

# The Killing Game: Copper, A Possible Bactericide for Contact Surfaces

---

A Microbiology Paper  
Presented To  
The Junior Science, Engineering, and Humanities Symposium

---

By  
Michael T. Stockwell  
Senior  
Tuscumbia High School  
526 School Rd.  
Tuscumbia, MO 65082

Mrs. Constance Wyrick  
Science Research Advisor  
Tuscumbia, MO 65082

## Abstract

**Name:** Michael T. Stockwell

**School:** Tuscumbia High School

**Research Sponsor:** Mrs. Constance Wyrick

**Title:** The Killing Game: Copper, A Possible Bactericide for Contact Surfaces

Antibiotic resistant bacteria are becoming more prevalent across the country not only in hospitals, but also in community settings like schools. These infections are often spread by common contact surfaces such as door handles and push plates. Copper has been suggested to have antimicrobial properties and has been suggested as a replacement surface for stainless steel. The purpose of this project was twofold: First, to determine if copper or brass could be used to limit bacterial growth on contact surfaces in public schools and secondly to determine the viability of bacteria on metal surfaces over time. It was hypothesized that: both the copper and brass would show antimicrobial properties, copper would be most effective of the three types of metal, and over 90% of the bacteria placed on the copper and brass plates would be killed within 30 minutes of contact

To conduct the study, stainless steel, brass, and copper plates were tested for bacteria after a 24-hour exposure time on the doors of the restrooms at several schools. Bacterial samples were collected from a 100 cm<sup>2</sup> area on each plate for comparison. In addition, a known quantity of a common gram negative bacterium (*E. coli*) and a common gram positive bacterium (*S. epidermidis*) will be placed on each the different types of plates to determine the antimicrobial properties of the copper, brass, and stainless steel under controlled conditions over time.

Based on this study, the following conclusion can be drawn. The hypothesis stating that both the copper and brass would show antimicrobial properties was accepted. The hypothesis stating that copper would be the most effective of the three metals was rejected; brass was comparable to copper's effectiveness. The hypothesis stating that over 90% of the bacteria placed on the copper and brass plates would be killed within 30 minutes of contact was accepted; over 99% of the bacteria were killed within 30 min.

## **Background Information**

### *Antibiotic Resistance*

For thousands of years many different species of bacteria have thrived on our planet. Their rapid multiplication, high mutation rate, and ability to add genes from other sources make bacteria tough survivors. These same factors also make bacteria that much harder for humans to control in the ever widening battle against illness. In the struggle to survive many bacteria have become resistant to commonly used antibiotics. They accomplish this by several methods including: barring the drug from entering the cell, using enzymes to inactivate the drug, pumping out the drug as soon as it enters, or changing the target of the drug. (Berkowitz, 2)

Recently, antibiotic resistant bacteria have become more prevalent for several reasons. First, in many different hospital units, such as intensive care, neonatal, oncology, etc. broad-spectrum antibiotics are often administered to the patients. These patients are often very susceptible to infection and provide the perfect setting for bacteria to grow and develop resistance (Capriotti). Secondly, hospitals overall are generally under budgeted for the spread of in-hospital infections. A study performed on a group of 28 hospitals found that the average annual cost of healthcare-associated infections was \$594,683 while the average budget for prevention was \$129,000. (Anderson, 1) This leads to lax rules and procedures to prevent the spread of infection. Lastly, outside of hospitals there is little done to maintain good hygienic conditions. Public places like schools are breeding grounds for infection that can be easily transmitted to hundreds of students.

### *Copper as an Antimicrobial*

Copper is number 29 on the periodic table, one of the transition metals, and is one of the essential trace elements for humans. It has a reddish color and a high metallic sheen. Copper has

been used for thousands of years by different cultures because of its many advantages. It is valued for its conductivity (second only to silver), malleability, ability to resist wear, and many other properties. (Weiner, 759) Copper also has many useful alloys such as brass. Brass is tougher than copper, resists corrosion better, and is cheaper than pure copper. Because of these properties brass is much more widely used throughout the world.

