Abstract

The U.S Military requires improved sterility and disinfection strategies in austere medical settings. Inorganic transition metal ion disinfectants bind to various bacterial constituents to affect viability. Unfortunately, the reactivity of metal ions reduces their efficiency as a disinfectant in real-world applications. Zeolites are crystalline high internal surface area microporous aluminosilicates formed by interlocked TO_4 tetrahedra (T-Si, Al), which show widespread applications in many industrial processes such as catalysis, adsorption, and separation. Zeolites can also store transition metal ions in their supercage cavities through electrostatic interactions and ion exchange. A novel compound nanozeolite (NZ), 30nm Faujasitic zeolite crystals, has been developed and is commercially available and may be able to serve as a reservoir of metal ions, directly delivering them to microbial targets. We hypothesized that NZ impregnated with metal will have an increased antimicrobial effect in a shorter duration due to the effective zeolite-bacteria surface interactions, faster ion-release kinetics, and bacterial penetration. This project utilizes EPA-recognized disinfectant testing strains, *Pseudomonas aeruginosa* (ATCC®15442 and *Staphylococcus aureus* (ATCC®6538) to test the antimicrobial efficacy of NZ formulations encapsulated with Ag^{+}, Zn^{2+}, and Cu^{2+}.

Methods

In-vitro susceptibility dilution assays were performed with *P. aeruginosa* (ATCC®15442) and *S. aureus* (ATCC®6538) to determine the minimal bactericidal concentrations of NZ with silver and zinc (NZ-AgZn), and nanozeolites with silver and copper (NZ-AgCu). Controls for these experiments were bacteria suspended in phosphate-buffered saline (PBS) alone and NZ synthesized without addition metals (NZ-NaFAU30) at concentrations of up to 1,000 parts per million (ppm). Bacteria in the mid-log phase were reconstituted in 1X PBS to a starting concentration of $1x10^4$ - $1x10^5$ Colony Forming Units (CFU/mL). Suspensions were then challenged with concentrations of NZ-AgZn and NZ-AgCu between 1.3 – 1,000 ppm for 2 hours at 37˚C with 200 rpm shaking. Following treatment, bacteria were serially diluted in 1X PBS and spread onto tryptic soy agar plates using sterile glass beads. After a 16-18-hour incubation at 37°C, plates were counted with an INTERSCIENCE Scan®500 automatic colony counter to determine bacterial log reduction in CFUs. The CFU data was entered into GraphPad Prism software. Statistical analysis was conducted using ANOVA and Dunnett's Post-Hoc tests to compare the treatment concentrations against the controls; A p-value < 0.05 was considered statistically significant. Experiments were performed three independent times with three technical replicates per experiment.

Figure 1. Schematic of framework structure of faujasitic zeolite and with Siver ions

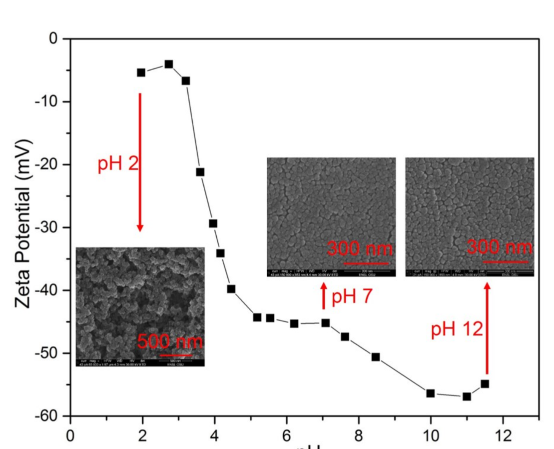

Figure 2. Zeta potential as a function of pH, SEM show particle packing at different pH. Graph provided by Dutta, P. (Zeovation)

