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Examining and comparing the effect of metalsat different concentrations on the inhibition of Escherichia coli

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Abstract

Even in trace amounts, heavy metals can cause harm to the ecosystem. In reaction to heavy metals, bacteria express a specific set of genes. Among them are genes associated with heat shock, SOS, and oxidative stress regulons. Several genes unique to cadmium stress are upregulated in Escherichia coli cells when exposed to this metal, a phenomenon not seen in response to other stressors. We searched for genes that were induced in the presence of cadmium, but not by heat shock or oxidative stress, using random in vivo translational gene fusion. This allowed us to identify these genes and learn about their regulation. The need to discover substitutes for conventional medicine has grown in tandem with the rise of antibiotic resistance. Metalworking is an integral part of Indian culture. Silver cups and copper cutlery are standard issue at my home.From ancient times, metals have been used therapeutically in Ayurveda for their health advantages.I wanted to do additional research and examine the inhibitory levels of different metals at different concentrations on bacteria because, according to previous studies, these metals have antibacterial characteristics.

Keywords: Escherichia coli, Heavy Metals, Bacteria, Gene Fusion, Antibacterial Characteristics, Extracellular Polymeric Substances.

1. Introduction

Microorganisms have recently received attention for heavy metal removal as a result of the requirement for ecologically acceptable method. Metal ions have a tendency to react with the polysaccharides, proteins, lipids, and functional groups found in the bacterial cell wall. There are a number of benefits to using adsorbents, including their low production of biological and chemical sludge, their accessibility, abundance, and affordability. Additionally, adsorbents can recover biosorbents and metals, and they do not produce secondary contaminants. Bacteria are a dependable and effective biosorbent due to their many desirable traits, including a high surface-to-volume ratio, the ability to produce macromolecules known as extracellular polymeric substances (EPSs), and their widespread presence in a variety of environments.

On their own, suspended biofilms can't absorb contaminants continuously, and they could leak out of the reactor, reducing efficiency and causing operational issues. As a result, biofilm preservation is best accomplished using fixed beds. Materials having porous architectures and adsorption capabilities for pollutants and biofilms are good choices. The zeolite structure's negative charge is balanced by bonding with exchangeable cations, leading to a strong propensity for metal cation transfer.

The gastrointestinal microbiome of humans and other animals, the environment, and contaminated food all contain E. coli and its diverse species group of gram-negative bacteria. Although the majority of E. coli strains pose little threat to people, there are a few that are opportunistic pathogens that can inflict a variety of ailments

in both animals and humans, including diarrhea, UTIs, respiratory disorders, pneumonia, and more. Due to its extensive presence in the digestive systems of mammals, reptiles, wild birds, the environment, and food, E. coli faces several challenges and evolves in response to natural selection.

Due to its capacity to accumulate genes giving resistance to numerous clinically-relevant antimicrobial drugs, E. coli antibiotic resistance is becoming an ever-greater worry for public health professionals. Carrying ARGs and HMRGs on the same mobile components is commonly linked, according to studies. Heavy metal resistance was not previously identified in samples from the Salish Sea environment that had been examined for antibiotic resistance in an earlier exploratory investigation. The samples in question had come from both water and animals, and some of them had come from Superfund sites in the area.

2. Background Information

One kind of bacterium is the rod-shaped, gram-negative Escherichia coli (E. coil). Many animals have this bacterium in their intestines, and it helps with digestion quite a bit. The majority of dangerous strains are hazardous because they produce Shiga. Indigestion of spoiled food is a potential entry point for certain E. coil strains into the human body. Human diarrhoea can result from bacterial infections that impact the small intestine. While in most cases this isn't a serious health risk, and at most can cause dehydration, nevertheless in some cases this can lead to severe and life-threatening situations.[1]

Antibiotics are used for treating bacterial infections. Resistance to antibiotics in bacteria occurs when the bacteria develops the ability to defeat the medicine. This resistance can end up becoming death threatening, limiting medical options to treat/defeat the bacteria[2] and threatening our ability to treat common infections with new resistance mechanism emerging and spreading. These issues make it important for us to find alternatives to general medicine.[3] Escherichia coli has been of concern for a long time, this bacteria shows great potential to produce resistant genes through horizontal gene transfer[4].

