Antimicrobial Testing on Copper versus Copper Alloys

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1. Summary

Copper and copper alloys are known as metals with antimicrobial properties. Esco conducted the antimicrobial test on pure copper and some copper alloys in a microbiology laboratory. Six microorganisms were tested against one type of pure copper and seven types of copper alloy. In this study, we found that pure copper is more effective than copper alloy in rendering microorganisms inactive.

2. Introduction

Numerous instances of research prove that copper and copper alloy have antimicrobial properties. Microorganisms deactivate within hours of exposure to copper or copper alloys. The EPA said that copper and copper alloys can claim to have antimicrobial properties if they kill more than 99.9% of microorganisms in 2 hours.

Many factors affect antimicrobial properties, such as the purity of the copper, the copper content of the alloy, the temperature, and the humidity. To deactivate the microorganisms, there must be contact between the microorganism and a copper surface called 'contact killing'. The mechanisms of 'contact killing' are still unclear at this time, but some research mentions that copper surfaces release a copper ion that affects the cells and is followed by the oxidation of cell content

Due to the antimicrobial properties of copper, the substance is widely used by the healthcare industry. Hospital uses it for door handles, touch plates, bed rails, call buttons, toilet seats, etc. EPA-approved Antimicrobial Copper applications include: doors (lock sets, handles, levers, push plates, kick plates, mop plates, stretcher plates, stops, pulls, closures), cabinet and casework surfaces (knobs, pulls, handles), tables, countertops, back-splashes, casework, switch and wall plates, exit devices, hand rails, stair rails, grab bars, lockers, shelving and trash receptacles, plumbing (handles, spouts, control levers), windows (levers, hinges, pull handles), and elevator cabs. Now, some laboratory equipment manufacturers use copper to make equipment such as CO_2 incubators.

As one of many laboratory equipment manufacturers, Esco uses pure copper (C1100) to build CO_2 incubators. This material helps customers reduce cleaning time because of its antimicrobial properties. Microorganisms attach to the surface chamber of the incubator and are deactivated in mere hours. The objective of this study is to test the effectiveness of copper and some copper alloys to kill a drop of microorganisms over a certain amount of time.

3. Material and Equipment

Materials used in this study:

a. Copper Alloys, coded: C2680, C68800, C61100, C5100, C5210, C19400, and C19210
b. Pure copper (C1100)
c. Microorganisms in suspension: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Candida albicans,* and *Bacillus atrophaeus*

d. Stainless steel as the control
e. Tryptone Soya Broth (TSB) medium
f. Tryptone Soya Agar (TSA) medium
g. Sterile DI-water for dilution
h. Sterile 0.85% NaCl solution
i. Acetone
j. 70% IPA

Equipment used in this study:

a. Tweezers
b. Pipette with tip
c. Test tube
d. Beaker, glass
e. Tissue
f. Vortex
g. Digital thermometer
h. Esco BSC Model LA2-4A1

4. Method

This test was conducted according to Michels *et. al* (2009) with some modifications, as well as Grass et. al (2011).

a. Pure copper and copper alloys were cut into 1 x 1 cm squares called coupons.
b. Vortex pure copper and copper alloy samples were placed in the acetone for 30 s.
c. The samples were sterilized by immersion in the 70% IPA.
d. Coupons were exposed to 20 µL bacterial suspension.
e. Coupons were incubated for 6 hours inside a BSC.
k. The coupons were diluted in the sterile 0.85 % NaCl solution at 0, 2, 4, and 6 hour incubation time periods.
f. Coupons were placed on the TSA and incubated at 37 °C for 48 hours before checking the result.
g. Repeated steps a-f with copper, but with incubation times of 0, 1, 2, 4, and 6 hours.