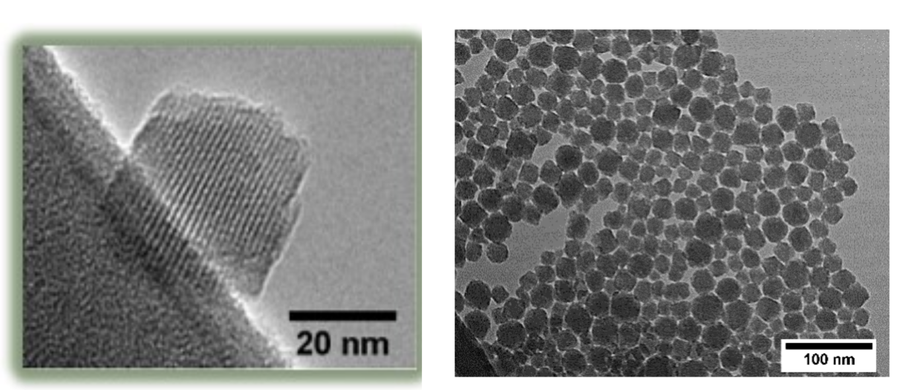

Figure 3. High-resolution TEM of Nanozeolite particle. Photo provided by Dutta, P. (Zeovation)

References:

Dutta, P., & Wang, B. (2019). Zeolite-supported silver as antimicrobial agents. *Coordination Chemistry Reviews*, 383, 1–29. https://doi.org/10.1016/J.CCR.2018.12.014

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Zhang, H., Kim, Y., & Dutta, P. K. (2006). Controlled release of paraquat from surface-modified zeolite Y. *Microporous and Mesoporous Materials*, 88(1–3), 312–318. https://doi.org/10.1016/J.MICROMESO.2005.09.026

Experimental Schematic

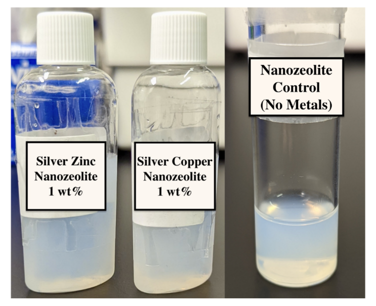


Figure 4. Photo of NZ samples suspended in water at 10,000 ppm concentration. 1%wt is equal to 10,000 ppm.

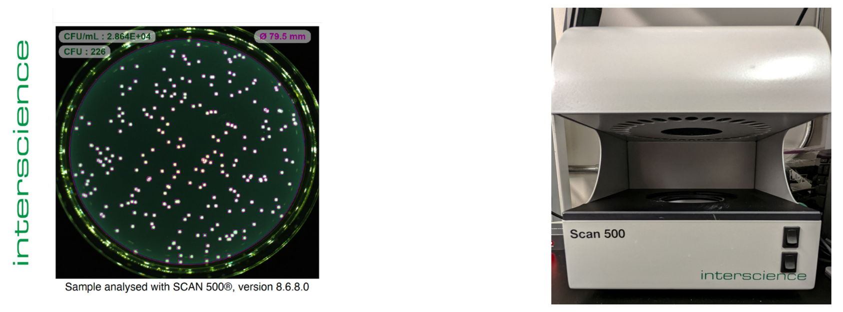


Figure 5. Photo of Untreated sample *P. aeruginosa* on SCAN®500 (left) Photo of Scan®500 machine from Interscience (right).