Copper is shown to have immense benefits to one's health. Ayurveda recommends the storage of water in copper tanks, even before understanding the science behind it.The mechanism of action of copper is still debatable various theories include the interaction of the particles with the cell/plasma membrane making it prone to damage from copper ions through its destruction. Another method includes the generation of reactive oxygen species byundergoing reduction resulting in multiple irreversible damages. The final mechanism is the damage of the membrane and infiltration through the release of copper ions.[5] Silver can be seen being used all over, from making utensils to jewelry. Silvers mechanism of action in bacteria is through making holes in the bacterial membrane and binding to cell components making it nearly impossible for the cell to performs its functions.[6]

The metal, zinc while essential to the microorganism in small quantities, it is equally destructive in high quantities,inhibiting the growth of the bacteria. Its mechanism is through changing the conformation of calcium

[1]"E. Coli: What is It, How Does It Cause Infection, Symptoms & Causes." *Cleveland Clinic*, my.clevelandclinic.org/health/diseases/16638-e-coli-infection. Accessed 4 Jan. 2023.
[2]"What Exactly is Antibiotic Resistance?" *Centers for Disease Control and Prevention*, 5 Oct. 2022, www.cdc.gov/drugresistance/about.html. Accessed 4 Jan. 2023.
[3]"Antibiotic Resistance." *World Health Organization (WHO)*, 31 July 2020, www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. Accessed 4 Jan. 2023.
[4]"Antimicrobial Resistance in Escherichia Coli." *PubMed*, Accessed 4 Jan. 2023.
[5]"Copper As an Antimicrobial Agent: Recent Advances - RSC Advances (RSC Publishing) DOI:10.1039/D1RA02149D." *RSC Publishing Home – Chemical Science Journals, Books and Databases*, 19 May 2021, pubs.rsc.org/en/content/articlehtml/2021/ra/d1ra02149d. Accessed 4 Jan. 2023.

[6]"Silver Turns Bacteria into Deadly Zombies." *Science | AAAS*, www.science.org/content/article/silver-turns-bacteria-deadly-zombies. Accessed 4 Jan. 2023.

binding sites, by acting as a competitor.[7] Zinc can be seen inhibiting the SOS response produced by the cell when there is damage to the DNA.[8]

Due to the metals effect on E.coil and their repeated use in daily life, I decided to move forward with the above metals at different concentrations to find the minimum concentration for the optimum zone of inhibition. Due to the lack of resources in the school lab an alternative of metal compounds were taken. At lower concentrations studies show that metals can help in the development of the bacteria rather than the inhibition, hence multiple concentrations were taken.[9]

3. **Research Question:** How effective are Copper sulphate pentahydrate, Sliver nitrate extrapure, and Zinc nitrate hexahydrate at 0.3 moles, 0.5 moles and 1.0 moles on the inhibition of bacteria (Escherichia coli) measured by the zone of inhibition in cm?

Experimental Hypothesis 1: Copper sulphate pentahydrate, Sliver nitrate extrapure, and Zinc nitrate hexahydrateshow inhibition on Escherichia coli

Null Hypothesis 1: Copper sulphate pentahydrate, Sliver nitrate extrapure, and Zinc nitrate hexahydrateshow no significant signs of inhibition on Escherichia coli

Experimental Hypothesis 2: Inhibition of E. coil increases as the concentrations of Copper sulphate pentahydrate, Sliver nitrate extrapure, and Zinc nitrate hexahydrateincrease.