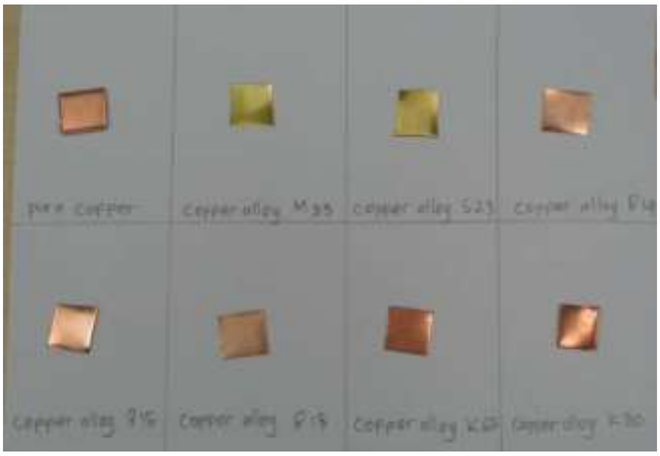

Figure 1. Coupons of pure copper and copper alloys

Figure 2. Copper with microorganism suspensions

5. Result

This test was done in the Biological Safety Cabinet, in which the temperature and humidity were 27 – 29° C and 40 – 50%, respectively. The results of this study are as follows.

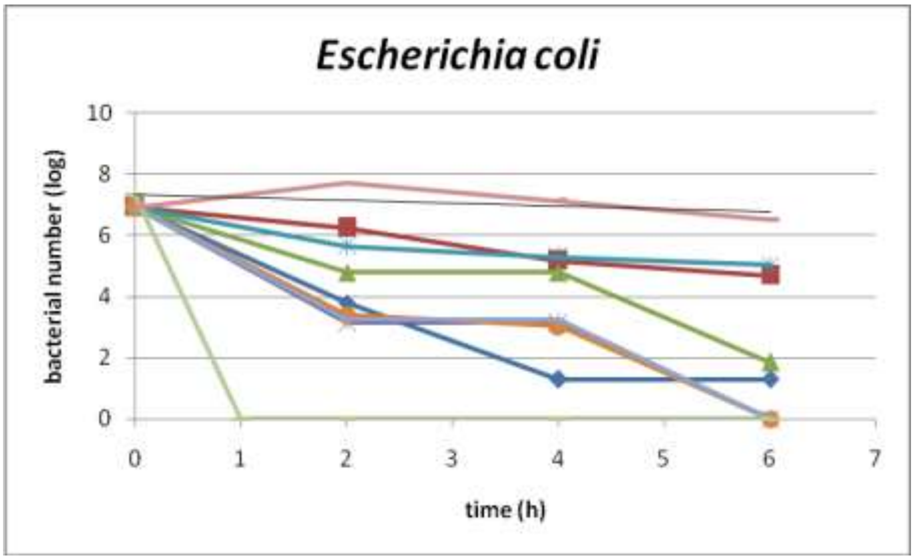

Figure 3. *Escherichia coli* reduction by copper and copper alloy

1. C2680; 2. C68800; 3. C61100; 4. C5100; 5. C5210; 6. C19400; 7. C19210; 8. C1100; 9. Stainless steel

Escherichia coli is a microorganism commonly found in water, soil, and waste of living matter. This microorganism is widely used as a cleanliness indicator for water and food. In this experiment, *E. coli* was dropped on sheets of pure copper and copper alloy. Total reduction was achieved by pure copper (C1100) after one hour incubation. After two hours of incubation, three logs of reduction were reached by copper alloys C5100, C19400, and C19210, while total reduction by these three copper alloys was over six hours of incubation.

