Cu Later: Balancing Copper Ion Release and Antibacterial Performance of Copper Alloys Against *Escherichia coli* K-12

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BACKGROUND

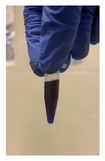
Copper-containing materials are widely used for their ability to passively inhibit bacterial growth through the release of **Cu^{2+} ions**, which disrupt **cellular membranes, proteins,** and **DNA**. However, excessive copper ion release can create **environmental** and **toxicological concerns**, especially in **aquatic systems**. Because copper is commonly used as an alloy rather than in pure form, alloying elements such as **zinc** or **tin** can alter **surface chemistry** and **copper ion release**, meaning **antibacterial effectiveness** may not scale directly with copper content.

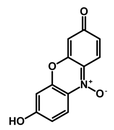
This study uses Escherichia coli K-12 as a **model organism** to examine how **copper alloy composition** influences bacterial response and **copper ion leaching**, providing context for designing **antimicrobial materials** that balance **effectiveness** with **environmental safety.**

Copper ions interacting with E. coli

- *Copper ions penetrate the bacterial cell surface*
- *The cell membrane breaks down and loses integrity*
- *Internal contents leak out as vital processes fail*
- *The bacterium can no longer survive*

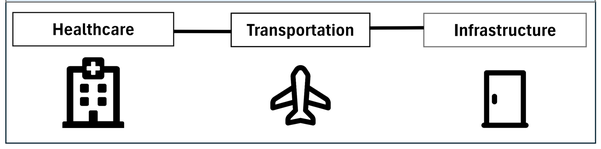
Resazurin Sample in Lab





- *Resazurin is a redox indicator whose chemical structure allows it to change color in response to bacterial metabolism.*
- *A color shift from blue to pink indicates viable, metabolically active cells.*

Copper-Based Antimicrobials in Public Environments

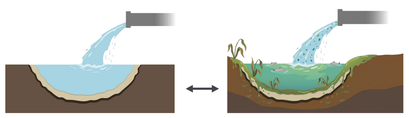


PROBLEMS

- Copper content **varies** widely among commercial alloys
- Antibacterial performance is often **assumed** to scale directly with copper percentage
- Excessive copper ion leaching poses **environmental** and **ecological** risks
- Comparative data evaluating efficacy versus ion leaching is **limited**
- The relationship between copper alloy composition and **environmentally** relevant Cu^{2+} release thresholds remains **poorly characterized**.

PURPOSE

Balancing Antibacterial Efficacy with Environmental Safety



Primary: Do alloys with higher copper content show stronger antibacterial effects on *E. coli* K-12, measured by resazurin reduction?

Secondary: How does bacterial presence influence copper ion leaching from alloys containing different copper percentages over time?

Goal: Investigate and pin-point an optimal window of bacteria inhibition and **Cu^{2+}** release.

HYPOTHESIS

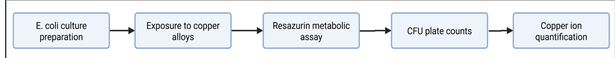
If the copper content of a metal alloy **increases**, then antibacterial activity against E. coli K-12 will **increase** due to enhanced Cu^{+2} ion release.

However, **brass** will achieve antibacterial effects while releasing **lower** concentrations of copper ions than **pure copper.**

All Images, Diagrams, and Visuals were created by the researcher through BioRender.com or PowerPoint software.
All Graphs were coded by researcher in IntelliJ software.

METHODOLOGY

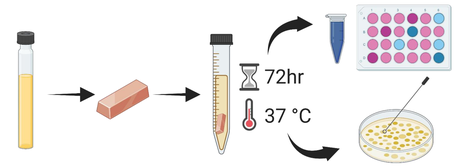
Experimental Outline



Independent Variable:	Percent of copper in metal (zinc, brass, bronze, copper)
Dependent Variable:	Bacteria viability, CFU/mL, metabolic activity, and Cu^{2+} concentration
Biological Control	*E. coli* + LB broth
Chemical Control	Metal + LB broth

Zinc was used as a non-Cu metal control to isolate copper-specific antibacterial and leaching effects.