Null Hypothesis 2: Copper sulphate pentahydrate, Sliver nitrate extrapure, and Zinc nitrate hexahydrate show no significant inhibition on E. coil as the concentration increases.

Experimental Hypothesis 3: Copper sulphate pentahydrate shows a greater inhibition compared to Sliver nitrate extrapure and Zinc nitrate hexahydrate.

Null Hypothesis 3: Copper sulphate pentahydrateshows no significant difference

Variables:

Independent Variable	Copper sulphate pentahydrate Sliver nitrate extrapure Zinc nitrate hexahydrate
Dependent Variable	Zone of Inhibition in cm

4. **Controlled Variables**

Variable Controlled	Reason	Method
Concentration of Agar produced	The concentration of the agar has an impact on the rate of metal diffusion and bacterial growth.	The same proportion as recommended by the manufacturer's instructions
E. coil spread on the plate	The findings may change depending on how much spread there is and how much bacteria form.	Equal amounts of bacteria were collected using a micropipette for each petri dish.

[7]"Zinc Treatment is Efficient Against Escherichia Coli α-haemolysin-induced Intestinal Leakage in Mice." *PubMed Central (PMC)*, www.ncbi.nlm.nih.gov/pmc/articles/PMC5374507/. Accessed 4 Jan. 2023.

[8]"Zinc Blocks SOS-induced Antibiotic Resistance Via Inhibition of RecA in Escherichia Coli." *PLOS*, 22 May 2017, journals.plos.org/plosone/article?id=10.1371/journal.pone.0178303. Accessed 4 Jan. 2023.

[9]"Metal Homeostasis and Resistance in Bacteria." *PubMed Central (PMC)*, www.ncbi.nlm.nih.gov/pmc/articles/PMC5963929/. Accessed 19 Apr. 2023.

Radius of wells	For the metal solution to diffuse and distribute equally	To verify that the diameter of each well is the same, one cork borer is used
Time of bacterial growth	Similar rates of reproduction and bacterial populations	After adding the extracts, the all petri dishes are incubated for 48 hours with the bacteria
Volume of metal solution poured into well	A metal solution with varying concentrations could change the results and interfere with the inhibition, distorting the values.	Equal amounts of solution were collected with a micropipette for each petri dish.
Incubation temperature	Bacterial growth is temperature-dependent.	48 hours of incubation at 27°C for all petri dishes.
Autoclave conditions	To avoid any contamination	Sterilize using an autoclave at 121°C and 15 psi.

5. **Material Required:**

Apparatus	**Quantity**	**Uncertainty**
Measuring cylinder- 100ml	1	± 0.1 mL
Measuring cylinder- 10ml	3	± 0.02 mL
Weighing digital balance	1	±0.05 g
Spatula	1	-
Conical flask	1	-
Heating plate 10 x 12 1500W	1	-
Petri dish	30	-
Oven	1	
Laminar airflow chamber	1	-
Glass rod- stirrer	1	-
Cork borer	1	-
Autoclave	1	
Inoculation loop	1	-
Spirit lamp/candle	1	-
Spirit (20ml)	20ml	-
Match box	1	-
Micropipette	1	± 0.2 µl
Micropipette Tips	9	-
Vernier calliper	1	±0.5 mm
Test Tubes	3	-
HIMEDIA Nutrient agar	52.65g	-
Distilled Water	2000 ml	-
Copper sulphate pentahydrate	5g	-
Sliver nitrate extrapure	5g	-
Zinc nitrate hexahydrate	5g	-
Methanol	150ml	-
Gloves	1 Pair	-

Microorganism Required:

