Evaluating the efficacy of Cu and Ag ions for the inactivation of *Legionella pneumophila*

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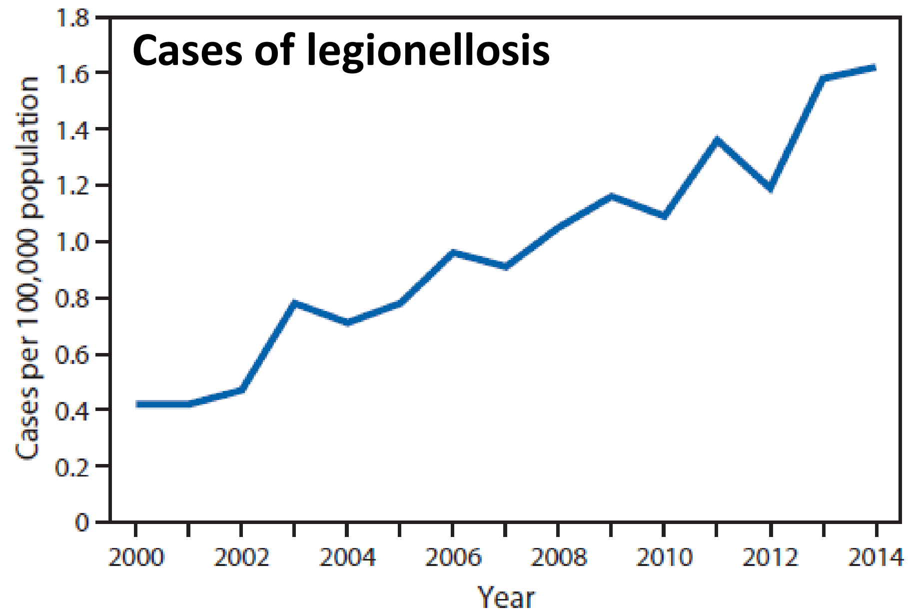
Legionella

- Opportunistic drinking water pathogen
  - > 50 species of *Legionella*
  - ***Legionella pneumophila (Lp)*** causes most infections, specifically serogroup 1
  - Infection through inhalation of contaminated aerosols
  - Legionnaires Disease, Pontiac Fever
  - **First discovered in 1977 after an outbreak at an American Legion conference in Philadelphia, PA**
- Ideal growing temperatures: 25 – 42°C
- **Gram-negative bacteria**
- Planktonic or in biofilms



Garrison et al., 2016

Legionella Control in Premise Plumbing Systems	
Chemical treatment technologies: • Chlorine-based disinfection • Copper-silver ionization (CSI) • Ozonation	**Physical treatment technologies:** • Thermal inactivation • Filtration • Ozonation
Emerging treatment technologies: • Ultraviolet (UV) irradiation • UV light emitting diodes (LEDs) • Innovative point-of-use (POU) filters	**Other strategies:** • Superheat-and-flush disinfection • Shock hyperchlorination

Carlson et al., 2020

Audience Questions

- 1. When was *Legionella* first discovered?
  a) 1977 after an outbreak at an American Legion conference in Philadelphia, PA
  b) 1776 at an American Legion conference in Philadelphia, PA
  c) 1884 in the one of the first microbiology labs

- *2. Legionella* is Gram-negative bacteria.
  a) True
  b) False

Using copper-silver ionization (CSI) to control *Legionella*

- **CSI:** the use of positively-charged copper (Cu) and silver (Ag) ions as a bactericidal agent
  - Use of copper and silver salts (for example $CuSO_4$ and $AgNO_3$, respectively)
  - Electrolytic production of Cu and Ag ions via CSI units

Triantafyllidou et al., 2016

- Suggested mechanisms of action:
  - Cu destroys cell wall permeability.
  - Ag disrupts protein and enzyme synthesis.

CSI in buildings

- Often installed in hot water loops.
- Evaluated in hospitals or nursing homes with Cu and Ag ions being produced electrolytically (picture on top right).
- Levels of Cu and Ag ions produced through these units can vary.
  - Target levels set by manufacturer and literature.
  - Cu: MCL of 1.3 mg L^{-1}, SMCL of 1.0 mg L^{-1}
  - Ag: SMCL of 0.1 mg L^{-1}
- Various levels of success.
  - Controlling *Lp* vs eradicating *Lp.*
- **Aesthetic concerns**.

States et al., 1998

Triantafyllidou et al., 2016

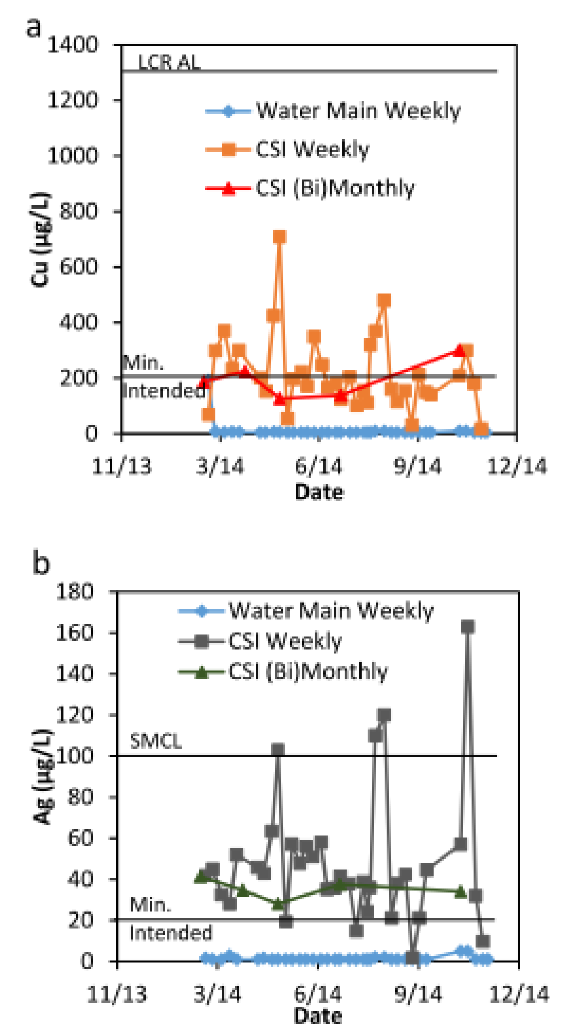
CSI in buildings (cont.)

- Control of ion levels is difficult (Triantafyllidou et al., 2016, States et al., 1998).
- Regular monitoring may be needed for ion levels and voltage output.
- Importance of maintaining electrodes to prevent scaling.
- Other secondary treatments (e.g. water softeners) can affect ion concentration.

Sample Month	Mean Concentration µg/L Copper	Silver
1994		
January*	<100	<1
January*	<100	<1
February	140	12
March	156	26
April	91	3
May	91	3
June	210	4
July	121	15
August	372	50
September	259	14
October	502	65
November	711	909
December	348	195
1995		
January	369	324
February	155	41
March	183	39
April	198	35
May	183	16
June	854	229
July	422	37
1996		
January	121	30

*Samples collected befor
†Calculated from spread-

Adapted from States et al., 1998



Triantafyllidou et al., 2016

CSI in buildings (cont.)

- Reduction of *Lp* positive sites but not eradication (States et al., 1998, Liu et al., 1994).

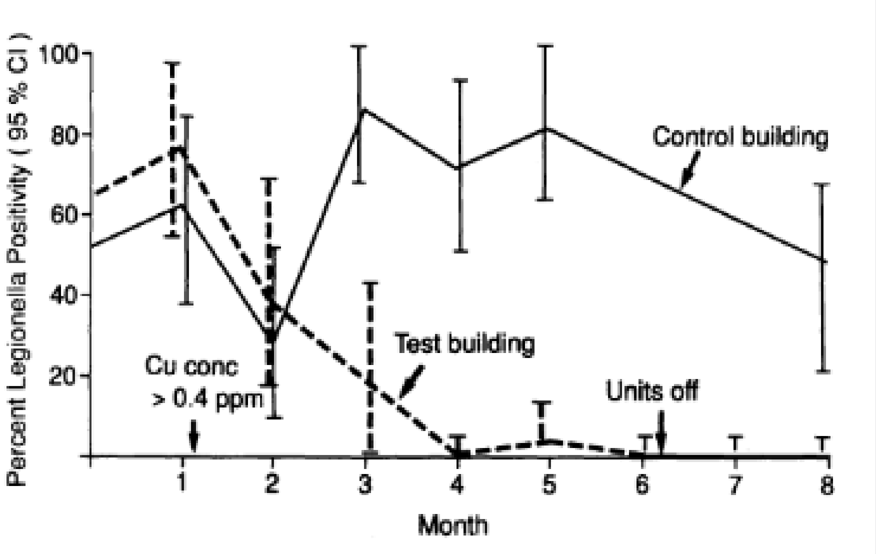
Sample Month	*L. pneumophila* Positive Sites *percent*	*L. pneumophila* Mean Concentration *cfu/mL*†	Mean Concentration *µg/L* Copper	Mean Concentration *µg/L* Silver
1994				
January*	100	30	<100	<1
January*	100	57	<100	<1
February	30	1	140	12
March	14	<1	156	26
April	9	1	91	3
May	26	2	91	3
June	35	4	210	4
July	30	4	121	15
August	23	1	372	50
September	14	1	259	14
October	4	2	502	65
November	23	5	711	909
December	18	2	348	195
1995				
January	9	1	369	324
February	13	1	155	41
March	9	2	183	39
April	18	<1	198	35
May	5	1	183	16
June	17	2	854	229
July	13	2	422	37
1996				
January	9	1	121	30

*Samples collected before a copper-silver ionization system was activated
†Calculated from spread-plate samples

States et al., 1998

CSI in buildings (cont.)

- Reduction of *Lp* positive sites but not eradication (States et al., 1998, Liu et al., 1994).
- Reduction of *Lp* positive sites relatively quick (<1 month).
- Size and complexity of building water systems influences ion concentrations (Liu et al., 1994).