Assay Overview



1. Alloy exposure = antibacterial effects
2. Cu^{2+} quantification = Cu^{2+} released
3. Resazurin assay = cell viability
4. CFU plate count = viable cells following alloy exposure

Scale of Metabolic Activity

1 Alloy Exposure

E. coli Exposure to Copper Ions

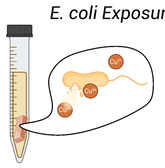


Illustration of the proposed mechanism by which copper inhibits E. coli, through surface driven Cu^{2+} ion release

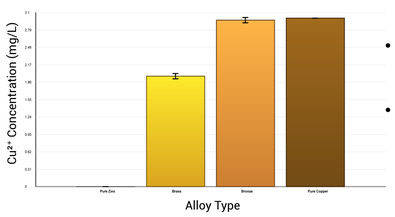
- Pure **copper**, **zinc**, **brass**, and **bronze** samples used
- Identical surface areas and exposure conditions (1 x 0.5 cm)

Use: $V_1C_1 = V_2C_2$

- To calculate amount in each treatment for same OD600 of 0.1
- *E. coli* K-12 cultures + LB broth incubated with each metal (= 10 mL)

2 Copper Ion Measurement

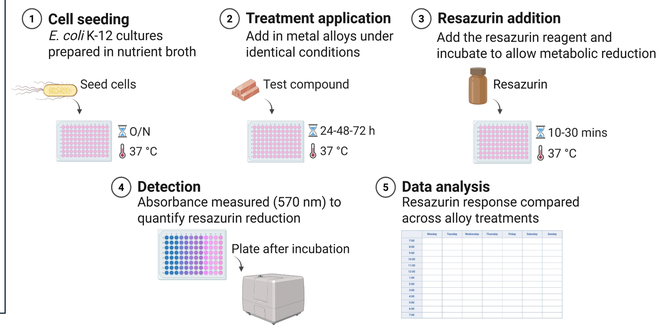
Copper Ion Release by Alloy Type



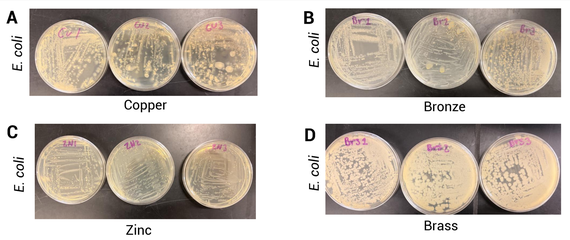
- Post-exposure (72 h) solutions analyzed for **Cu^{2+} concentration**
- Ion release normalized across alloys

Utilized colorimetric strips correlating to milligrams per liter (mg/L) for **quantification**

3 Resazurin Assay



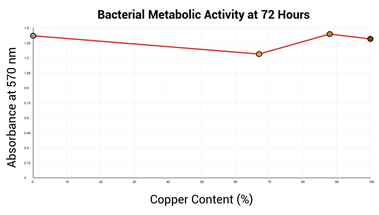
4 CFU Plate Count



Serial **dilutions** plated to determine Colony Forming Units per milliliter
Mean CFU/mL calculated from replicates

DATA & STATISTICAL ANALYSIS

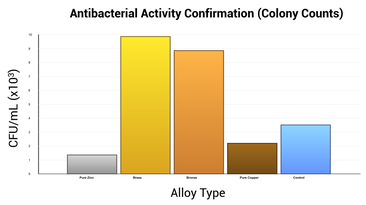
Quantification of Assay Data

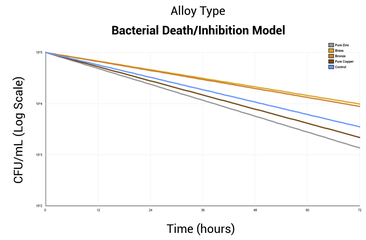


Resazurin Metabolic Activity (72-hour validated data)

- Pure copper and zinc produced the **strongest suppression of metabolic activity**
- Brass and bronze showed **moderate metabolic activity**, not proportional to copper percentage
- Antibacterial inhibition **did not increase linearly** with copper content

This divergence supports metabolic suppression as a **distinct but complementary** measure of antibacterial activity.