Microorganism	Escherichia coli

Metal Compound	0.3 moles	0.5 moles	1.0 moles
Copper sulphate pentahydrate (5ml)	$\frac{5 \times 0.3 \times 249.68}{1000} = 0.37$	$\frac{5 \times 0.5 \times 249.68}{1000} = 0.62$	$\frac{5 \times 1.0 \times 249.68}{1000} = 1.25$
Zinc nitrate hexahydrate (5ml)	$\frac{5 \times 0.3 \times 297.5}{1000} = 0.45$	$\frac{5 \times 0.5 \times 297.5}{1000} = 0.74$	$\frac{5 \times 1.0 \times 297.5}{1000} = 1.49$
Sliver nitrate extrapure (1ml)	$\frac{1 \times 0.3 \times 169.87}{1000} = 0.05$	$\frac{1 \times 0.5 \times 169.87}{1000} = 0.08$	$\frac{1 \times 1.0 \times 169.87}{1000} = 0.17$

6. **Making metal solutions:**

1. Measure 0.37g, 0.62g and 1.25g of Copper sulphate pentahydrate using a digital weighing scale to produce 0.3 moles, 0.5 moles and 1.0 moles respectively and mix it thoroughly with 5 ml of water using a glass rod.
2. Measure 0.45g, 0.74g and 1.49g of Zinc nitrate hexahydrate using a digital weighing scale to produce 0.3 moles, 0.5 moles and 1.0 moles respectively and respectively and mix it thoroughly with 5 ml of water using a glass rod.
3. Measure 0.05g, 0.08g and 0.17g of Sliver nitrate extrapureusing a digital weighing scale to produce 0.3 moles, 0.5 moles and 1.0 moles respectively and mix it thoroughly with 1 ml of water using a glass rod.
4. Pour all 9 solutions in 9 separate test tubes and 1 test tube with 5ml of distilled water

Formula: $\frac{\text{Amount of ml required} \times \text{moles} \times \text{molar mass of metal compound}}{1000}$

Making Agar Solution:

1. Utilizing the measuring scale, weigh 14g of agar.
2. Combine 500 ml of water with the agar.
3. Fill the conical flask with the solution.
4. On a heating plate, heat the mixture to 100°F until bubbles appear on top.
5. Wrap aluminum foil and paper around the solution and fasten it with a rubber band.

Making Escherichia coli culture:

1. To make nutrient broth, combine 1.3 g of the substance with 100 ml of distilled water.
2. Use an autoclave to sterilize nutrient broth at 121°C and 15 psi.
3. Take one loop of culture from the master culture plate and incubate it for growth for 24 hr.
4. The culture is centrifuged after 24 hours, and the sedimented culture is diluted with water before being inoculated.

Main procedure:

1. Indicate the metal, moles, and trial on the petri dishes.
2. Newspaper-wrapped petri dishes
3. Autoclave the petri plates, agar solution, glass spreader, and cork borer.
4. Place the petri dish in the Laminar airflow chamber after washing and sanitizing your hands.
5. Fill the petri dishes halfway with the agar solution.
6. Give the solution 30 minutes to dry.
7. Pour 100 mL of the E. coil on top of the agar using the micropipette.
8. Apply the glass spreader to the E. coil.
9. To remove three evenly sized pieces of hardened agar, drill three holes in the cork.
10. Using the micropipette, insert 100 mL of the labelled solution into the perforations.

11. Put the petri plates in the incubator at 27°C after 10 minutes.
12. After 48 hours, indicate the inhibition zone.
13. Place the cover of the petri dish on top, then use a marker to label the clear spaces.
14. Measure the clean area's diameter using a Vernier caliper
15. Calculate the well's area using a Vernier caliper

7. Considerations:

- Light a candle in the air chamber while working to prevent contamination with airborne microbes.
- Wear a lab coat throughout the entire procedure.
- Clean hands with Sprite or methanol, wear gloves, and prevent contact with bacteria by utilising the air chamber.
- Use gloves when handling all three metal compounds, they are irritants and are harmful if orally indigested
- Keep Zinc nitrate hexahydrate away from flame
- Minimum amount of sliver nitrate was used because of its price on the market
- Autoclave all apparatus after procedure
- Animals were not harmed in the process
- All apparatus was autoclaved and disposed properly for the limitation of contamination