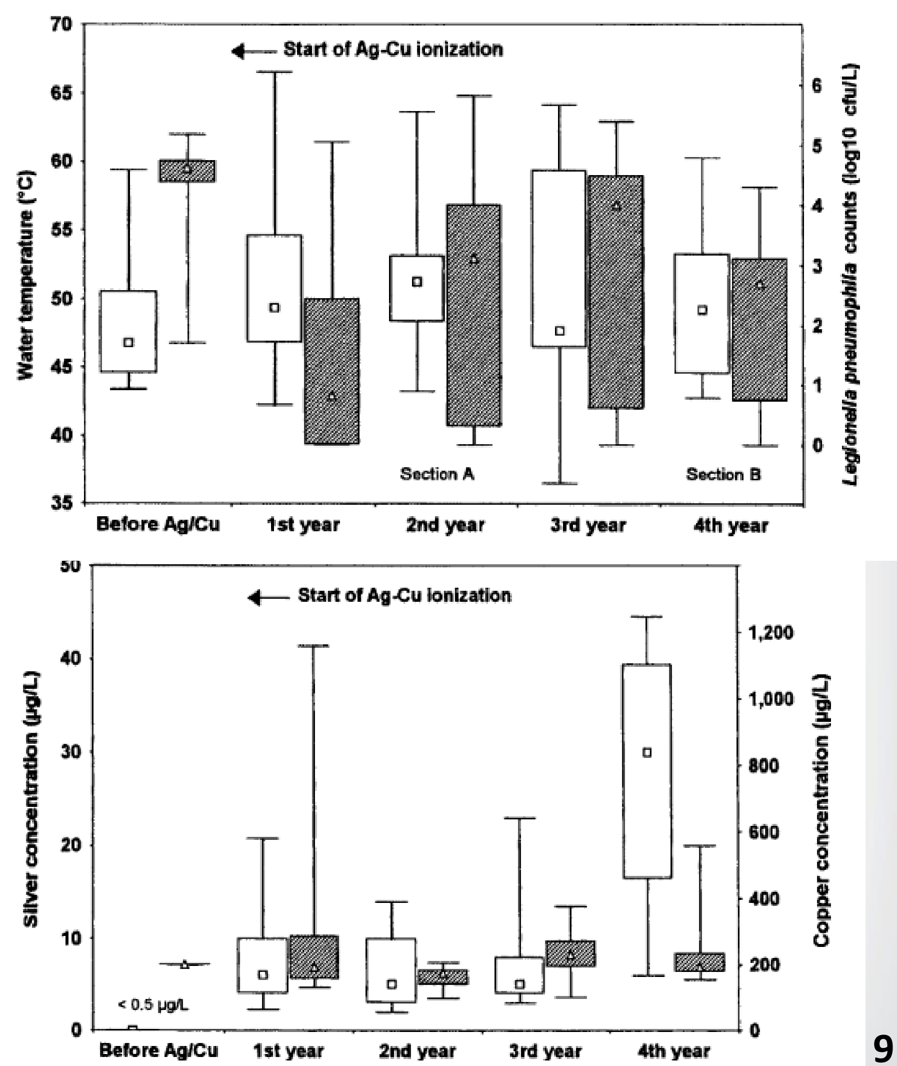


Liu et al., 1994

CSI in buildings (cont.)

- **Long-term success is difficult to achieve (e.g. years).**
  - *Lp* resistance to Cu and Ag ions (Rohr et al., 1999).

- Building/municipal water chemistry influences effectiveness (Lin et al., 2002).
  - Effectiveness negatively influenced by high pH (>8.5).



CSI in buildings (cont.)

- Buildings have seen reduction in *Lp* positive sites at various levels of Cu and Ag.

Cu (ppm)	Ag (ppm)	Result	Study
0.14	0.012	Reduction in *Lp* positive sites	States et al., 1998
0.4	0.04	Reduction in *Lp* positive sites	Liu et al., 1994
0.2	0.006	3.8 $\log_{10}$ but decreased over time	Rohr et al., 1999
0.27	0.03	Saw no reduction in *Lp*	Lin et al., 2002
0.36	0.033	Reduction in *Lp* positive sites	Walraven et al., 2016

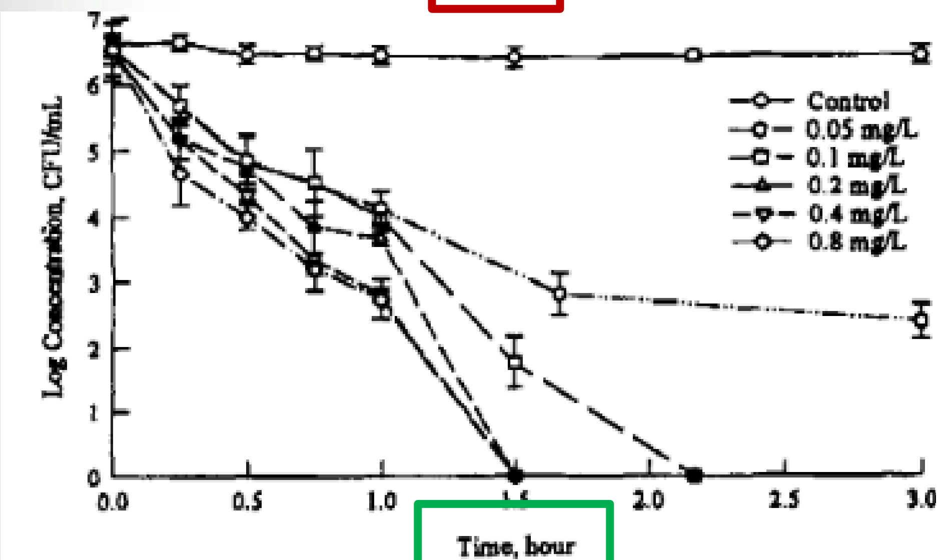
*Mean or median ion values reported.

- Electrolytic production of ions.
- Cu and Ag levels are not reported but success of CSI is, often as the reduction in *Lp* positive sites (Mietzner et al., 1997, Stout et al., 2003).

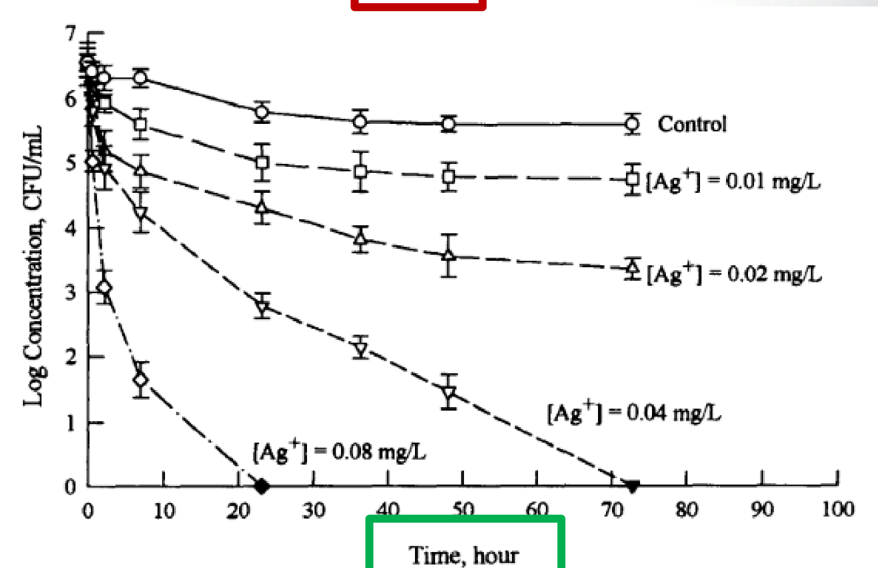
Individual effects of Cu and Ag

- Both Cu and Ag are independently effective against Lp
- Cu is faster (hours vs days)
- Deionized water buffered to pH 7 and Cu/Ag salts

Cu



Ag



Lin et al., 1996

Combined effects of Cu and Ag

Additive and synergistic effects have been observed

- 0.02 ppm Cu, 0.02 ppm Ag → additive
- 0.02 ppm Cu, 0.04 ppm Ag → additive
- 0.04 ppm Cu, 0.02 ppm Ag → synergistic
- 0.04 ppm Cu, 0.04 ppm Ag → synergistic

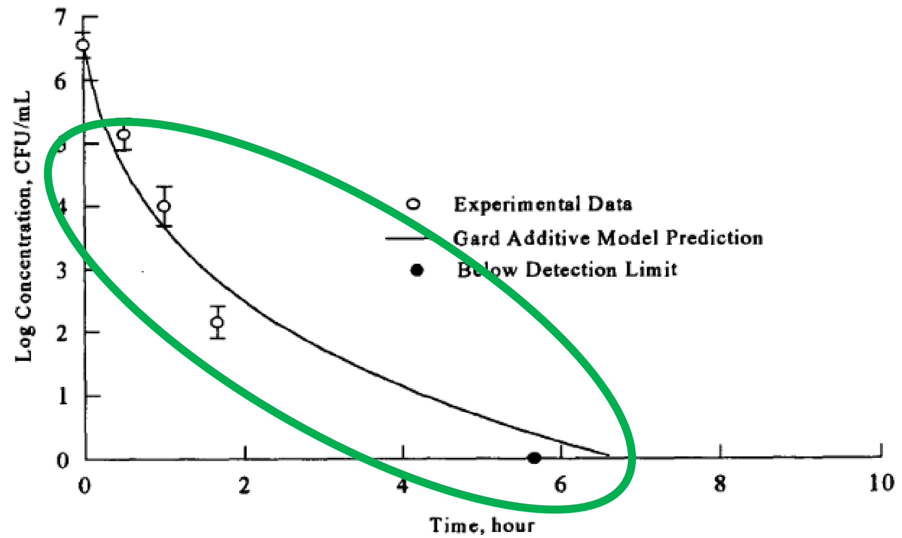


Fig. 7. Inactivation of *L. pneumophila* with 0.02 mg/l of copper and 0.04 mg/l of silver ions.