Ancient people used copper's properties to disinfect water by running it through copper pipes (Michels, 1). Only recently has it been suggested as a modern antimicrobial agent. Copper's potential as an antimicrobial agent has begun to attract the attention of both academia and industry. The results of some preliminary studies support copper's proposed effectiveness against bacterial and viral infections. There is now a rush to conduct field studies to determine its effectiveness in "real world" settings.

Question Posed: Can copper metal be used in a real world setting to inhibit the growth of bacteria on commonly used contact surfaces.

## **Purpose**

The purpose of this project was twofold:

- To determine if copper or brass could be used to limit bacterial growth on contact surfaces in public schools.
- To determine the viability of bacteria on metal surfaces over time.

## **Hypothesis**

It was hypothesized that:

- Both the copper and brass would show antimicrobial properties
- Copper would be most effective of the three types of metal
- Over 90% of the bacteria placed on the copper and brass plates would be killed within 30 minutes of contact

## **Experimental Design**

To conduct this study, the Copper Development Association was contacted, and they donated the copper and brass used in this study. Dr. Westenberg, professor of microbiology at the Missouri University of Science and Technology, was also contacted and asked to act as a qualified scientist. He critiqued the experimental design, allowed the use of his lab, and provided the bacterial cultures used in the study. In addition, all of the microbiological materials were provided by Dr. Westenberg. Principals and superintendents of the three schools used in this study (Tuscumbia, Russellville, and Eldon) were then contacted asked for permission to conduct the study at their respective schools.

Push plates into the restrooms were identified as one of the most used contact surfaces in the schools. These were stainless steel plates that many students touched each day and could be easily replaced and swabbed for bacteria. There were a total of nine doors with push plates available between the three schools for the study.

This experiment was run over a three-day time period, in which each of the three types of metal (copper, brass, and stainless steel) had a 24-hour exposure period with each door tested. To insure there were no skews in data from sickness or infection by students, all of the surfaces were cycled randomly through the three-day period. Every morning a metal plate was placed on each of the restroom doors in the study. The plates were then disinfected with ethanol, and they were left on the door throughout the day. The custodial staff was instructed not to sanitize any of the doors for the duration of the study. The next morning (24 hours later) bacterial samples were collected, using a sterile swab, from a defined 100 cm<sup>2</sup> area on each plate for comparison, and the swab was then placed into 1mL of a saline solution. (A plexiglass template was used to make sure the same area of the plates was swabbed each day.) After collection, the plate was then

switched on the door and the process repeated until samples were collected from each type of metal on the doors. After all of the samples were collected, they were taken to the microbiology lab at the Missouri University of Science and Technology. 100 microliters of each of the samples was placed onto two agar petri plates. The plates were incubated at 37 degrees Celsius for 48 hours, and the colonies were counted.

In addition to the field aspect of the study, there was also a study conducted under controlled laboratory conditions with a known quantity of nonpathogenic bacterial samples. A common gram negative bacterium (*Escherichia coli* 25922) and a common gram positive bacterium (*Staphylococcus epidermidis* 12228) were used to test the antimicrobial effectiveness of each type of metal. A  $10^5$ /mL solution of each bacterium was placed on a  $100\text{cm}^2$  area of each the different types of metal and run in duplicate. Each type metal (stainless steel, brass, and copper) was tested for three time periods (30 min, 60 min, and 120 min). The bacteria was then collected using a sterile swab and placed into 1mL of a saline solution. 100 microliters of this solution was placed onto bacterial plate. The plates were incubated at 37 degrees Celsius for 48 hours. Results were compared within groups and overall between the different types of surfaces.

In addition, several controls were run to compare against the experimental groups. First, the two bacteria were tested on a plexiglass surface for two hours to determine the number of CFUs on a nonmetal surface. Secondly, 10 microliters of each of the two original bacterial solutions were plated to determine a base count for the two solutions. Lastly, a  $100\text{cm}^2$  area of each type of metal was swabbed after cleaning to validate that the cleaning was sufficient.