Qualitative data (Images in Appendeix- ii)

- After 24 hours of theE. coilbeing added to the nutrient broth, a cloudy white formation can be observed to show the growth of the bacteria
- The diffusion of the copper solution in the agar solution could be seen due to its blue tinted color
- Clear zones of inhibition could be seen just after 24 hours
- No solution was left after 48 hours and clear zones of inhibition of all three metals, increasing as the concentration of the solution can be seen
- Copper sulphate pentahydrate showed the highest zone of inhibition compared to the other metal compounds

8. Quantitative data:

The central tendency for the scattered data is provided to us by the trial's average.

$$\text{Mean} = \frac{\Sigma x}{n}$$

Σx: total of all values

n: quantity of values in the data collection

Our understanding of the data's distribution is provided by standard deviation

$$S = \sqrt{\frac{\sum(X - \overline{X})^2}{N - 1}}$$

S: The sample's standard deviation

X: Each value in the data set

$\overline{X}$: Mean of the values in data set

N: Number of values in data set

Sample Calculation:

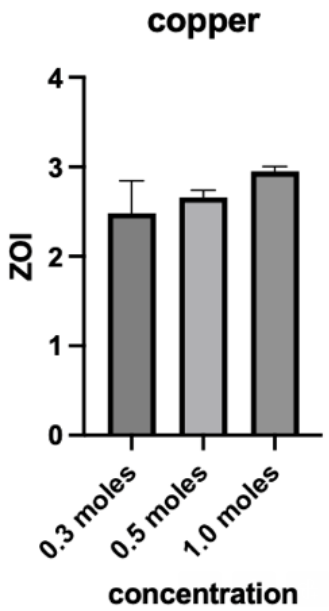
Mean: 2.2+2.3+2.0+2.8+2.8+2.8/6= 2.4833

Standard Deviation: $\sqrt{\frac{(2.2-2.4833)^2+(2.3-2.4833)^2+(2.0-2.4833)^2+(2.8-2.4833)^2+(2.8-2.4833)^2+(2.8-2.4833)^2}{6-1}}$

9. Data Analysis:

The statistical analysis made use of one-way ANOVA and the Tukey multiple comparisons test. "One-way ANOVA ("analysis of variance") compares the means of two or more independent groups in order to determine whether there is statistical evidence that the associated population means are significantly different[10]."“Tukey's multiple comparison test is one of several tests that can be used to determine which means amongst a set of means differ from the rest.[11]”

Copper sulphate pentahydrate (5ml) ±0.5mm			
Conc.	0.3 moles	0.5 moles	1.0 moles
Trial 1	2.2	2.6	3.0
Trial 2	2.3	2.6	2.9
Trial 3	2.0	2.7	2.9
Trial 4	2.8	2.8	2.9
Trial 5	2.8	2.65	3.0
Trial 6	2.8	2.6	3.0
Mean	2.48333333	2.65833333	2.95
STD. Dev	0.36009258	0.0801041	0.05477226



ANOVA summary Copper	
F	7.193
P value	0.0065
P value summary	**
Significant diff. among means (P < 0.05)?	Yes
R squared	0.4896

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
0.3 moles vs. 0.5 moles	-0.1750	-0.4979 to 0.1479	No	ns	0.3620
0.3 moles vs. 1.0 moles	-0.4667	-0.7896 to -0.1438	Yes	**	0.0051

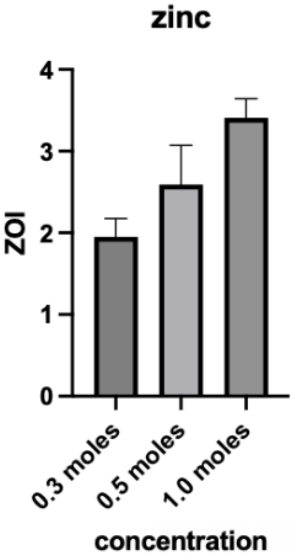
[10]"SPSS Tutorials: One-Way ANOVA." *LibGuides at Kent State University*, 1202, libguides.library.kent.edu/spss/onewayanova. Accessed 19 Apr. 2023.