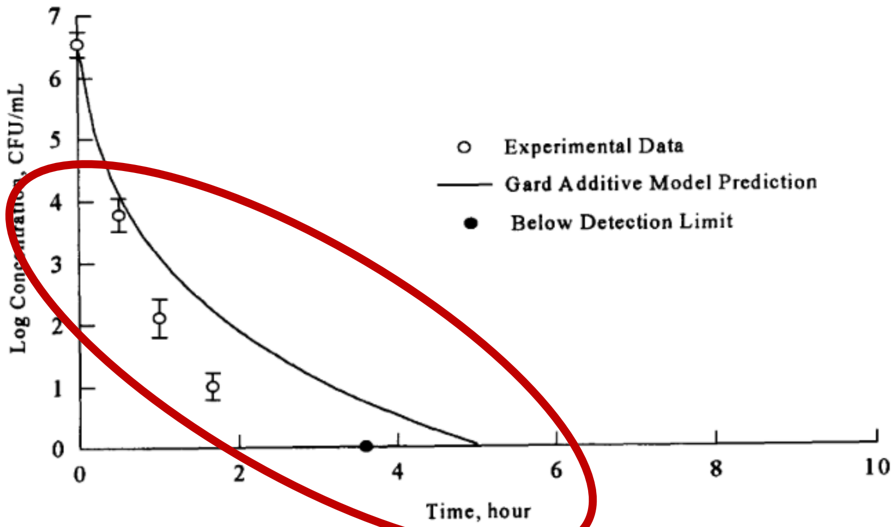


Fig. 8. Inactivation of *L. pneumophila* with 0.04 mg/l of copper and 0.02 mg/l of silver ions.

Lin et al., 1996

Combined effects of Cu and Ag

- With levels of 0.4 and 0.04 ppm of Cu and Ag, respectively, a 3-$\log_{10}$ reduction was observed after 24 hours of contact (0 ppm Cl-) (Landeen et 1989).
- Chlorine works synergistically with Cu and Ag ions (panels on right) (Landeen et 1989).
- Test solution: well water
- Cu and Ag ions produced electrolytically

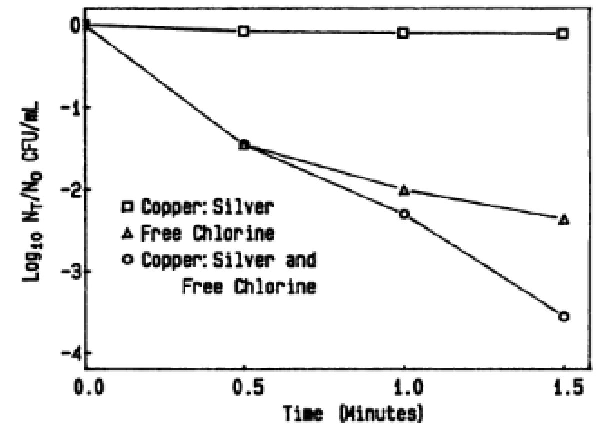


FIG. 4. Reduction of *L. pneumophila* by exposure to electrolytically generated copper and silver (400 and 40 μg/liter) and/or free chlorine (0.4 mg/liter).

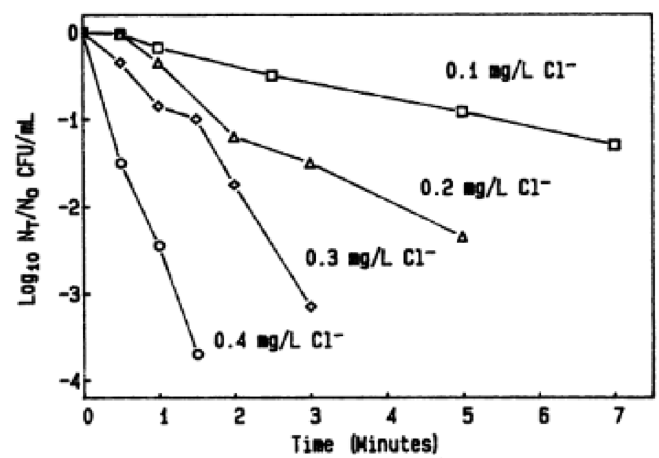


FIG. 3. Reduction of *L. pneumophila* by exposure to electrolytically generated copper and silver (400 and 40 μg/liter) and various concentrations of free chlorine.

Landeen 1989

Strengths and weaknesses of CSI

Strengths

- Not influenced by temperature.
- No harmful DBPs.
- Relatively affordable.
- Easy installation.
- Quick and effective in some studies.
- Effective against other bacteria.

Weaknesses

- Less effective at higher pH.
- Possible corrosion.
- Can form complexes (and hence reduces effectiveness).
- Control of electrolytically-produced ion levels is difficult.
- *Lp* resistance to Cu and Ag ions has been suggested.
- Aesthetic problems (color, taste, odor, staining).
- Scale build-up.
- Monitoring ion levels in real-time is difficult.

Current knowledge gaps

- In case studies, electrolytically-produced Cu and Ag levels fluctuate making it difficult to isolate what Cu and Ag levels are effective.
- Cu and Ag ions have rarely been evaluated alone (i.e. they are always evaluated in conjunction with one another).
- For lab studies, test solutions are inconsistent across studies making results comparisons of needed Cu and Ag ions difficult.
- Little is known regarding how other water chemistry parameters influence the effectiveness of Cu ang Ag ions.
- Variability across *Lp* serogroups in response to Cu ang Ag ions?

Research question

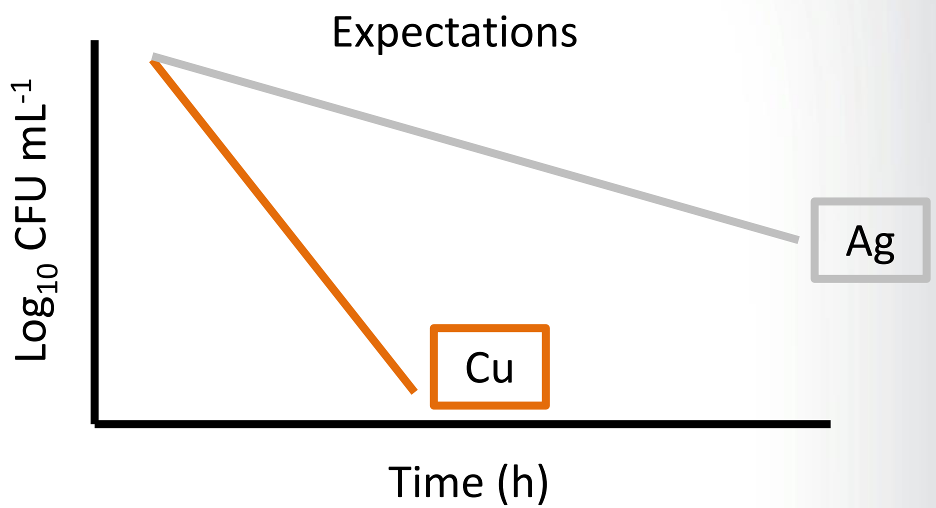
- What individual concentrations of Cu and Ag are required for *Lp* inactivation?
  - Isolate the effects of Cu and Ag on the inactivation of *Lp.*
  - Test different *Lp* strains and serogroups.

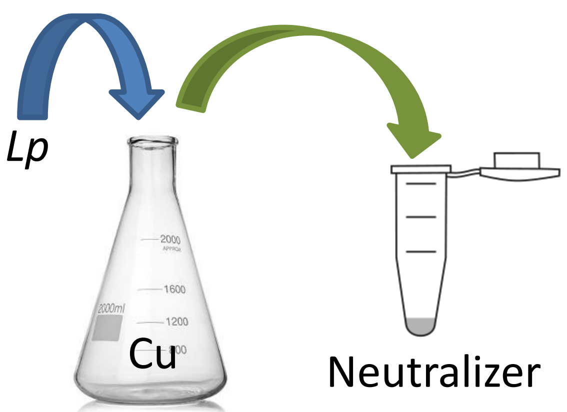
Longer-term goals:

- Scaling these bench-scale experiments up to a pilot study in drinking water test loop.
  - Applying optimized Cu and Ag concentrations to control L. pneumophila.
- Characterize the effects of the Cu and Ag ions on various drinking water quality parameters.
  - pH, chlorine, and phosphate.

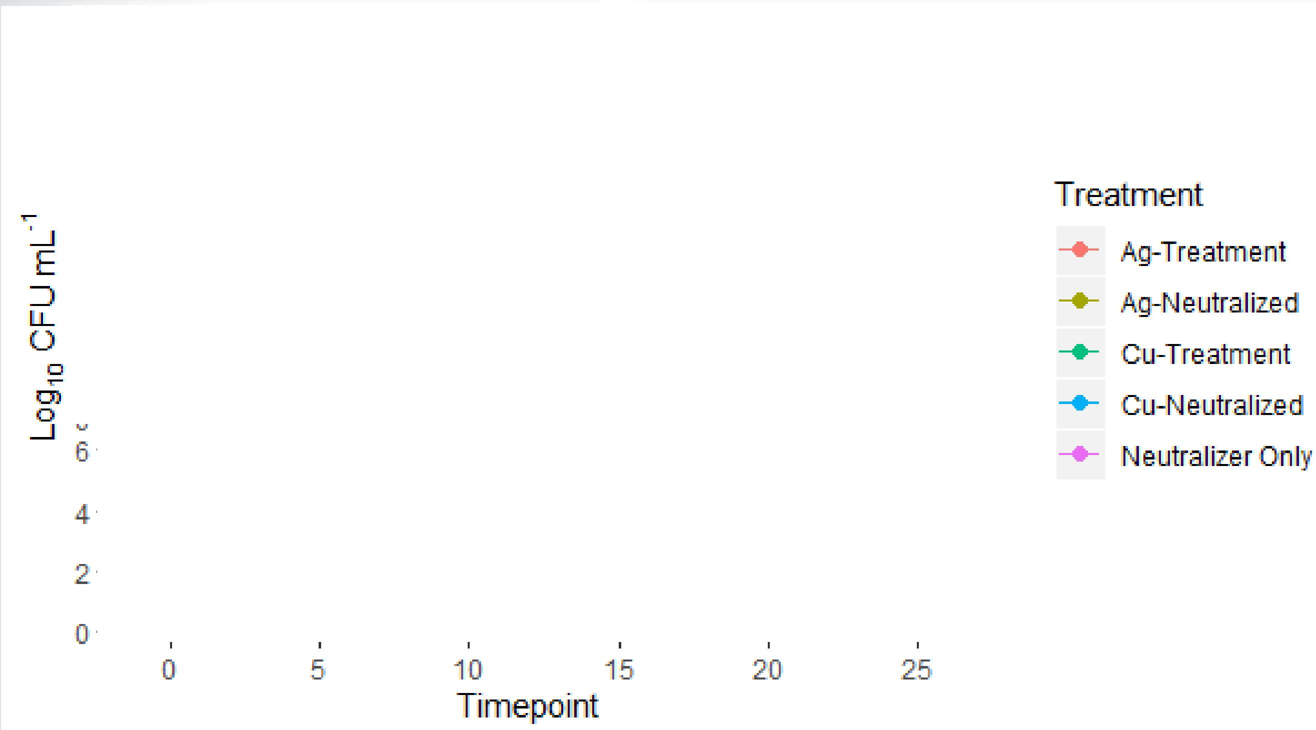
Experimental conditions

- Test strain: *L. pneumophila* sg-1 strain
  - – Drinking water isolate
- Test media: "DIC10" buffer
  - → 10 mg/L inorganic carbon, pH 8, filter-sterilized
- Ion solutions filtered at 0.22 um to obtain total and dissolved concentrations
  - – Start and end ion concentrations via ICP analysis
- 22°C
- Timepoints: 0, 2, 5, and 24 hours