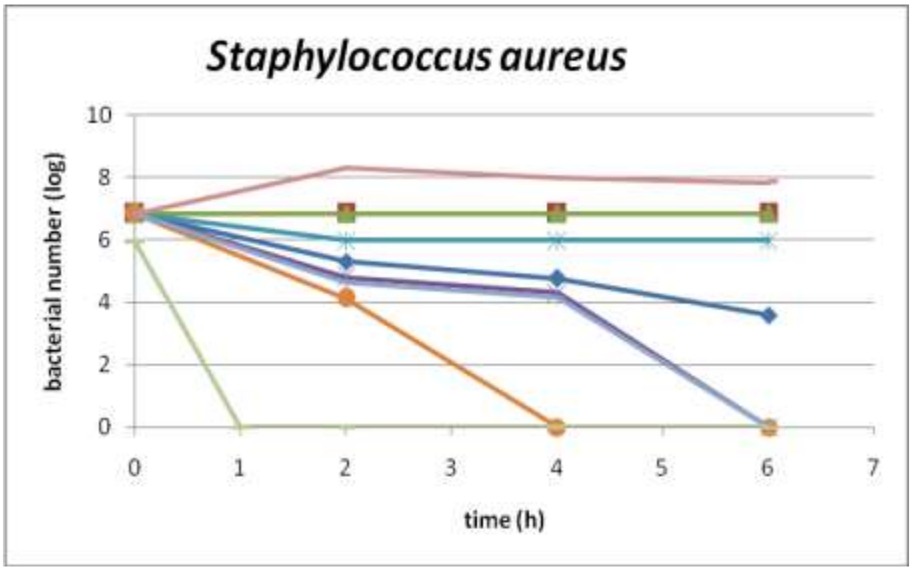

Figure 4. *Staphylococcus aureus* reduction by copper and copper alloy

1. C2680; 2. C68800; 3. C61100; 4. C5100; 5. C5210; 6. C19400; 7. C19210; 8. C1100; 9. Stainless steel

Staphylococcus aureus is not always pathogenic, but it is a common cause of skin infections, respiratory disease, and food poisoning. After incubating for one hour, 100% reduction was reach with pure copper (C1100). While after two hours incubation, this microorganism had two logs of reduction with copper alloys C5100, C19400, and C19210. Then, after six hours incubation, this microorganism was killed by those three copper alloys.

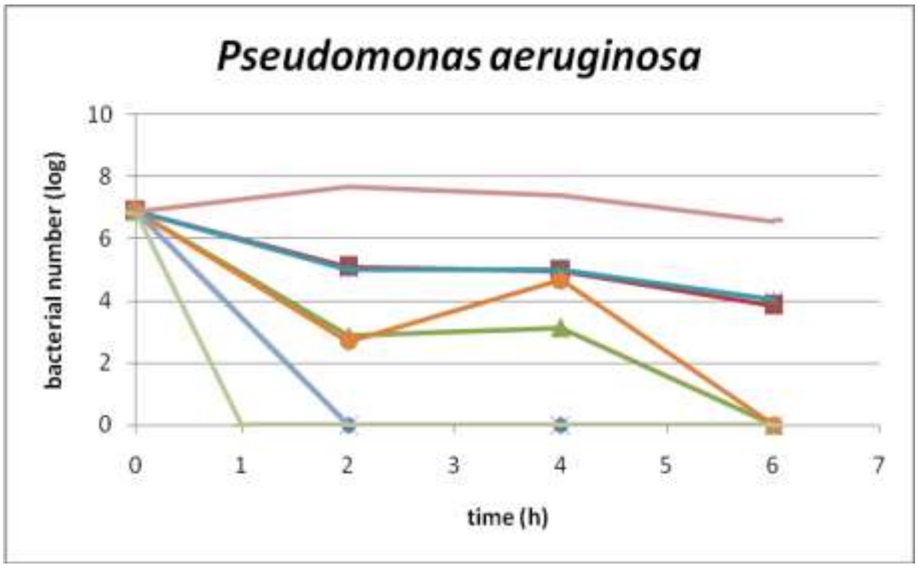

Figure 5. *Pseudomonas aeruginosa* reduction by copper and copper alloy

1. C2680; 2. C68800; 3. C61100; 4. C5100; 5. C5210; 6. C19400; 7. C19210; 8. C1100; 9. Stainless steel

Pseudomonas aeruginosa is found in soil, water, and skin flora. These microorganisms can cause diseases in animals, including humans. Total reduction of this microorganism was achieved by pure copper (C1100) after one hour incubation. After two hours of incubation, this microorganism was killed by copper alloys C2680 and C19210 as well. After six hours of incubation, copper alloys C61100 and C19400 reduced this entire microorganism.