[11]*Wiley-Blackwell*, www.blackwellpublishing.com/specialarticles/jcn_8_304.pdf. Accessed 19 Apr. 2023.

0.5 moles vs. 1.0 moles	-0.2917	-0.6146 to 0.03123	No	ns	0.0797

From the ANOVA summary, the F-value is 7.193, and the P-value is 0.0065. Since the P-value is less than 0.05, there is a significant difference between the means of the different concentrations. The results of Tukey's test show that there is no significant difference between the mean values of 0.3 moles and 0.5 moles. However, there is a significant difference between the mean values of 0.3 moles and 1.0 moles, as well as between 0.5 moles and 1.0 moles. The adjusted P-value for the significant differences is less than 0.05, indicating that the differences are statistically significant. In conclusion, the data analysis suggests that the concentration of the copper sulphate pentahydrate has a significant effect on the amount of the substance, and there are significant differences between the means of the different concentrations. We reject null hypothesis 1 and null hypothesis 2 and accept experimental hypothesis 1 and experiment hypothesis 2. Compared the other metals copper shows a greater inhibition, hence null hypothesis 3 in rejected and experimental hypothesis is accepted.

Zinc nitrate hexahydrate (5ml) ±0.5mm			
Conc.	0.3 moles	0.5 moles	1.0 moles
Trial 1	2.3	2.2	3.7
Trial 2	1.8	2.5	3.35
Trial 3	2.1	1.9	3.7
Trial 4	1.7	3.15	3.2
Trial 5	2.0	2.85	3.2
Trial 6	1.8	2.95	3.3
Mean	1.95	2.59166667	3.40833333
STD. Dev	0.2258318	0.4789746	0.2332738

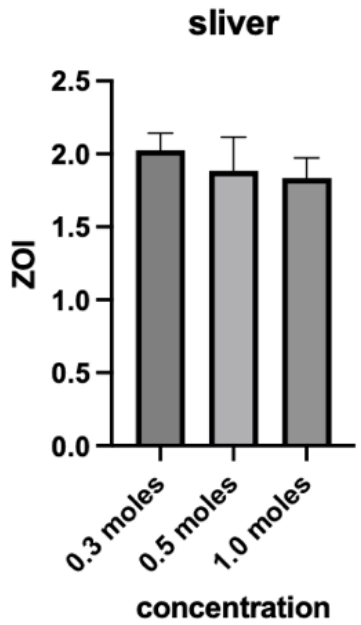


ANOVA summary Zinc	
F	28.72
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.7929

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
0.3 moles vs. 0.5 moles	-0.6417	-1.143 to -0.1407	Yes	*	0.0120
0.3 moles vs. 1.0 moles	-1.458	-1.959 to -0.9573	Yes	****	<0.0001
0.5 moles vs. 1.0 moles	-0.8167	-1.318 to -0.3157	Yes	**	0.0020

From the ANOVA summary, the F-value is 28.72, and the P-value is less than 0.0001. Since the P-value is less than 0.05, there is a significant difference between the means of the different concentrations. The results of Tukey's test show that there is a significant difference between the mean values of all three concentrations. The adjusted P-value for all the significant differences is less than 0.05, indicating that the differences are statistically significant. In conclusion, the data analysis suggests that the concentration of the zinc nitrate hexahydrate has a significant effect on the amount of the substance, and there are significant differences between the means of the different concentrations. We reject null hypothesis 1 and null hypothesis 2 and accept experimental hypothesis 1 and experiment hypothesis 2.