Results – Experiment I



(h)

- Neutralizer: combination of 14% sodium thiosulfate and 10% sodium thioglycolate (Landeen 1989)

Ion	Target Concentration	Actual Concentration
Cu	0.1 ppm	0.097 ppm
Ag	0.01 ppm	0.009 ppm

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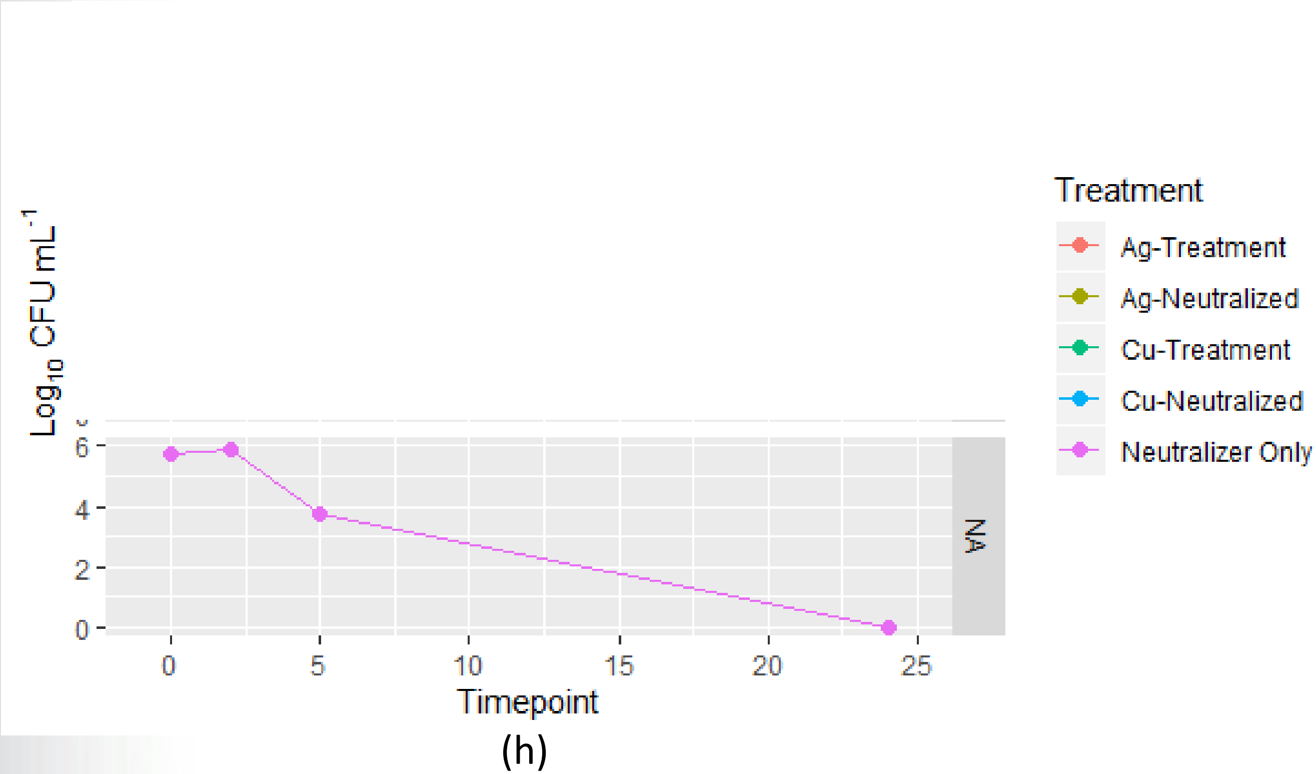

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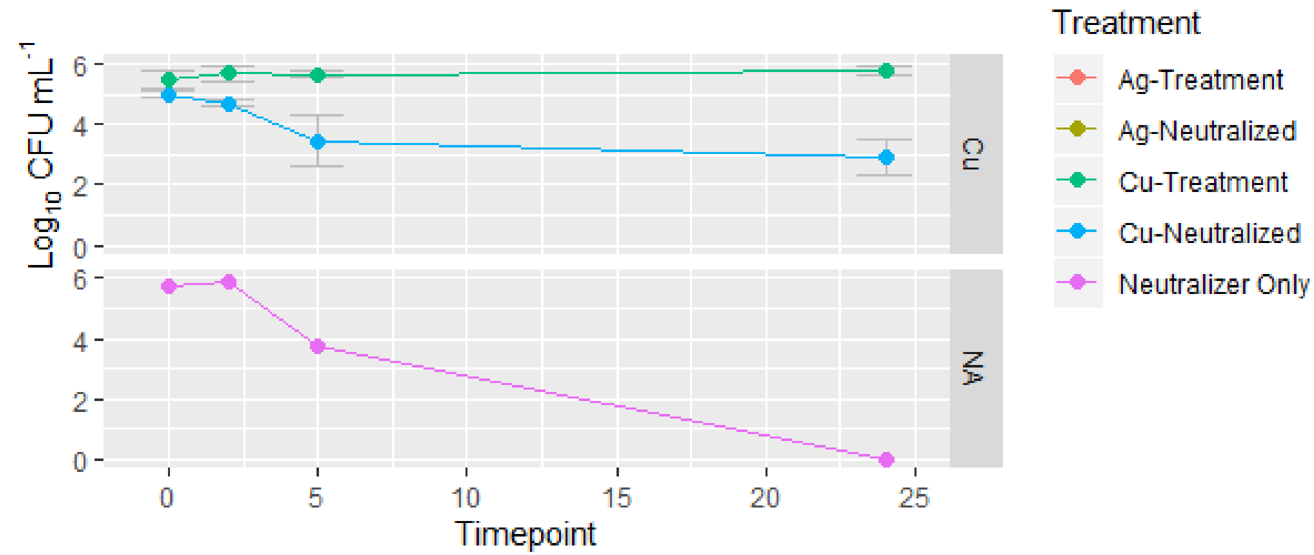

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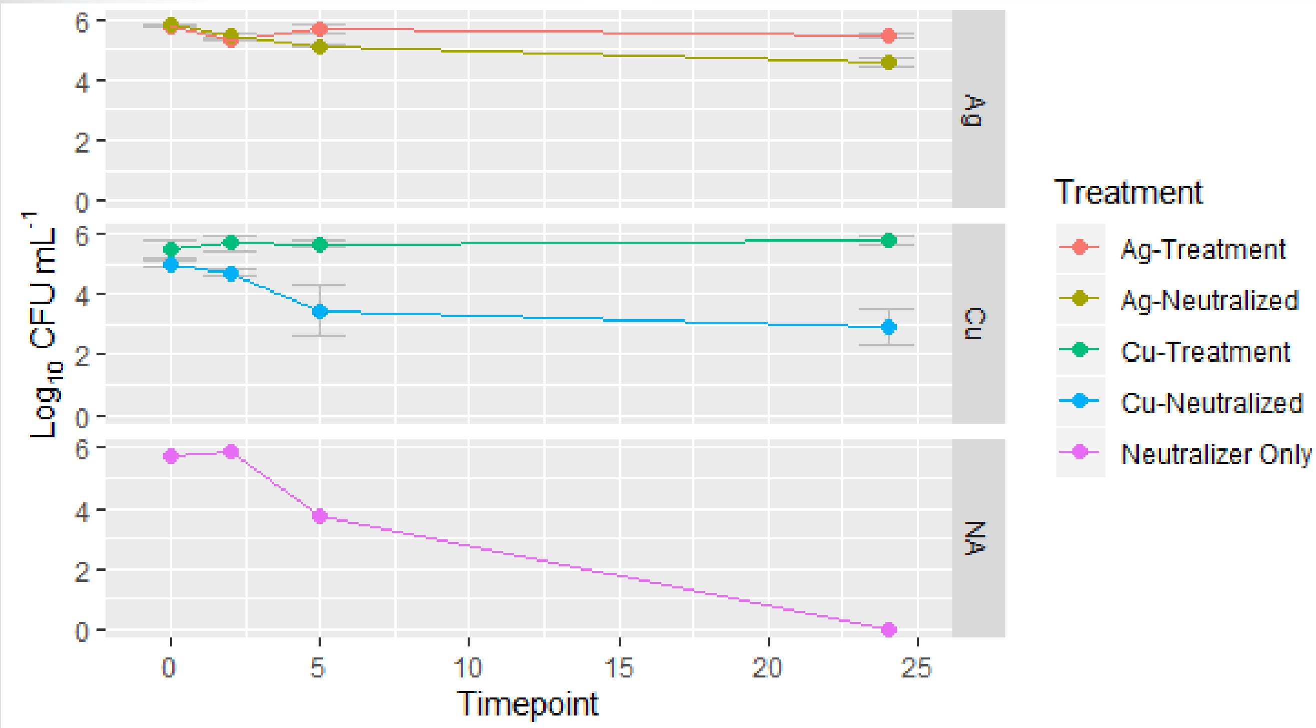