## Data Table

### Bacteria on Surfaces Over Time

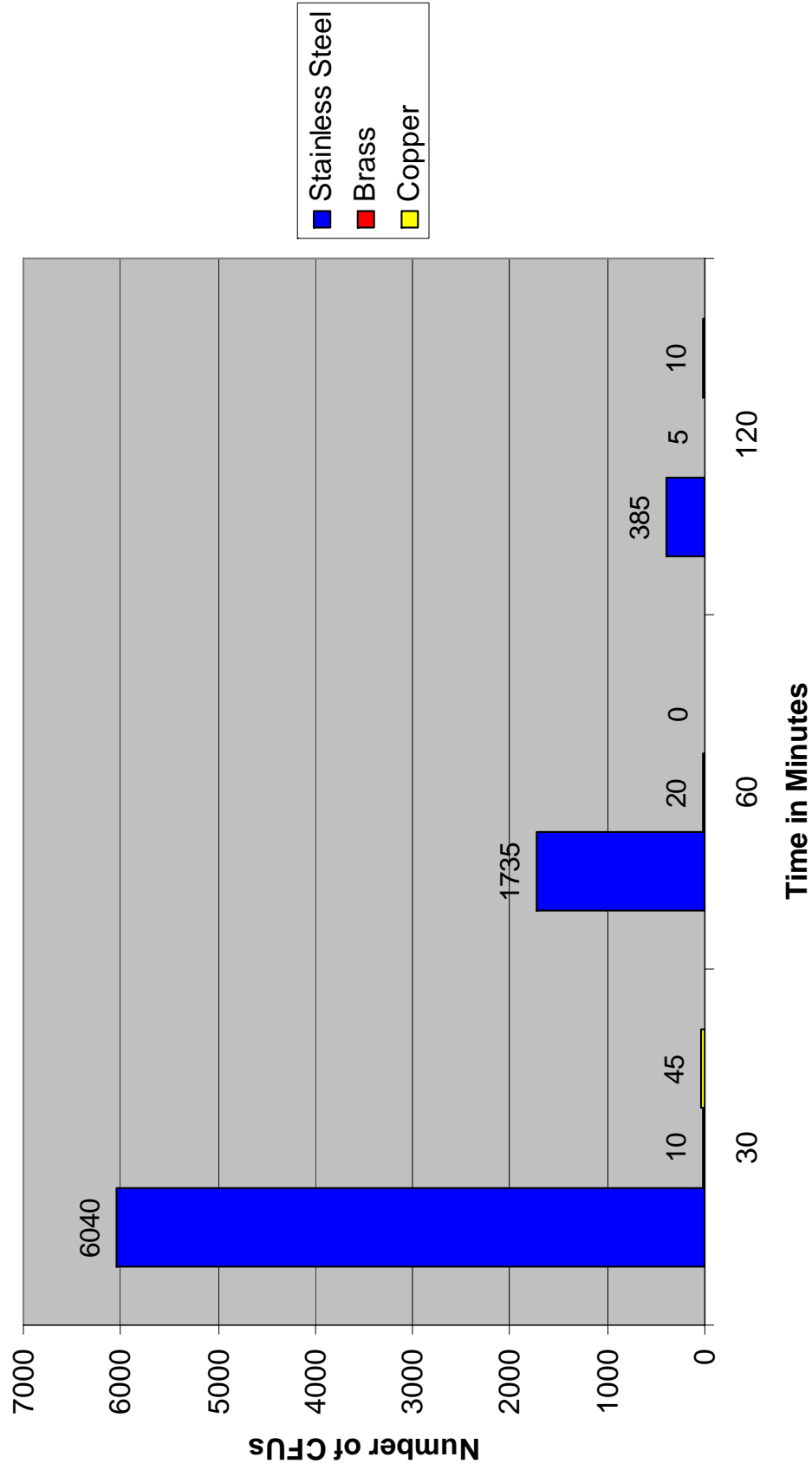
		30 min	CFUs/mL 60 min	120 min
<b>S. epidermidis</b>		(Initial CFU/mL= 26190)		
<b>Stainless Steel</b>				
	1	4330	1450	410
	2	7750	2020	360
	<b>Average</b>	<b>6040</b>	<b>1735</b>	<b>385</b>
<b>Brass</b>				
	1	10	40	10
	2	10	0	0
	<b>Average</b>	<b>10</b>	<b>20</b>	<b>5</b>
<b>Copper</b>				
	1	90	0	0
	2	0	0	20
	<b>Average</b>	<b>45</b>	<b>0</b>	<b>10</b>
<b>Plexiglass</b>		N/A	N/A	<b>15280</b>
<b>E. coli</b>		(Initial CFU/mL= 19410)		
<b>Stainless Steel</b>				
	1	12730	4700	N/A*
	2	11880	1430	2410
	<b>Average</b>	<b>12305</b>	<b>3065</b>	<b>2410</b>
<b>Brass</b>				
	1	10	0	0
	2	40	0	10
	<b>Average</b>	<b>25</b>	<b>0</b>	<b>5</b>
<b>Copper</b>				
	1	10	10	70
	2	0	0	10
	<b>Average</b>	<b>5</b>	<b>5</b>	<b>40</b>
<b>Plexiglass</b>		N/A	N/A	<b>7780</b>