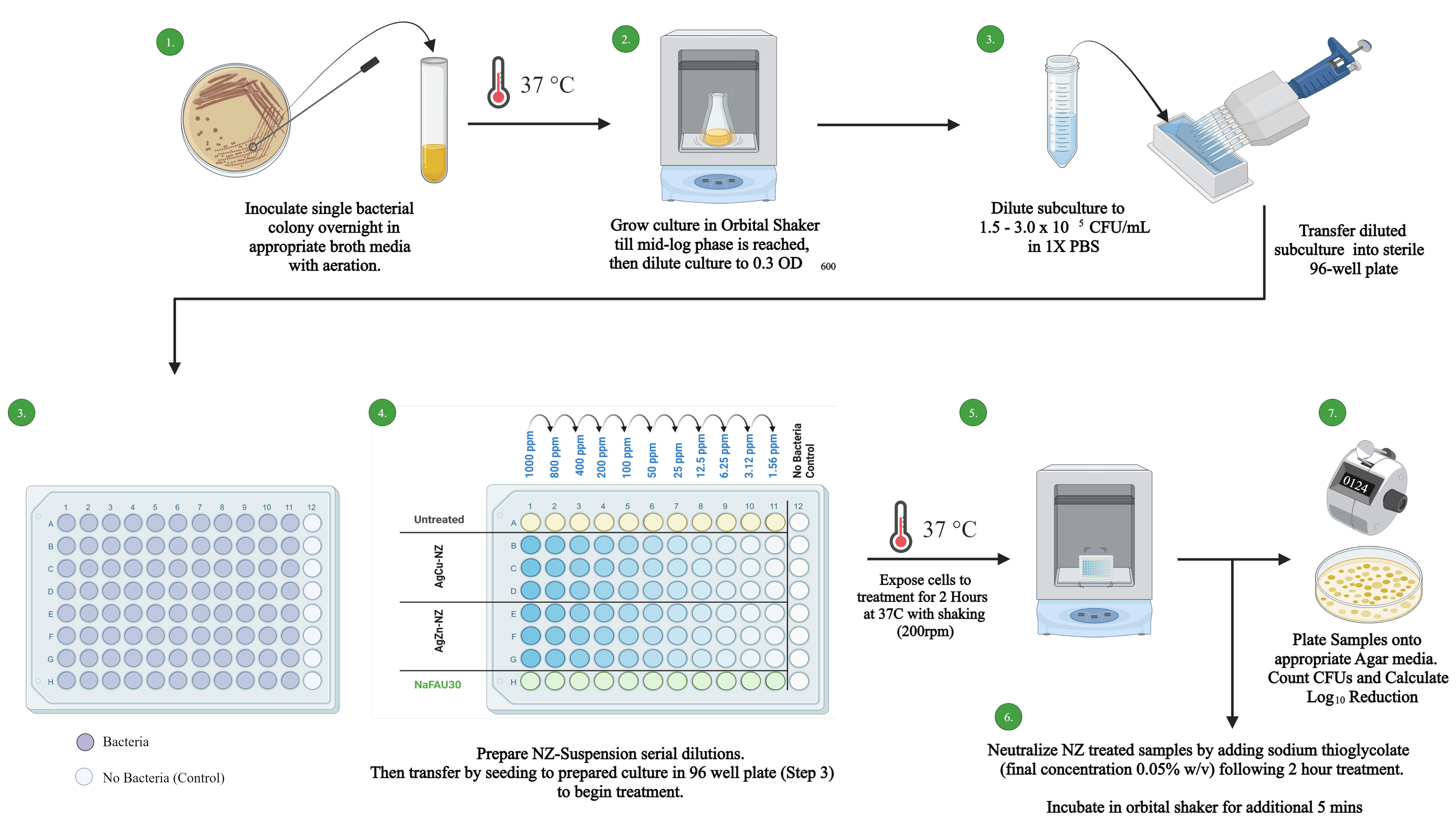
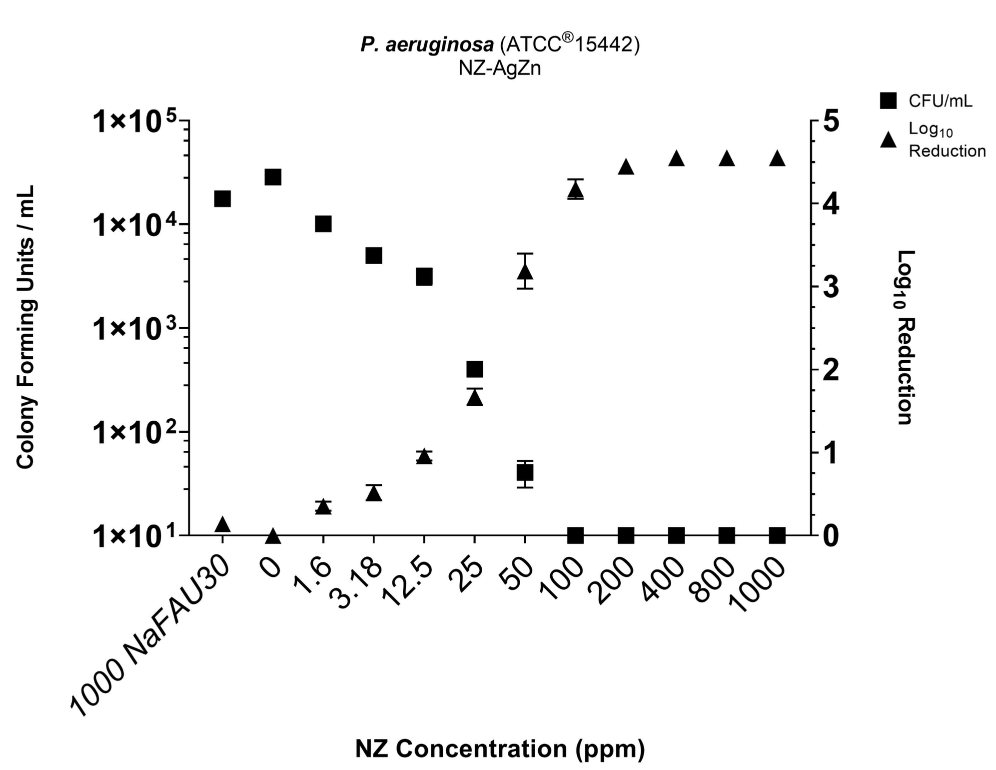