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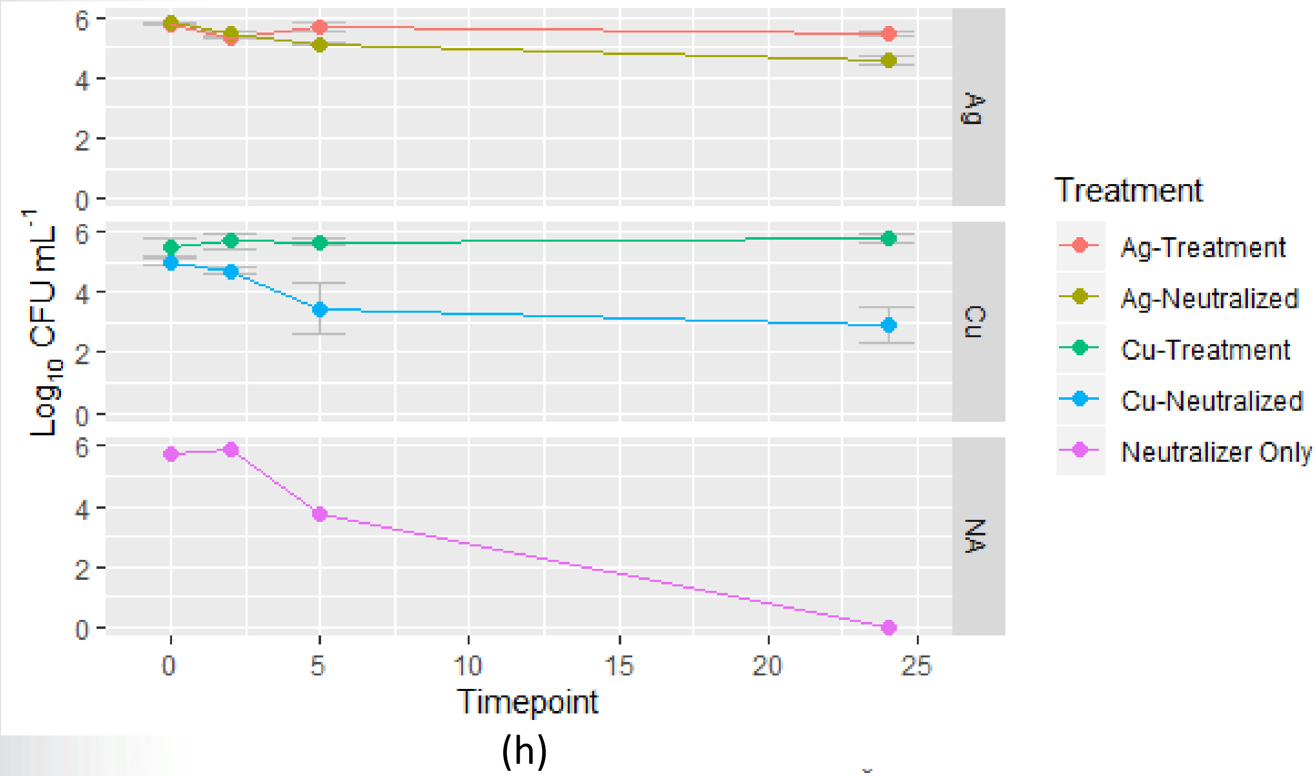

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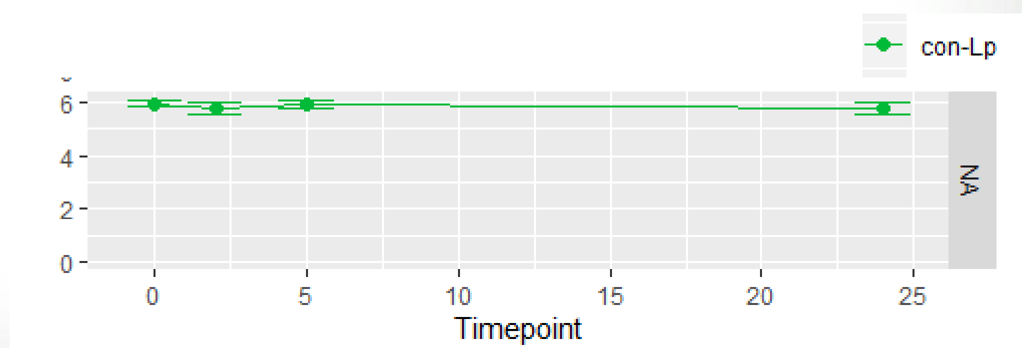

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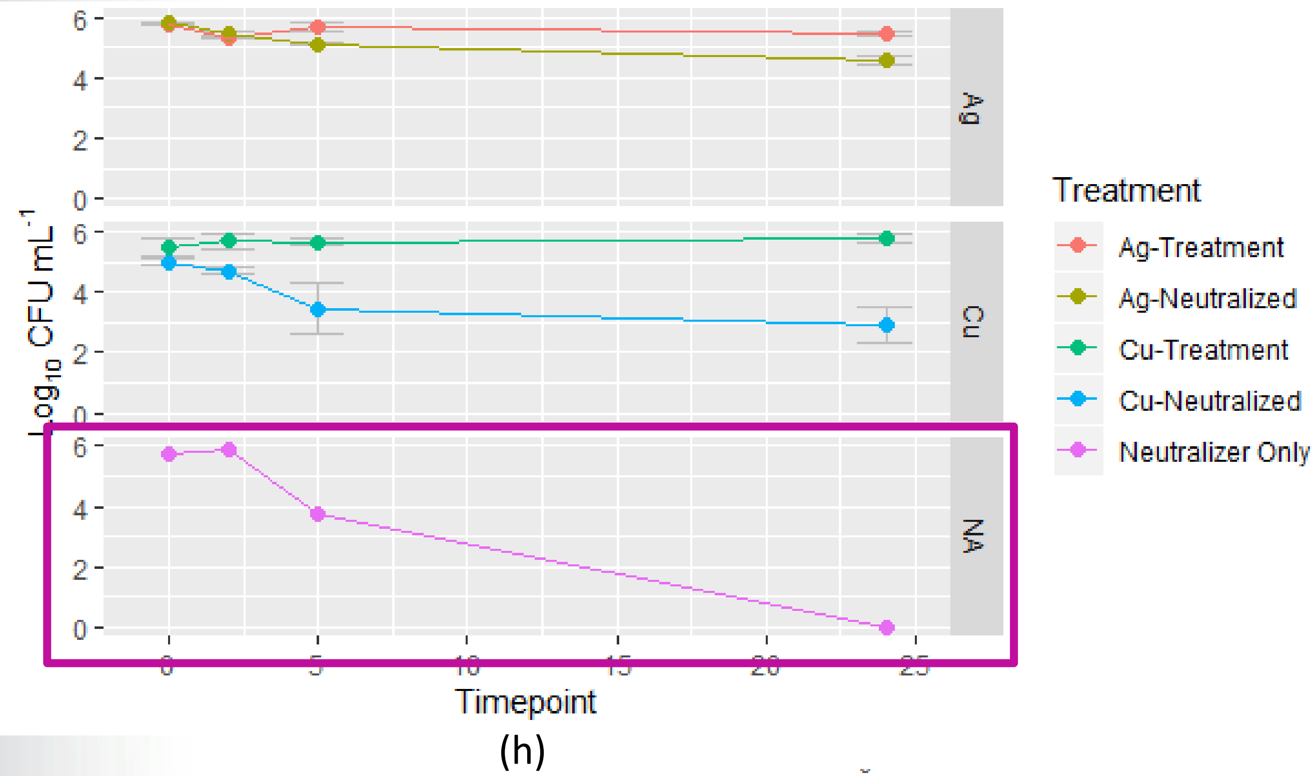

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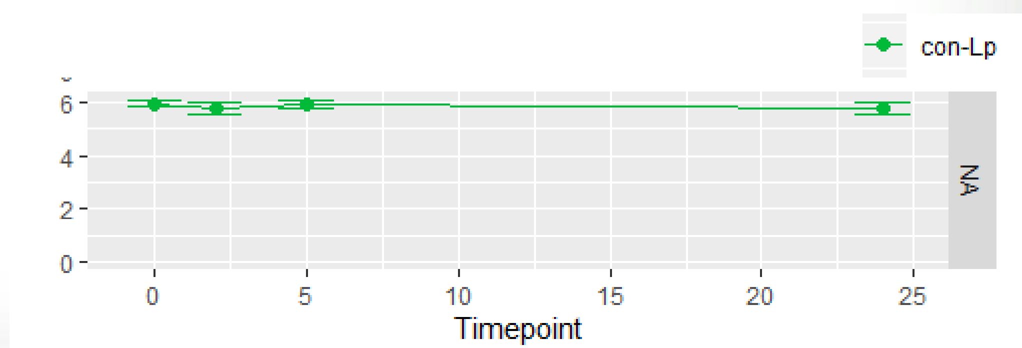