\* Agar plate contaminated by large foreign bacteria



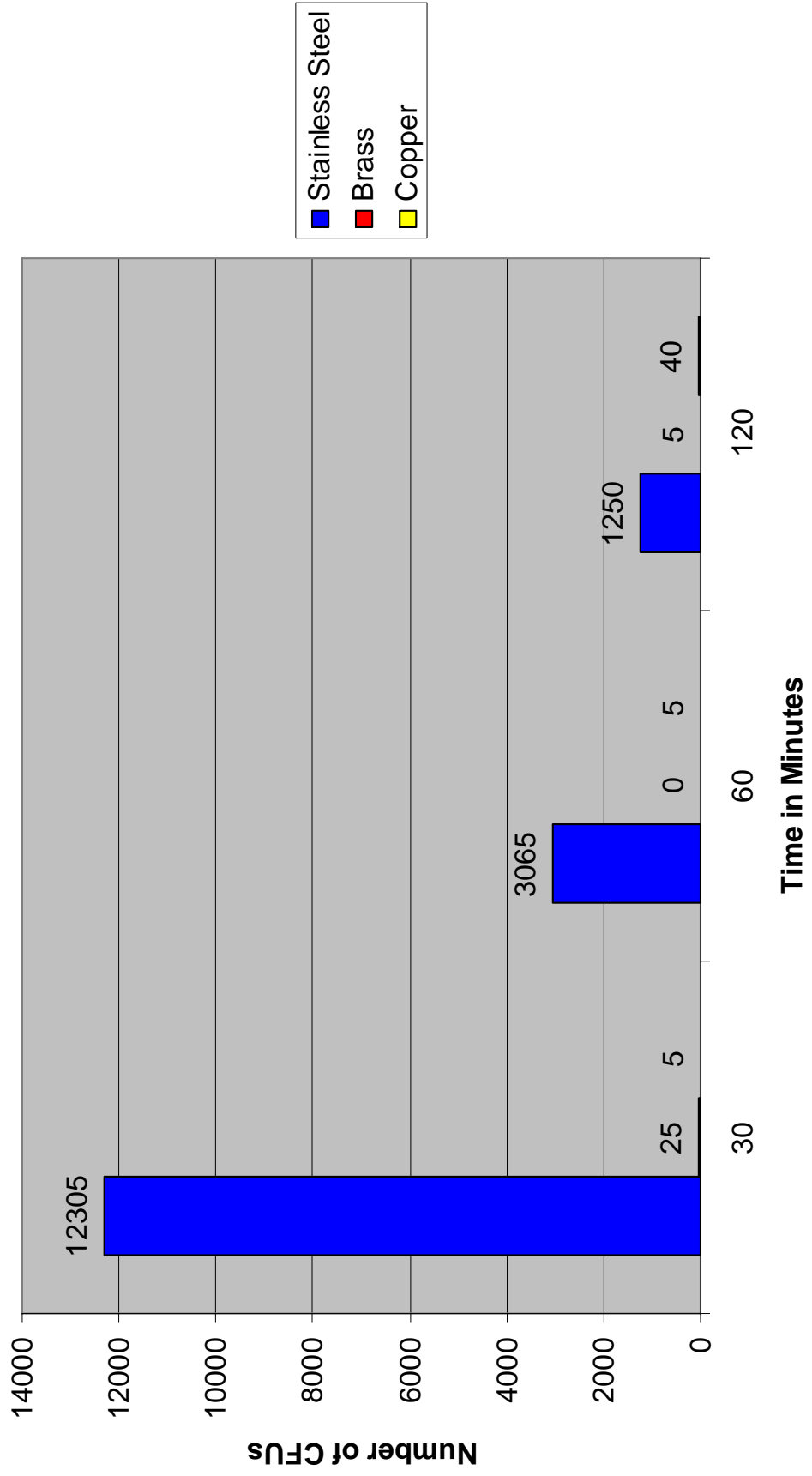
# CFUs on Metal Test Plates Over Time

*S. epidermidis*



# CFUs on Metal Test Plates Over Time

E. coli



**Data Table**  
**Bacterial Counts for Bathroom Push Plates (Trial One)**  
Area = 100 cm<sup>2</sup>

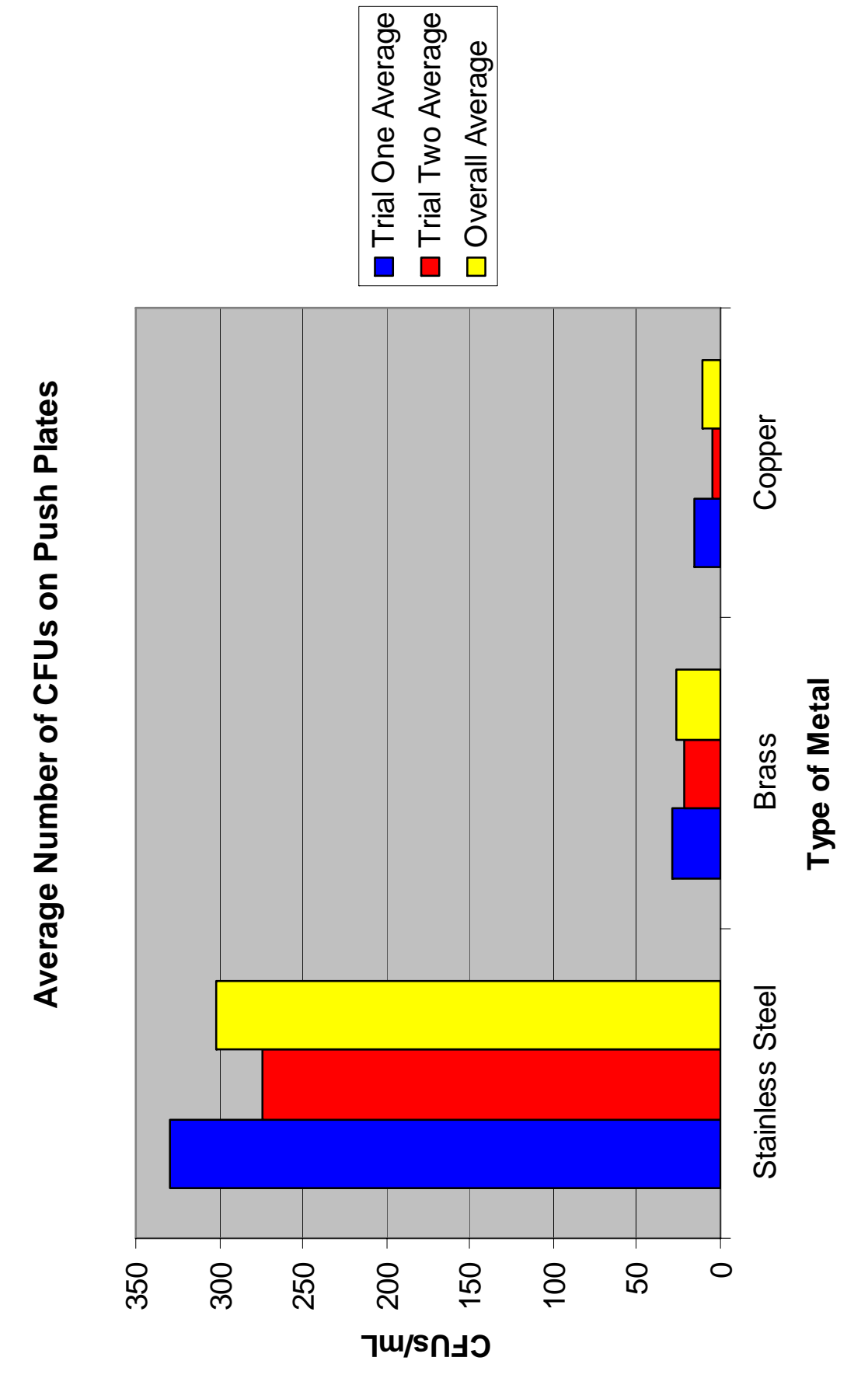
	CFUs/mL Stainless Steel			CFUs/mL Brass			CFUs/mL Copper		
	Sample 1	Sample 2	Average	Sample 1	Sample 2	Average	Sample 1	Sample 2	Average