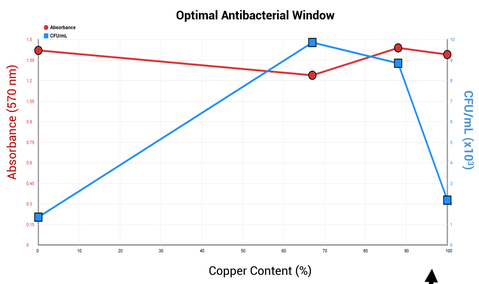




CFU Viability (Direct Measurement)

- CFU counts decreased by **multiple orders of magnitude** in zinc and pure copper relative to controls
- Brass and bronze showed **partial inhibition**, but retained higher CFU/mL than pure copper
- Log-scale visualization reveals non-linear antibacterial behavior across alloys
- **Lowest CFU/mL**: Zinc and pure copper
- **Intermediate CFU/mL**: Bronze
- **Highest CFU/mL among copper alloys**: Brass
- **Highest overall:** No-metal control

Statistical Validation of Antibacterial Differences



Key Insight: Antibacterial activity is maximized before Cu^{2+} release is maximized

Statistical Test Used

- One-way ANOVA comparing **log_{10}(CFU/mL)** across treatments **(n = 3)**
- Log transformation applied due to exponential bacterial growth and death

ANOVA Results

- **F = 78.30**
- **p = 0.00005**

Interpretation

- Alloy composition had a **highly significant effect** on bacterial viability
- Probability that observed differences occurred by chance is < 0.01%

RESULTS

Comparative Antibacterial Performance Across Alloys

- Bacterial metabolic activity (resazurin, 72 h) differed significantly across metal treatments
- Pure copper and zinc produced the **lowest metabolic activity**, indicating strong inhibition
- Brass and bronze showed **moderate metabolic suppression**, greater than the control but weaker than pure copper
- CFU/mL counts confirmed **reduced bacterial viability** in all metal treatments relative to the no-metal control
- Zinc and pure copper produced the **lowest CFU/mL values overall**
- Brass exhibited the **highest CFU/mL among copper-containing alloys**
- Copper ion (Cu^{2+}) release increased with alloy copper percentage
- Pure copper released the **highest Cu^{2+} concentrations**, bronze intermediate, brass lowest

DISCUSSION

- Antibacterial performance did **not increase linearly** with copper percentage (partially correct hypothesis)
- Strong metabolic suppression occurred without the highest Cu^{2+} release, indicating a **correlation** between ion concentration and antibacterial effect
- Brass and bronze demonstrated measurable antibacterial activity while releasing **substantially less Cu^{2+}** than pure copper (hypothesis)
- Zinc's strong CFU reduction despite zero Cu^{2+} release indicates that **non-copper mechanisms** (metal stress or surface interactions) can inhibit bacterial viability
- Differences between resazurin suppression and CFU viability confirm that **metabolic inhibition and cell death are related but distinct outcomes**
- These findings support evaluating copper alloys using an **optimization framework** rather than maximizing copper content alone

CONCLUSION

- **Primary Question:** Alloy composition significantly influenced antibacterial activity; however, antibacterial effects did **not increase linearly** with copper percentage. Strong inhibition was observed in some intermediate copper-containing alloys as well as in pure copper.
- **Secondary Question:** Copper ion (Cu^{2+}) release increased with alloy copper content, with pure copper releasing the highest concentrations, bronze intermediate, brass lower, and zinc producing no detectable Cu^{2+} under experimental conditions.
- **Hypothesis Evaluation:** The hypothesis was **partially supported**. Higher copper content increased Cu^{2+} release, but antibacterial activity did not consistently scale with ion concentration. Brass and bronze produced meaningful antibacterial effects while releasing less Cu^{2+} than pure copper.
- **Overall Conclusion:** These findings support the presence of an **optimized antibacterial window**, in which effective bacterial inhibition can be achieved while minimizing copper ion leaching, with implications for safer and more environmentally responsible antimicrobial material selection.

APPLICATIONS

Healthcare

Identify effective copper materials for hospital surfaces to prevent bacterial contamination.

Public Infrastructure

Design of antimicrobial metals for high-contact surfaces (e.g., railings, doorknobs, transit poles).

Environmental Toxicology

Assessing how copper pollution from pipes, roofing, or industrial runoff alters microbial communities responsible for nutrient cycling in water and soil.

KEY REFERENCES

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