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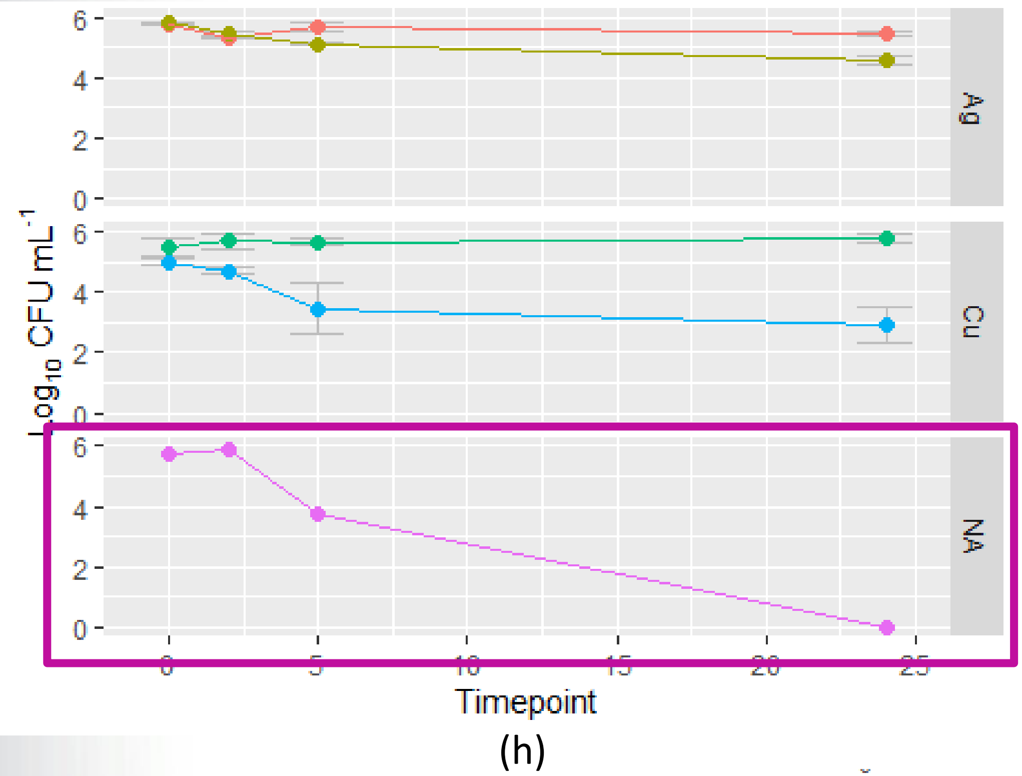
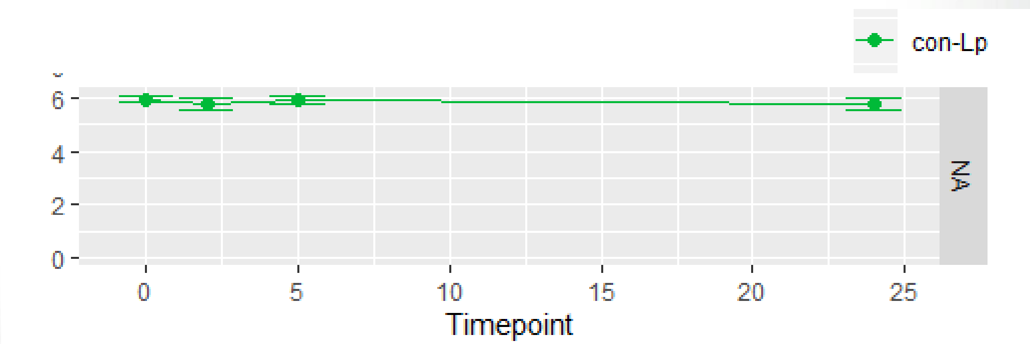


From this experiment:

- Toxicity of neutralizer?
  - *Previous work has not shown a neutralizer control.*
- Ion levels too low to see inactivation within 24 hours



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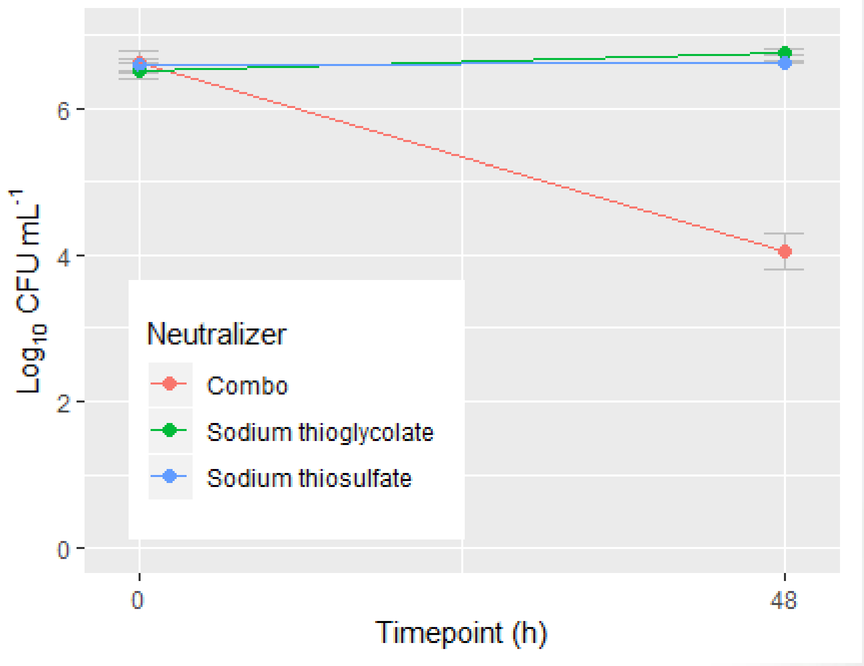
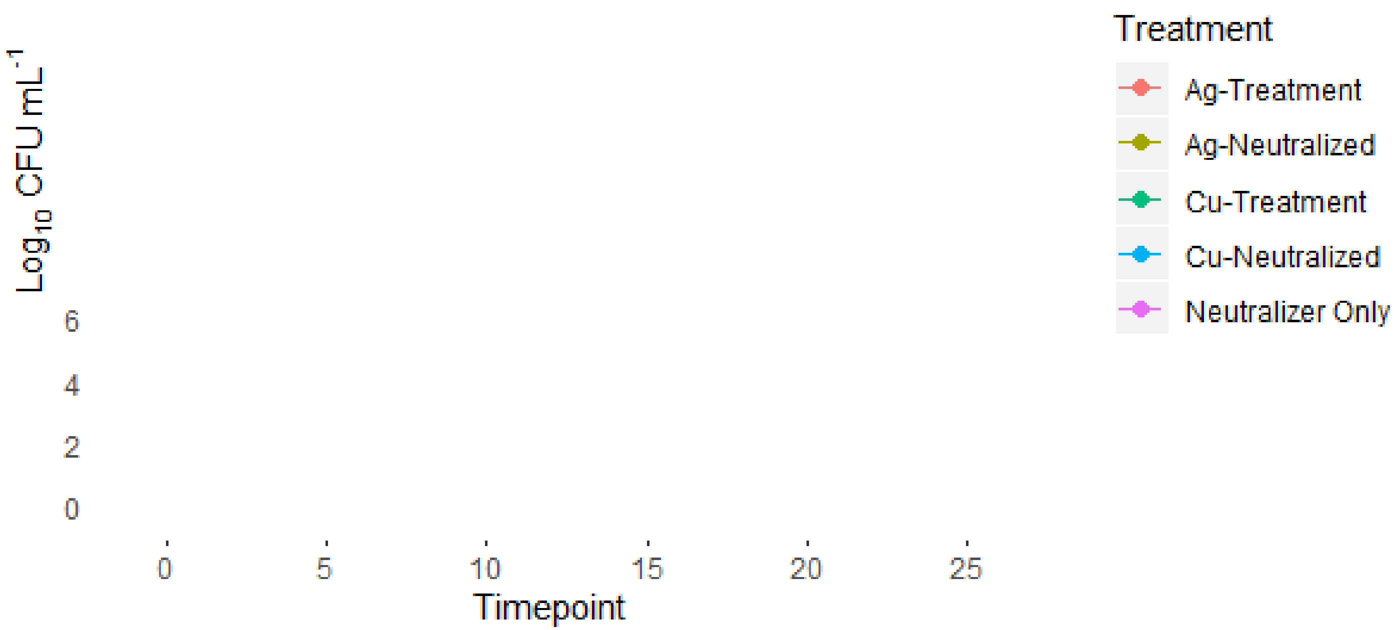
Results – Holding Experiment

- Two timepoints: 0 and 48 h
- Combination of sodium thiosulfate and sodium thioglycolate is toxic.
- No toxic effects when used independently.
- → Sodium thiosulfate as neutralizer.



Results – Experiment II

- Neutralizer:
10% sodium thiosulfate



Ion	Target Concentration	Actual Concentration
Cu	0.3 ppm	0.28 ppm
Ag	0.03 ppm	0.029 ppm

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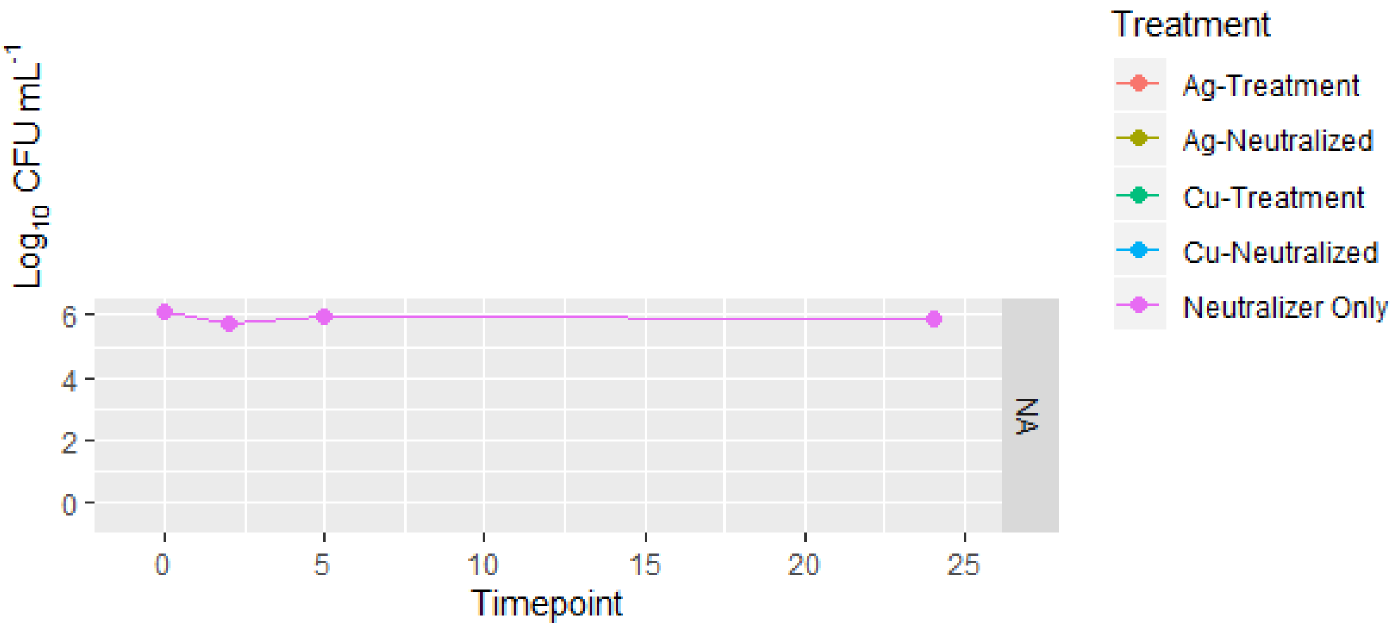

(h)