<b>Tuscumbia</b>									
Door 1	0	0	<b>0</b>	0	0	<b>0</b>	100	0	<b>50</b>
Door 2	0	0	<b>0</b>	0	30	<b>15</b>	0	0	<b>0</b>
Door 3	0	190	<b>95</b>	0	10	<b>5</b>	0	20	<b>10</b>
Door 4	200	100	<b>150</b>	0	40	<b>20</b>	0	10	<b>5</b>
<b>Russellville</b>									
Door 1	500	60	<b>280</b>	100	0	<b>50</b>	0	30	<b>15</b>
Door 2	400	780	<b>590</b>	0	0	<b>0</b>	0	20	<b>10</b>
Door 3	400	2720	<b>1560</b>	100	20	<b>60</b>	0	10	<b>5</b>
<b>Eldon</b>									
Door 1	400	40	<b>220</b>	200	0	<b>100</b>	0	0	<b>0</b>
Door 2	100	50	<b>75</b>	0	30	<b>15</b>	100	0	<b>50</b>
<b>Totals</b>									
<b>Total Bacteria</b>	<b>2000</b>	<b>3940</b>	<b><u>2970</u></b>	<b>400</b>	<b>130</b>	<b><u>265</u></b>	<b>200</b>	<b>90</b>	<b><u>145</u></b>
<b>Average per Plate</b>	<b>222</b>	<b>438</b>	<b><u>330</u></b>	<b>44</b>	<b>14</b>	<b><u>29</u></b>	<b>22</b>	<b>10</b>	<b><u>16</u></b>