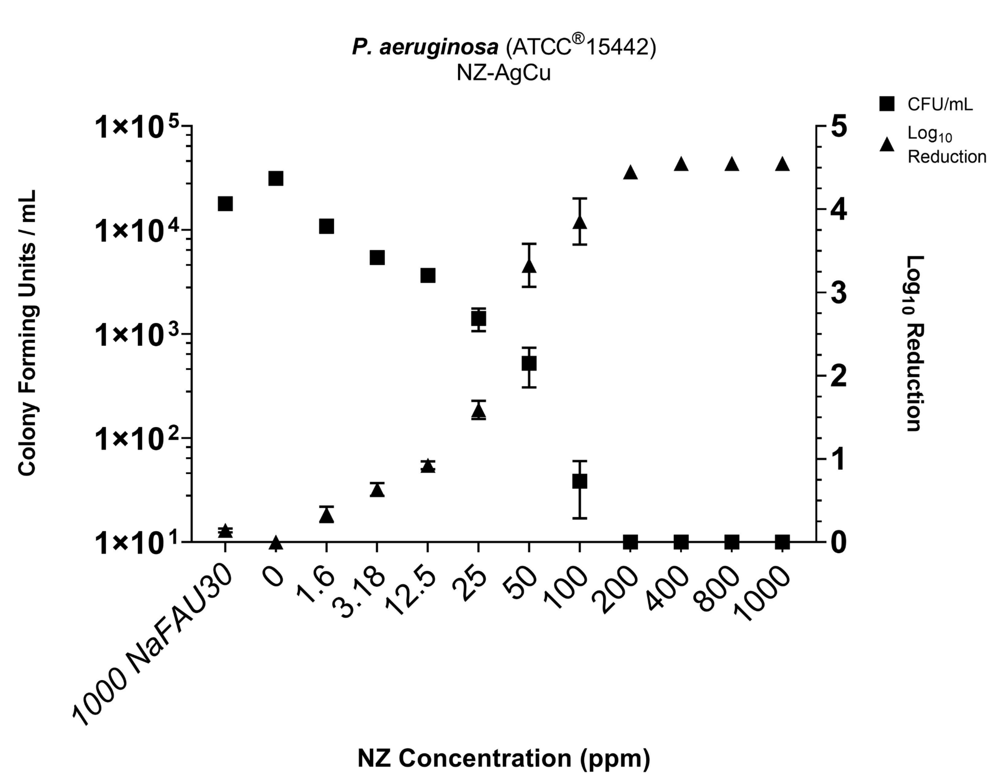
Figure 6. Experimental Schematic Diagram of *In-vivo* susceptibility dilution assay

Results



Concentration (ppm)	Significance	Adjusted P value
(NaFAU30) 1000 ppm	ns	>.999
1.6 ppm	ns	0.253
3.18 ppm	***	0.046
12.5 ppm - 1000 ppm	***	<.001