Sliver nitrate extrapure (1ml) ±0.5mm			
Conc.	0.3 moles	0.5 moles	1.0 moles
Trial 1	2.05	1.6	1.65
Trial 2	2.2	1.9	1.95
Trial 3	1.9	1.7	1.8
Trial 4	2.0	2.1	1.9
Trial 5	2.1	1.8	1.7
Trial 6	1.9	2.2	2
Mean	2.025	1.88333333	1.83333333
STD. Dev	0.11726039	0.23166067	0.14023789



ANOVA summary Sliver	
F	2.043
P value	0.1642
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.2141

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
0.3 moles vs. 0.5 moles	0.1417	-0.1138 to 0.3972	No	ns	0.3464
0.3 moles vs. 1.0 moles	0.1917	-0.06384 to 0.4472	No	ns	0.1596
0.5 moles vs. 1.0 moles	0.05000	-0.2055 to 0.3055	No	ns	0.8685

The ANOVA summary reports an F value of 2.043 and a p-value of 0.1642. Since the p-value is greater than 0.05, we can conclude that there is no significant difference among the means of the three concentrations. The R squared value is 0.2141, indicating that 21.41% of the variance in the data can be explained by the concentration of silver nitrate. Tukey's multiple comparisons test shows that there are no significant differences among any of the means at the 0.05 level of significance. The adjusted p-values for all comparisons are greater than 0.05, indicating that the differences observed in the means are likely due to random chance. In conclusion, based on the data provided, there is no significant difference among the means of the three concentrations of silver nitrate extrapure. We reject null hypothesis 1 and experimental hypothesis 2 accept experimental hypothesis 1 and null hypothesis 2.

10. Discussion

Due to the compound's antimicrobial effect, the area surrounding the disc is free of bacterial growth and is known as the zone of inhibition. better antibiotic activity and hence better efficiency are indicated by a broader zone of inhibition. According to earlier studies, Escherichia coli and other bacteria are resistant to the antibacterial effects of copper sulphate pentahydrate and silver nitrate extrapure. On the other hand, zinc nitrate hexahydrate has received less research on its antibacterial activity, yet it has been demonstrated to be somewhat efficient against bacteria.Given that the concentration is so low at 0.3 moles, it is anticipated that these compounds will not be very effective. However, it is anticipated that the compounds' potency would rise at 0.5 and 1.0 moles due to the increasing concentration.In a previous study, to assess the antibacterial activity of copper and zinc sulphates against Staphylococcus aureus and Escherichia coli, the researchers utilised the Kirby Bauer disc diffusion method. Similarly, to this investigation, it was discovered that copper sulphate was more successful at preventing the growth of Staphylococcus aureus and Escherichia coli than zinc sulphate. In the present investigation, it is shown that increasing concentrations leads in the increase of inhibition, similarly, the in vitro researchdemonstrated that increasing doses of copper and zinc sulphate solutions had strong antibacterial effect against these microorganisms[12].In another study sliver nanoparticles seem to show inihibtion, biologically prepared silver nanoparticles were found effective against biofilm-forming multidrug-resistant E. coli U12 on urinary catheters, this study not only validates the results gained from this investigation, but shows how this can be further researched for medical purposes.