\* CFU= Colony forming unit

**Data Table**  
**Bacterial Counts for Bathroom Push Plates (Trial Two)**  
Area = 100 cm<sup>2</sup>

	CFUs/mL Stainless Steel			CFUs/mL Brass			CFUs/mL Copper		
	Sample 1	Sample 2	Average	Sample 1	Sample 2	Average	Sample 1	Sample 2	Average
<b>Tuscumbia</b>									
Door 1	70	90	<b>80</b>	20	0	<b>10</b>	0	10	<b>5</b>
Door 2	150	2730	<b>1440</b>	10	0	<b>5</b>	10	0	<b>5</b>
Door 3	210	280	<b>245</b>	30	0	<b>15</b>	0	0	<b>0</b>
Door 4	70	150	<b>110</b>	0	0	<b>0</b>	0	0	<b>0</b>
<b>Russellville</b>									
Door 1	50	60	<b>55</b>	240	20	<b>130</b>	20	10	<b>15</b>
Door 2	170	210	<b>190</b>	0	0	<b>0</b>	0	10	<b>5</b>
Door 3	230	350	<b>290</b>	0	30	<b>15</b>	0	0	<b>0</b>
<b>Eldon</b>									
Door 1	0	20	<b>10</b>	0	20	<b>10</b>	0	40	<b>20</b>
Door 2	70	20	<b>45</b>	0	20	<b>10</b>	0	0	<b>0</b>
<b>Totals</b>									
<b>Total Bacteria</b>	<b>1020</b>	<b>3910</b>	<b><u>2465</u></b>	<b>300</b>	<b>90</b>	<b><u>195</u></b>	<b>30</b>	<b>70</b>	<b><u>45</u></b>
<b>Average per Plate</b>	<b>113</b>	<b>434</b>	<b><u>274</u></b>	<b>33</b>	<b>10</b>	<b><u>22</u></b>	<b>3</b>	<b>8</b>	<b><u>5</u></b>

\* CFU= Colony forming unit



## Statistics

Statistics were conducted using GraphPad QuickCalcs. An unpaired t-test was performed between the types of metals, which resulted in a two-tailed P value. Results were considered significant if  $p < 0.05$ .

<b>Stainless Steel and Brass</b>	p= 0.0156*
<b>Stainless Steel and Copper</b>	p= 0.0110*
<b>Brass and Copper</b>	p= 0.1260

\* Indicates Statistical Significance

## Discussion of Results

When running the study and comparing results several points of interest were noted:

- Although each plate was swabbed thoroughly, when comparing the number of colonies on the plexiglass to the number of colonies straight from the bacterial solution, it was determined that the swabbing was picking up about 50% of the bacteria actually on the plate.
- Not all schools had similar numbers of bacteria which could of have been caused by different student populations and age ranges.
- When comparing the number of CFUs on the restroom plates, there was a large difference in the number of colonies between stainless steel, and the brass and copper.
- When comparing the number of CFUs during the timed aspect, there was again a large difference in the number of colonies between stainless steel, and the brass and copper.
- Under laboratory conditions the copper and brass showed a serious reduction in number of bacterial colonies after even thirty minutes.
- Even though the stainless steel had a large number of colonies at thirty minutes, there was a reduction over time in the number of colonies. It would be good to determine the number of colonies after a longer period of time. It should be noted however, that although there was a reduction in the number of colonies, after two hours there were still hundreds of colonies on the plates. (On average it takes only 10-50 colonies to infect a human.)

## **Conclusions**

- The hypothesis stating that both the copper and brass would show antimicrobial properties was accepted
- The hypothesis stating that copper would be the most effective of the three metals was rejected, brass was comparable to copper's effectiveness
- The hypothesis stating that over 90% of the bacteria placed on the copper and brass plates would be killed within 30 minutes of contact was accepted, over 99% of the bacteria were killed within 30 min

## **Future Studies**

As I stated earlier, research into the antimicrobial properties of copper has just begun within the last few years. Organizations like the Copper Development Association are very excited about my research. As of now there have only been laboratory studies performed on copper. Although there is a study in progress in a hospital setting, there are no published field studies on copper's antimicrobial activity. Therefore I would like to publish my research that I have conducted. To do this I would have to add more doors from schools of different population sizes to compare the results. In addition, I would like to change contact surfaces not only on restroom push plates, but also on other commonly used surfaces in public areas such as: water fountains, cafeteria surfaces, staplers, etc.