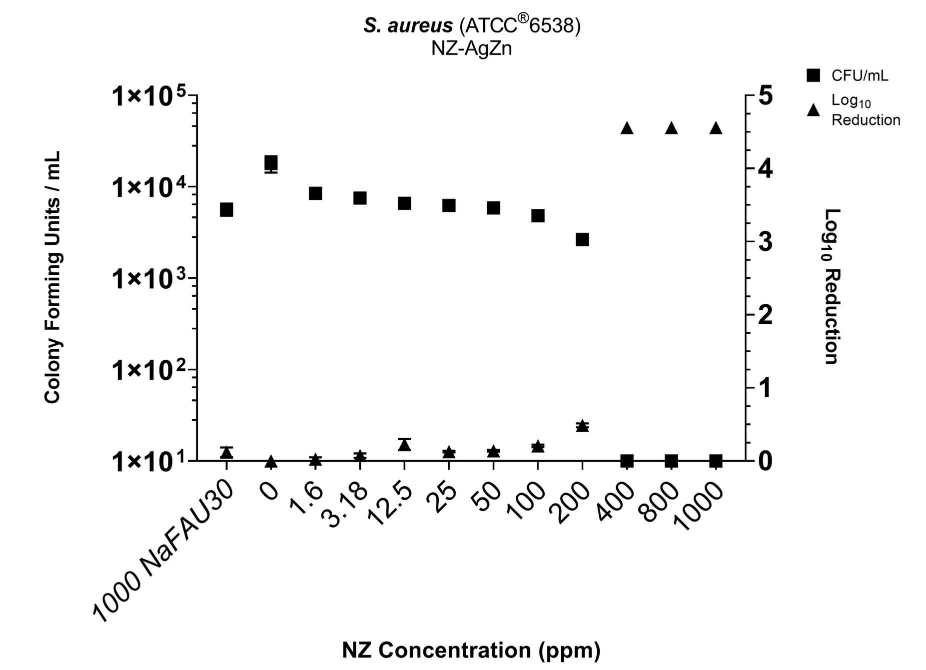
Figure 7. *P. aeruginosa* was challenged with NZ-AgZn at concentrations of 1.6 - 1000 ppm. A Graph is plotted with CFU/mL and Log_{10} reduction values following 2-hour treatment (Top). A two-way ANOVA was performed to analyze the effect of NZ-AgZn concentrations and CFU Log reductions using the untreated sample (0 ppm) as a control (Bottom). α = .05



Concentration (ppm)	Significance	Adjusted P value
(NaFAU30) 1000 ppm	ns	>.999
1.6 ppm	ns	0.632
3.18 ppm	*	0.03
12.5 ppm - 1000 ppm	***	<.001

Figure 8. *P. aeruginosa* was challenged with NZ-AgCu at concentrations of 1.6 - 1000 ppm. A Graph is plotted with CFU/mL and Log_{10} reduction values following 2-hour treatment (Top). A two-way ANOVA was performed to analyze the effect of NZ-AgCu concentrations and CFU Log reductions using the untreated sample (0 ppm) as a control (Bottom). α = .05

Results C



Concentration (ppm)	Significance	Adjusted P value
(NaFAU30) 1000 ppm	ns	.9886
1.6 ppm	ns	0.393
3.18 ppm	**	0.007
25 ppm - 1000 ppm	***	<.001

Figure 9. *S. aureus* was challenged with NZ-AgZn at concentrations of 1.6 - 1000 ppm. A Graph is plotted with CFU/mL and Log_{10} reduction values following 2-hour treatment (Top). A two-way ANOVA was performed to analyze the effect of NZ-AgZn concentrations and CFU Log reductions using the untreated sample (0) as a control (Bottom). α = .05

Conc

- Novel NZ formulations exhibited antibacterial efficacy v
- NZ-AgZn and NZ-AgCu reached complete kill of *P. aeru* of *S. aureus* at levels ≥ 200 ppm.
- When *S. aureus* and *P. aeruginosa* were challenged v biological or significant differences in log reductions wer
- This supports our hypothesis that Zn^{2+} or Cu^{2+} enc antimicrobial agents.
- Future studies will focus on identifying underlying me other antimicrobial NZ formulations.

Future D

This data illustrates the propensity and utility of self reaching potential for military applications in aust disinfection strategies will positively affect military bene will focus on developing and optimizing NZ antimicrob and Polyester), and understanding the mechanistic differ

Acknowle

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Discla

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