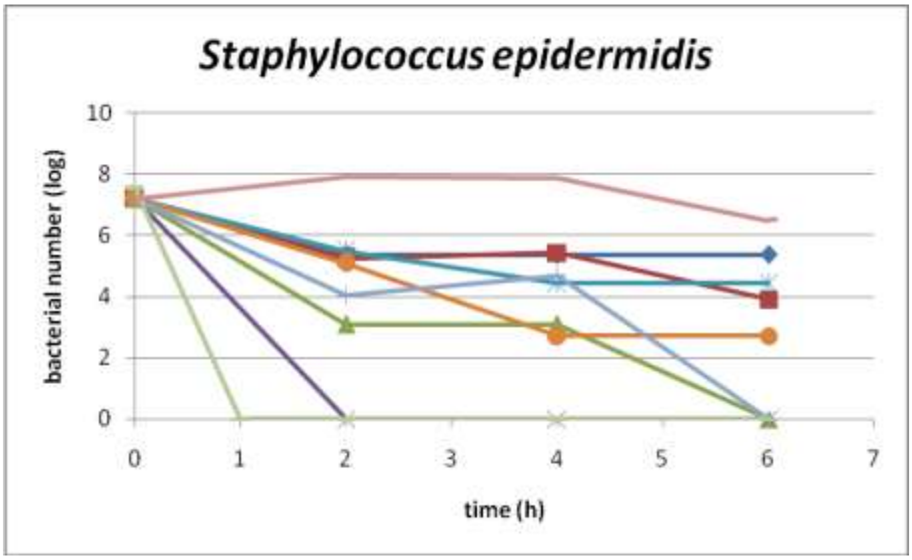

Figure 6. *Staphylococcus epidermidis* reduction by copper and copper alloy

1. C2680; 2. C68800; 3. C61100; 4. C5100; 5. C5210; 6. C19400; 7. C19210; 8. C1100; 9. Stainless steel

Staphylococcus epidermidis is a normal flora of human skin. It can also be found in the mucous membranes and in animals. The fastest reduction of this microorganism occurred with pure copper (C1100) with a log number of zero after one hour incubation. Total reduction was reach by copper alloy C5100 after two hours of incubation. After six hours, it was entirely reduced by copper alloys C61100 and C19210.

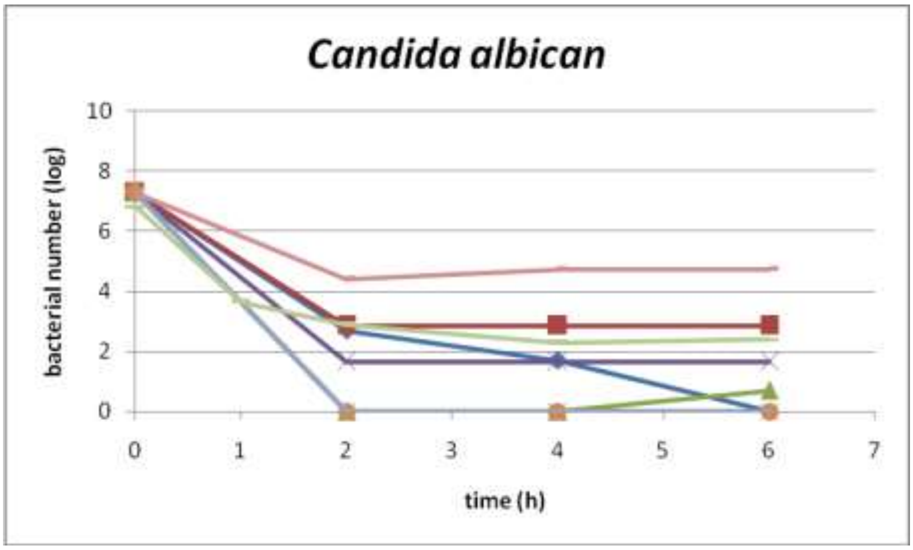

Figure 7. *Candida albican* reduction by copper and copper alloy

1. C2680; 2. C68800; 3. C61100; 4. C5100; 5. C5210; 6. C19400; 7. C19210; 8. C1100; 9. Stainless steel

Candida albicans is a normal flora of humans. It lives in human mouths and gastrointestinal tracks. It does not cause harmful effects, though it has shown overgrowth results in Candidiasis. In this experiment, copper alloys C61100, C5210, C19400, and C19210 completely reduced this microorganism when incubated for two hours. But until six hours of incubation, pure copper (C1100) reduced 4 logs only. This microorganisms was slightly hard to kill, because it's has cell wall contain of polysaccharides and proteins structural, which acts as barrier for copper ion to reach the cell.