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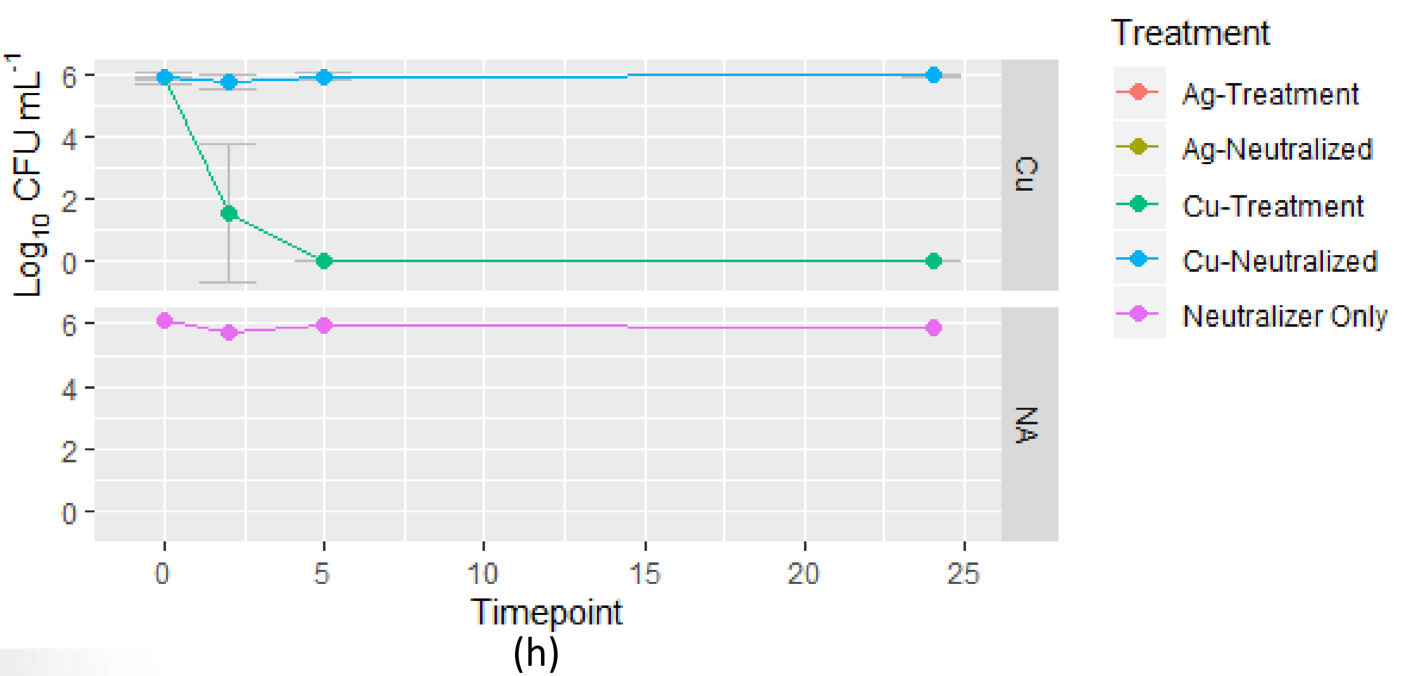

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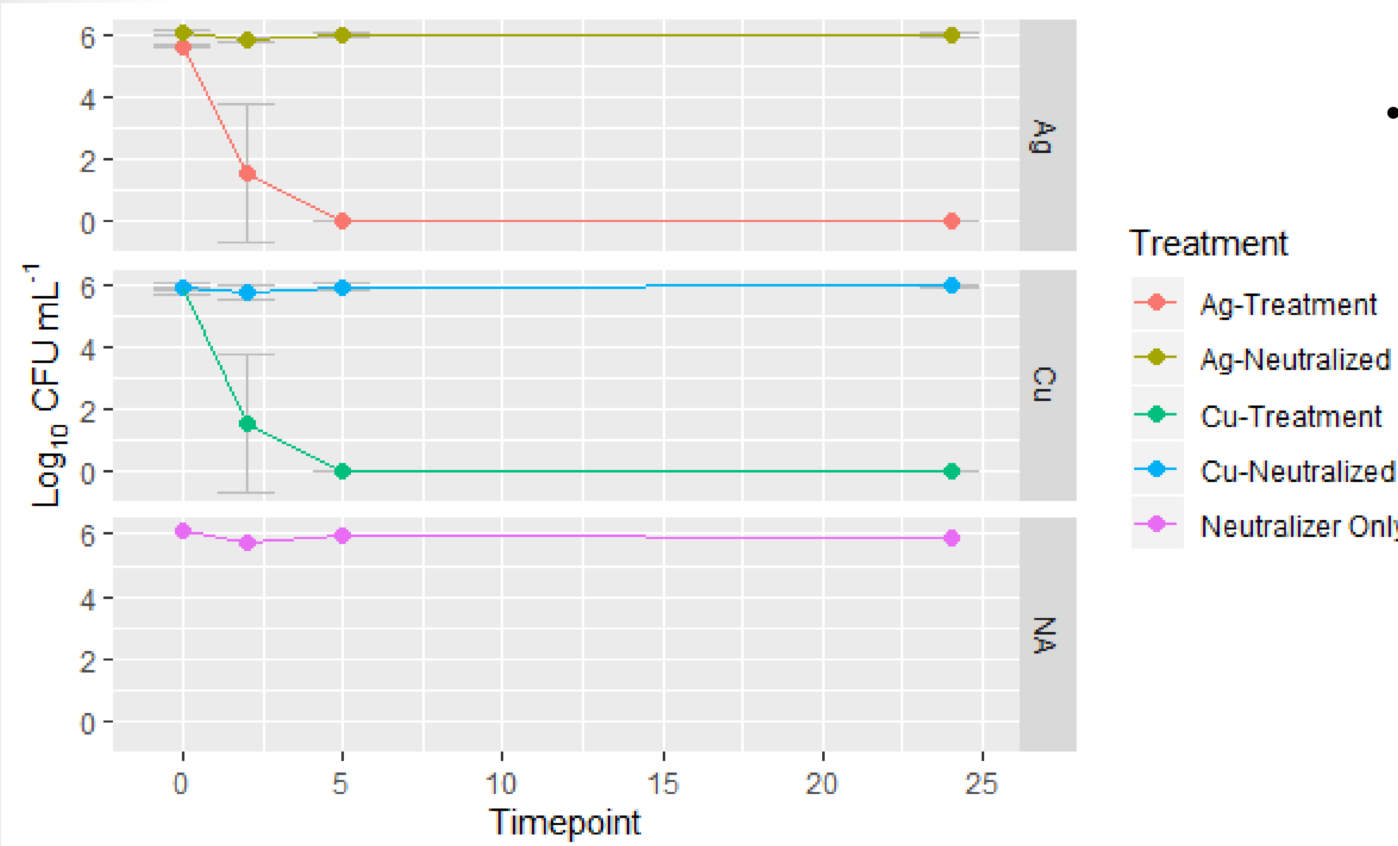

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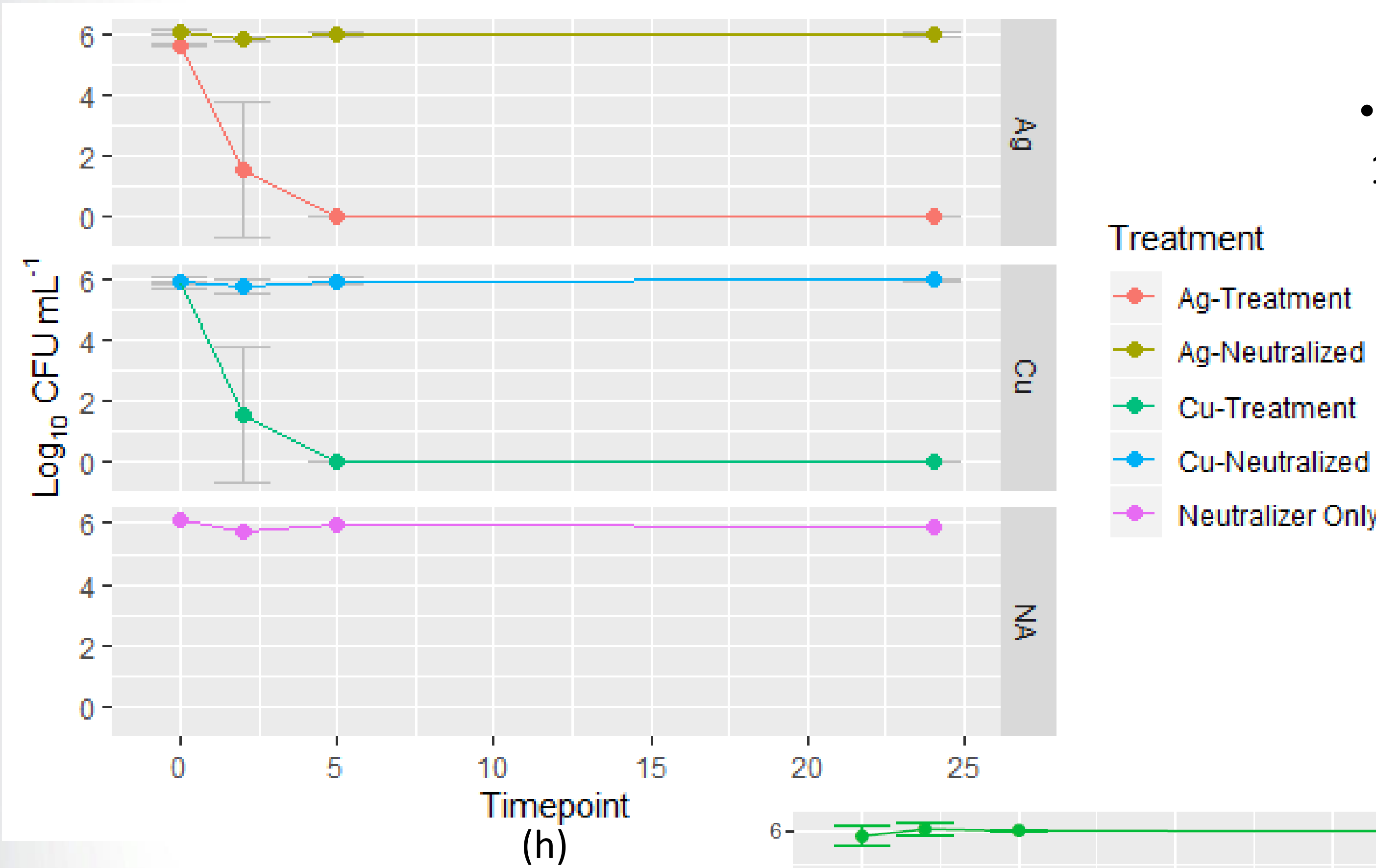