## **Bibliography**

Anderson, Deverick J., Kathryn B. Kirkland, Keith S. Kaye, Paul A. Thacker, Zeina A. Kanafani, Grace Auten, and Daniel J. Sexton. "Underresourced Hospital Infection Control and Prevention Programs: Penny Wise, Pound Foolish." Infection Control and Hospital Epidemiology 28 (2007): 767-773.

"Antimicrobial Properties and Applications." <http://www.cda.org.uk/antimicrobial/>.

Australia. Department of the Environment and Water Resources. Copper and Compounds

Berkowitz, Frank E. "Antibiotic Resistanc in Bacteria." Souther Medical Journal (1995).  
<http://search.ebscohost.com/login.aspx?direct=true&db=hch&AN=9508243078&site=ehost-live>.

Capriotti, Teri. "Multi-Drug Resistant Bacteria Create Continual Need for New Antibiotics." MEDSURG Nursing 10 (2001): 147-152.

"Copper Alloy Guide." Olin Brass. [www.olinbrass.com](http://www.olinbrass.com).

De Man, P, B Verhoeven, H Verbrugh, M Vos, and J Van Den Anker. "An Antibiotic Policy to Prevent Emergence of Resistant Bacilli." The Lancet 355 (2000): 973-978.

"Descriptions of Copper Alloys." Olin Brass. <http://www.olinbrass.com/alloy.html>.

Enright, Mark C. "Genome of an Epidemic Community- Acquired MRSA." The Lancet 367 (2006): 705-706.

Faundez, Gustavo, Miriam Troncoso, Paola Natarrete, and Guillermo Figueroa. "Antimicrobial Activity of Copper Surfaces Against Suspensions of Salmonella Enterica and Campylobacter Jejuni." BMC Microbiology.  
<http://www.biomedcentral.com/1471-2180/4/19>.

Hostynek, Jurij J., and Howard I. Maibach. "Review: Skin Irritation Potential of Copper Compounds." Toxicology Mechanisms and Methods.

Khachatourians, Geroge G. "Agricultural Use of Antibiotics and the Evolution and Transfer of Antibiotic-Resistant Bacteria." Candadian Medical Association Journal (1998).  
<http://search.ebscohost.com/login.aspx?direct=true&db=hch&AN=1281091&site=ehost-live>.

Kollef, Marin H., and Victoria J. Fraser. "Antibiotic Resistance in the Intensive Care Unit." Annals of Internal Medicine 134 (2001): 298-308.

"Lowering Infection Rates in Hospitals and Healthcare Facilities." Copper 1 (2007): 1-25.

Michels, H T., S A. Wilks, J O. Noyce, and C W. Keevil. "Copper Alloys for Human Infectious Disease Control."

Noyce, J O., H Michels, and C W. Keevil. "Inactivation of Influenza a Virus on Copper Versus Stainless Steel Surfaces." Applied and Environmental Microbiology (2007): 2748-2750.

Noyce, J O., H Michels, and C W. Keevil. "Potential Use of Copper Surfaces to Reduce Survial of Epidemic Meticillin-Resistant Staphylococcus Aures in the Healthcare Environment." Journal of Hospital Infection 63 (2006): 289-297.

Stewart, Philip S., and William Costerton. "Antibiotic Resistance of Bacteria in Biofilms." The Lancet 358 (2001): 135-138.

Weiner, Paul, and James M. Galligan. "Copper." The Encyclopedia Americana International Edition. Danbury, Connecticut: Grolier, 2001.



## **Acknowledgements**

I would like to thank the following people for their help with this project:

- Danielle McAuley, of the Copper Development Association, for providing the copper and brass used in this study and information on studies being conducted
- Dr. Westenberg, of the Missouri University of Science and Technology, for allowing me use of his lab and providing all of the microbiological materials used in this study.
- Russellville, Eldon, and Tuscumbia schools for allowing me to conduct my study
- Mrs. Connie Wyrick, my science research advisor