	76	0.5
60	0.5	508	0.5	384	0.5	64	0.5
76	0.5	516	0.5	372	0.5	60	0.5
68	0.5	616	0.5	412	0.5	60	0.5
64	0.5	524	0.5	396	0.5	60	0.5
84	0.5	620	0.5	380	0.5	56	0.5
80	0.5	572	0.5	428	0.5	72	0.5
68	0.5	636	0.5	512	0.5	36	0.5
116	0.5	628	0.5	404	0.5	60	0.5
56	0.5	552	0.5	396	0.5	52	0.5
68	0.5	616	0.5	536	0.5	64	0.5
132	0.5	596	0.5	488	0.5	60	0.5
68	0.5	600	0.5	476	0.5	56	0.5
140	0.5	760	0.5	432	0.5	64	0.5
108	0.5	580	0.5	476	0.5	76	0.5
116	0.5	628	0.5	528	0.5	60	0.5
72	0.5	568	0.5	456	0.5	56	0.5
148	0.5	536	0.5	500	0.5	68	0.5
144	0.5	492	0.5	476	0.5	60	0.5
100	0.5	604	0.5	448	0.5	84	0.5

Appendix G:

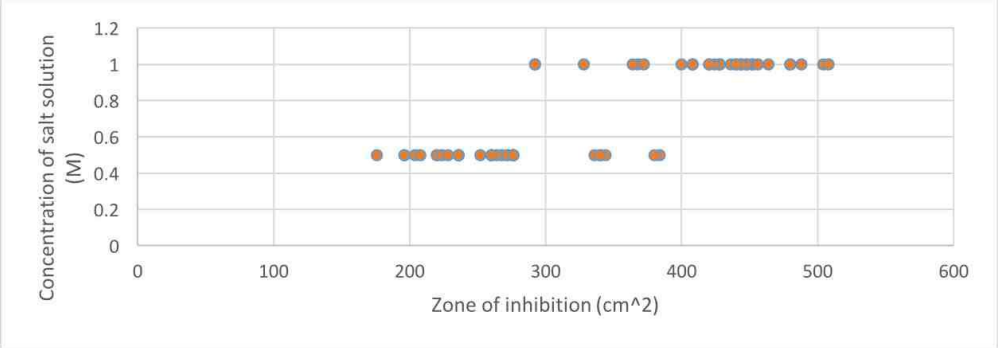
Graphs showing the spread of raw data for the zones of inhibition produced by two concentrations of each salt solution on the bacteria *S.albus* and *M.luteus*.

S.albus

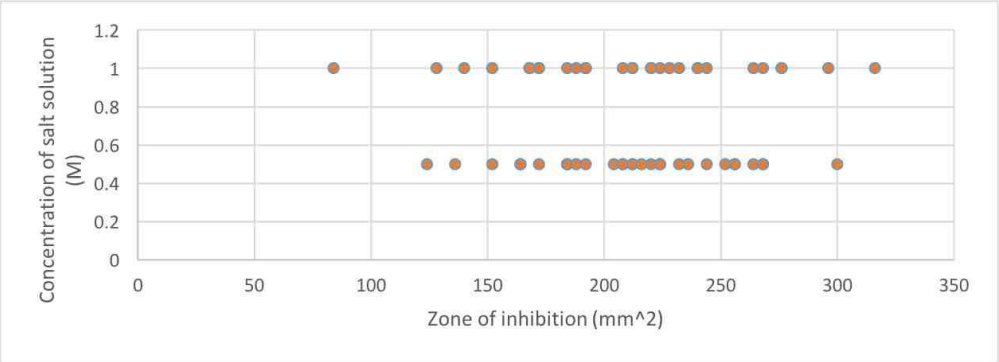
Copper chloride solutions:



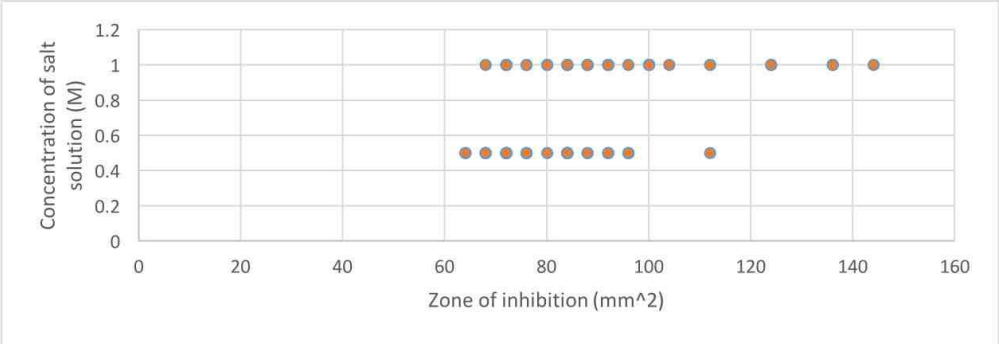
Copper Sulphate solutions:



Barium Chloride solutions:

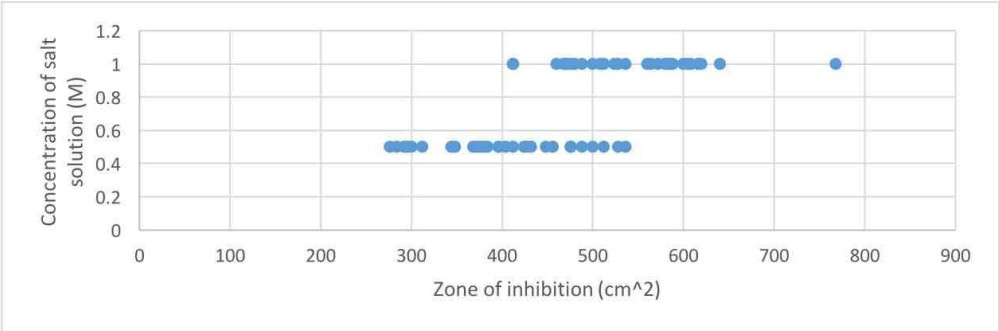


Silver nitrate solutions:

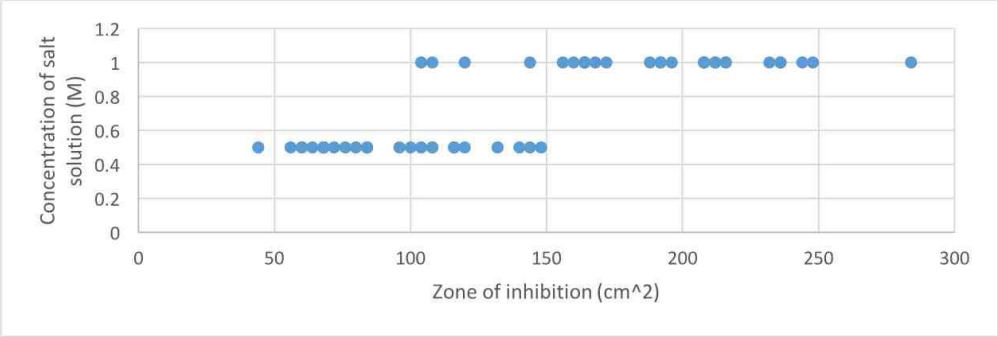


M.luteus

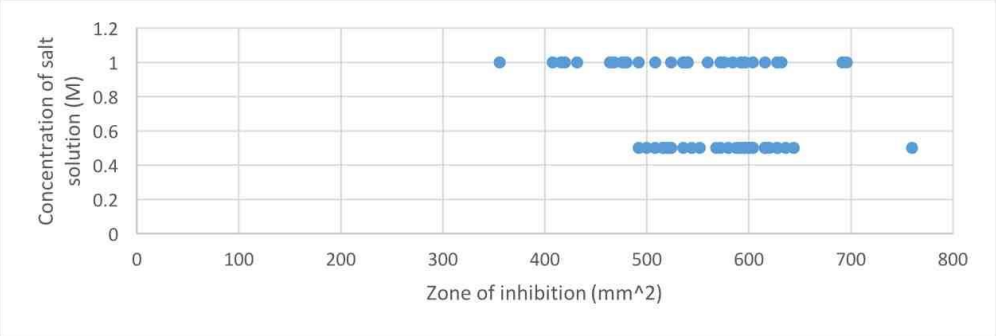
Copper sulphate solution:



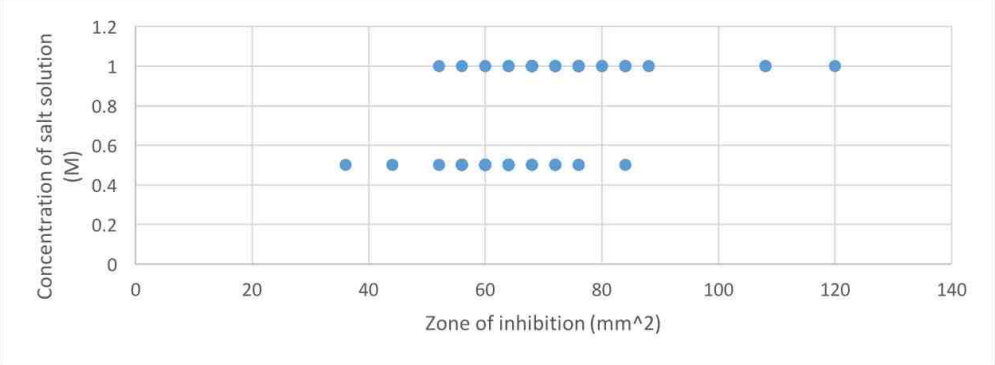
Barium Chloride solution:



Copper chloride solutions:



Silver nitrate solutions:



Appendix H:

Table of critical values

Degrees of Freedom	p=0.05	p=0.025	p=0.01	p=0.005
1	12.71	25.45	63.66	127.32
2	4.30	6.20	9.92	14.09
3	3.18	4.17	5.84	7.45
4	2.78	3.50	4.60	5.60
5	2.57	3.16	4.03	4.77
6	2.45	2.97	3.71	4.32
7	2.36	2.84	3.50	4.03
8	2.31	2.75	3.36	3.83
9	2.26	2.68	3.25	3.69
10	2.23	2.63	3.17	3.58
11	2.20	2.59	3.11	3.50
12	2.18	2.56	3.05	3.43
13	2.16	2.53	3.01	3.37
14	2.14	2.51	2.98	3.33
15	2.13	2.49	2.95	3.29
16	2.12	2.47	2.92	3.25
17	2.11	2.46	2.90	3.22
18	2.10	2.44	2.88	3.20
19	2.09	2.43	2.86	3.17
20	2.09	2.42	2.84	3.15
21	2.08	2.41	2.83	3.14
22	2.07	2.41	2.82	3.12
23	2.07	2.40	2.81	3.10
24	2.06	2.39	2.80	3.09
25	2.06	2.38	2.79	3.08
26	2.06	2.38	2.78	3.07
27	2.05	2.37	2.77	3.06
28	2.05	2.37	2.76	3.05
29	2.04	2.36	2.76	3.04
30	2.04	2.36	2.75	3.03
40	2.02	2.33	2.70	2.97
60	2.00	2.30	2.66	2.92
120	1.98	2.27	2.62	2.86
infinity	1.96	2.24	2.58	2.81

http://www.ruf.rice.edu/~bioslabs/tools/stats/ttable.html