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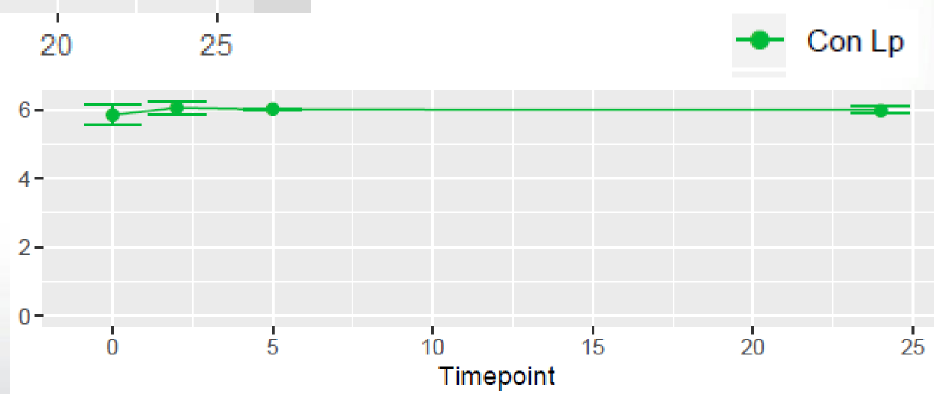

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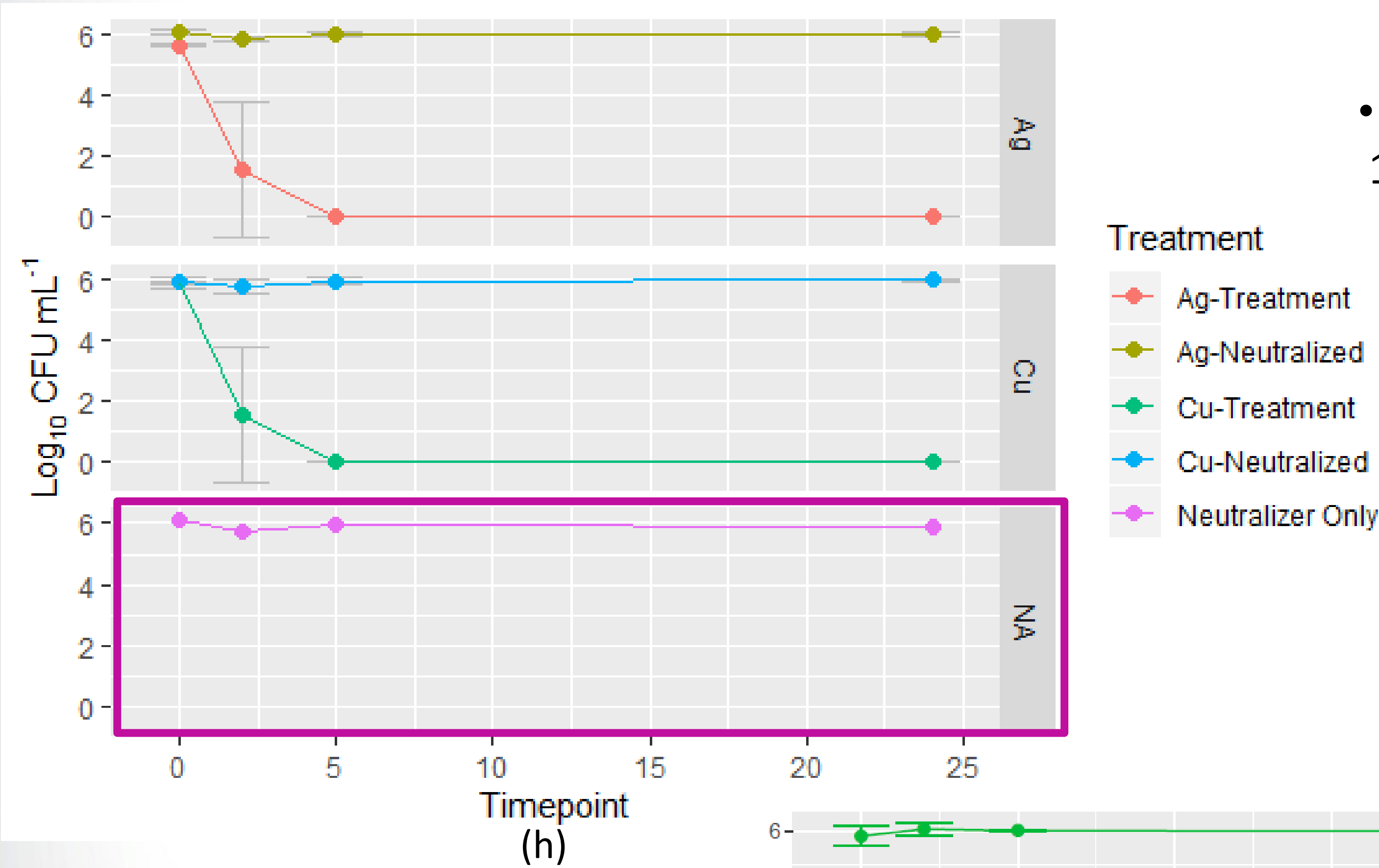

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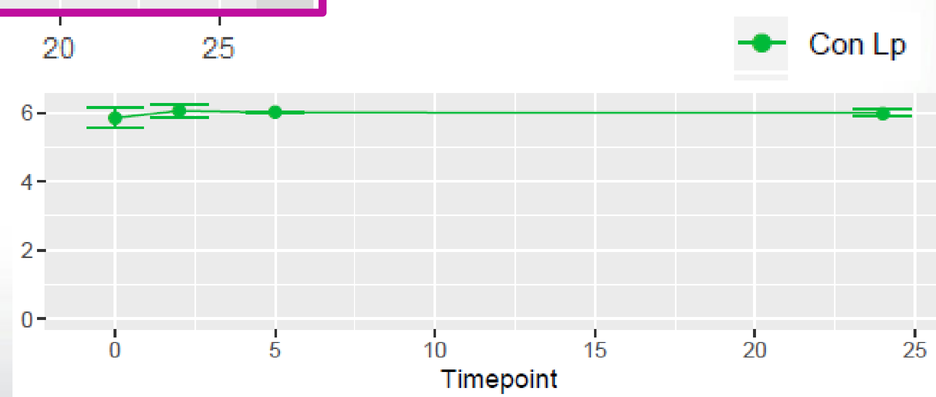

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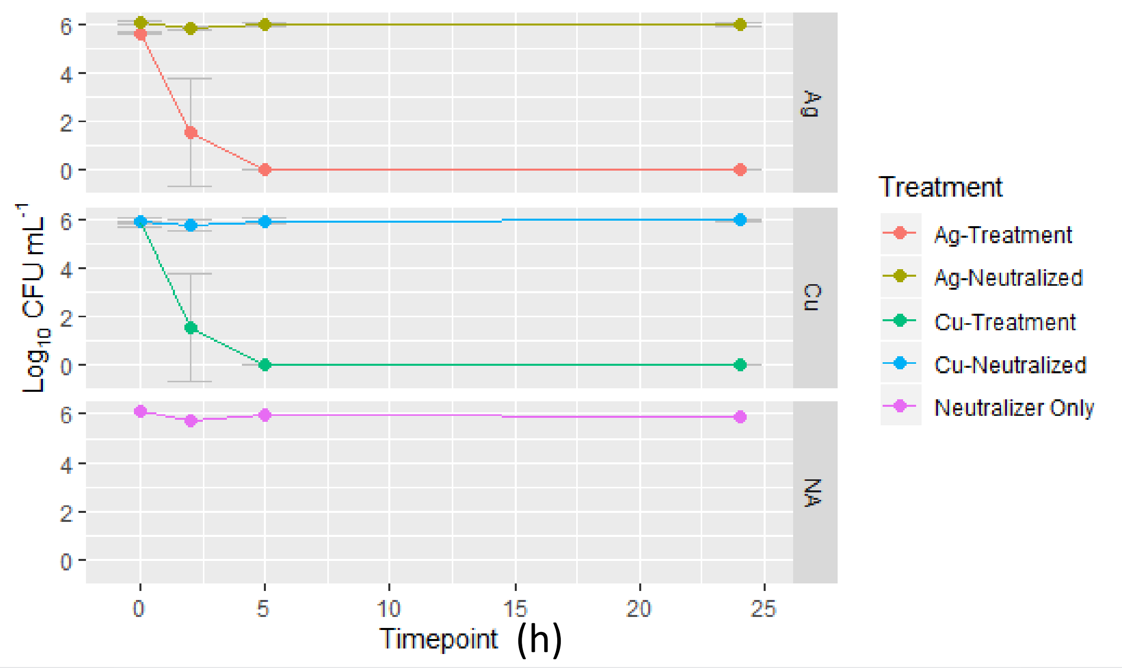
Summary of results

- Successfully achieved and maintained target concentrations of Cu and Ag.
- Cu and Ag successfully inactivated Lp after 5 hours (green and red lines).
- Sodium thiosulfate successfully neutralized Cu and Ag ions (blue and yellow lines).
- Sodium thiosulfate did not negatively impact Lp (purple line).



What's next for this research?

- Evaluate earlier timepoints.
- Evaluate different independent concentrations of Cu and Ag.
- Look at combined effects of Cu and Ag at various concentrations and and synergistic effects with different disinfectants, e.g. chlorine, monochloramine.
- Evaluate the effects of Cu and Ag on different *Legionella pneumophila* strains and serogroups.

- Pilot study using Cu and Ag in a drinking water test loop.
- Biofilm-associated *Lp*

THANK YOU

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L. pneumophila sg1
drinking water isolate
grown on BCYE agar plates

Agar art courtesy of Helen Buse

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