11. Evaluation

Zinc nitrate hexahydrate, copper sulphate pentahydrate, and extra pure silver nitrate can all have their bacterial inhibitory effects measured using the zone of inhibition test.This method determines the area surrounding a disc that has been treated with a substance that inhibits bacterial growth. More antimicrobial activity is usually indicated by an enlarged zone of inhibition. The ability to test numerous compounds at once and the method's relative ease of use are two of its main advantages. The minimum inhibitory concentration (MIC) of an antimicrobial agent—the concentration at which the chemical stops microbiological growth—can also be determined using agar well diffusion. Because this investigation was carried out in an exceedingly clean environment and proper disposal procedures were followed, no infections were transmitted. In this experiment, we used multiple trials to minimize the impact of random mistake. The zone of inhibition test can be useful for determining how effective certain chemicals are at inhibiting bacterial growth, but it is not without its limitations and possible downsides. To start, the chemical concentration, bacterial kind and amount, and incubation conditions are just a few of the factors that affect the test.Because of this, the accuracy and reliability of the test could be jeopardized if any of these components were to undergo modification. The second issue is that factors other than antibacterial activity, such pH or osmotic stress, could be responsible for the observed inhibition, and the zone of inhibition test does not disclose the exact mechanism by which the chemical works on the bacteria. Using substances like copper sulphate pentahydrate, silver nitrate extrapure, and zinc nitrate hexahydrate to prevent bacterial proliferation in real environments can have negative impacts on both human and environmental health, even though these compounds have demonstrated antibacterial efficiency in test

[12]"Antimicrobial Activity of Copper Sulphate and Zinc Sulphate on Major Mastitis Causing Bacteria in Cattle." *Pharmacy Journal | Pharmaceutical Journal | The Pharma Innovation Journal*, www.thepharmajournal.com/archives/?year=2020&vol=9&issue=4&ArticleId=4574. Accessed 19 Apr. 2023.

tubes. For example, if these pesticides are used excessively, heavy metals can accumulate in the ground and water, which can pose a threat to both humans and animals. It is important to consider the test's limitations and possible downsides, as well as the practical use of Copper sulphate pentahydrate, Silver nitrate extrapure, and Zinc nitrate hexahydrate, even though the zone of inhibition test can provide some details about their antibacterial activity. Wearing protective gear, such as gloves, masks, and lab coats, might lessen the likelihood of accidents caused by its improper use.

12. Conclusion

In conclusion, the zone of inhibition can be used to assess how well Escherichia coli is inhibited by Copper sulphate pentahydrate, Silver nitrate extrapure, and Zinc nitrate hexahydrate. Based on the results we can conclude that all the metals showed signs of significant inhibtion and as the concentration increases the inihibtion increases.The most effective inhibitor is shown by copper, followed by zinc and silver.The investigation was proved to be successful and can help offer substitutes for anti-bacterial medications.

Further Scope

The potential applications of Copper sulphate pentahydrate, Silver nitrate extrapure, and Zinc nitrate hexahydrate in a variety of sectors, such as medicine, agriculture, and water treatment, can all be explored in more detail. These substances might be employed in the creation of fresh antibacterial agents or the production of improved versions of current ones. The utilisation of metal nanoparticles for their antibacterial properties is one area of research that has received a lot of attention lately.[13] Due to their enhanced surface area and distinctive physical and chemical properties, copper, silver, and zinc oxide nanoparticles have been found to exhibit more antibacterial activity than their bulk counterparts. The antibacterial properties of Copper sulphate pentahydrate, Silver nitrate extrapure, and Zinc nitrate hexahydrate may be improved by the addition of metal nanoparticles.For instance, adding copper nanoparticles to a coating substance may enhance its antibacterial characteristics and aid in preventing the growth of bacteria on surfaces. Additionally, the use of metal nanoparticles in medicine has produced encouraging outcomes for the treatment of diseases brought on by bacteria that are resistant to common antibiotics. While copper nanoparticles have been studied for their possible utility in the treatment of urinary tract infections brought on by Escherichia coli, silver nanoparticles have been employed in wound dressings and medical devices to avoid infection. For the prevention and management of infectious diseases, the creation of novel and effective antimicrobial drugs that can successfully attack drug-resistant bacteria is crucial.

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Appendix- i(Data)

Copper sulphate pentahydrate Partial Inhibition (5ml)		
0.3 moles	0.5 moles	1.0 moles
3.8	4.9	4.2
3.9	4.6	4.5
3.65	4.05	4.25
3.9	4.1	4.75
4.05	4.3	5
4.05	4.7	5

Appendix-ii (Images)