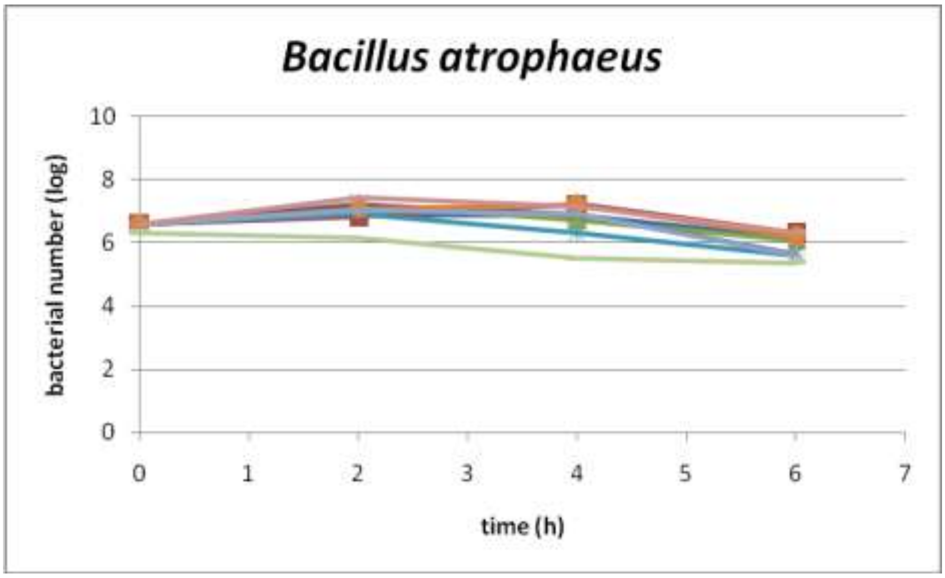

Figure 8. *Bacillus atrophaeus* reduction by copper and copper alloy

1. C2680; 2. C68800; 3. C61100; 4. C5100; 5. C5210; 6. C19400; 7. C19210; 8. C1100; 9. Stainless steel

Bacillus atrophaeus is a member of *Bacillus subtilis,* which is found in soil and human organs. This microorganism is the most difficult to kill because of its endospore. Endospore contain of many layer which resulting high resistant to high temperature, UV radiation, chemical disinfectant, and even freezing. The highest reduction occurred after six hours incubation, which showed 1 log reduction only.

The table below presents the entirely reduction of all pure copper (C1100) and copper alloys.

Table 1. Entirely reduction time of microorganisms (0 log) by pure copper (C1100) and copper alloys

Microorganisms	Time (h)							
	C2680	C68800	C61100	C5100	C5210	C19400	C19210	C1100
Escherichia coli	>6	>6	>6	6	>6	6	6	1
Staphylococcus aureus	>6	>6	>6	6	>6	4	6	1
Pseudomonas aeruginosa	2	>6	6	2	>6	6	2	1
Staphylococcus epidermidis	>6	>6	6	2	>6	>6	6	1
Candida albicans	6	>6	2	>6	2	2	2	>6
Bacillus atrophaeus	>6	>6	>6	>6	>6	>6	>6	>6

Pure copper (C1100) inactivates microorganisms most effectively because of its purity. Grass et al.

(2011) said that higher copper content results in higher contact-killing of microorganisms. This means that, in this study, copper alloy C19210 has the highest copper content (compared to other copper alloys) because it is the most effective at reducing microorganisms. This copper alloy can reduce *P. aeruginosa* and *C. albicans* after two hours of incubation, and it can reduce *E. coli, S. aureus,* and *S. epidermidis* after six hours of incubation. The second most effective copper alloy is C5100, which can reduce *P. aeruginosa* and *S. epidermidis* after two hours of incubation, and *E. coli* and *S. aureus* after six hours of incubation. The third most effective is C19400. This copper alloy can reduce *C. albicans* after two hours of incubation, *S. aureus* after four hours of incubation, and *E. coli* and *P. aeruginosa* after six hours of incubation.

6. Conclusion

Pure copper can deactivate *E. coli, S. aureus, P. aeruginosa,* and *S. epidermidis* in one hour. The most effective copper alloy is C19210, which can deactivate *P. aeruginosa* and *C. albicans* within two hours. Then, copper alloy C5100 can deactivate *P. aeruginosa* and *S. epidermidis* in two hours, and copper alloy C19400 can deactivate *C. albicans* in two hours.

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