Electronic Supplementary Material (ESI) for Environmental Science: Nano.

SUPPORTING INFORMATION

SYNTHESIS, CATALYTIC, ANTIMICROBIAL AND CYTOTOXICITY EVALUATION OF GOLD & SILVER NANOPARTICLES USING BIODEGRADABLE, π-CONJUGATED POLYAMIC ACID

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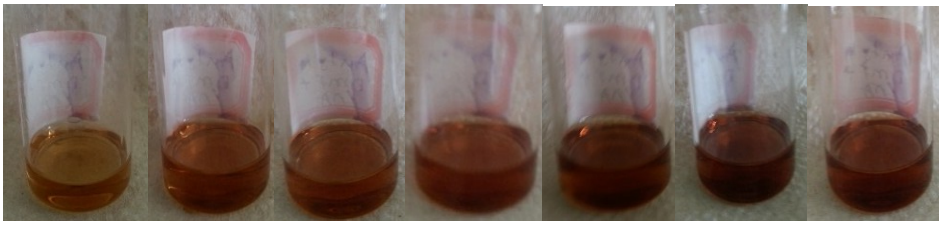
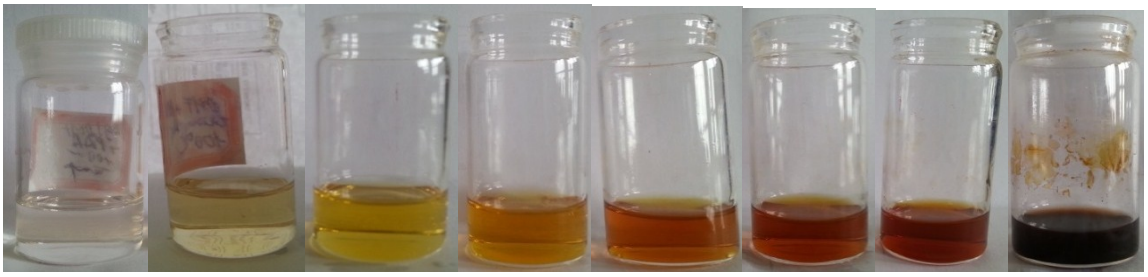
Fresh 1min 5min 10min 15min 20min 25min



30min 40min 50min 60min 75min 90min 120min

Figure S1A: Time-based pictogram of formation of AuNPs in the presence of PAA

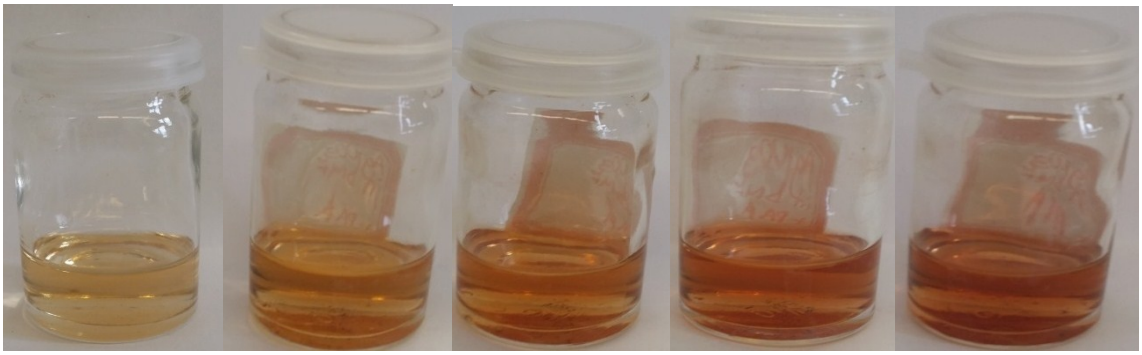


Fresh 5min 15min 25min 35min 45min 90min 200min

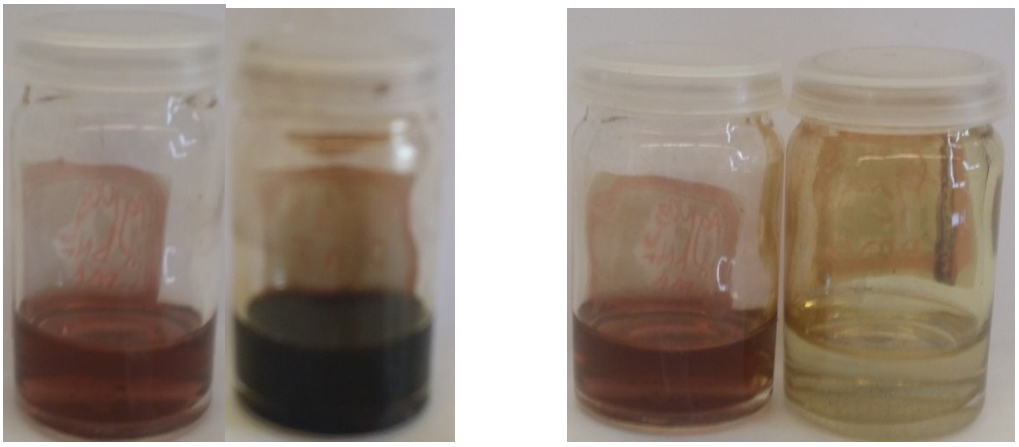
Figure S1B: AgNPs formation in DMF with PAA at 100℃

Fresh 5days

Figure S1C: *AgNPs in DMF with PAA at Room Temperature*



Fresh 15mins 30mins 50 mins 65mins



90 mins 120 mins Comparison of sample and control after 90 mins

Figure S2: *AgNPs in DMF with PAA at 70 ℃*

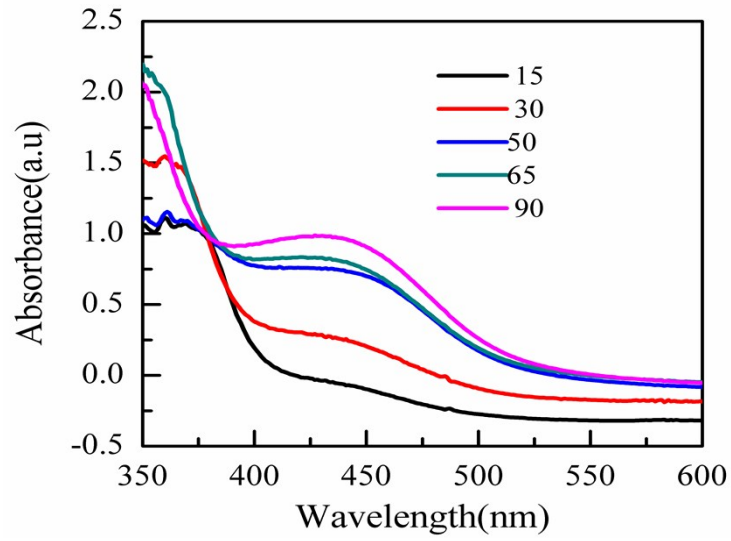
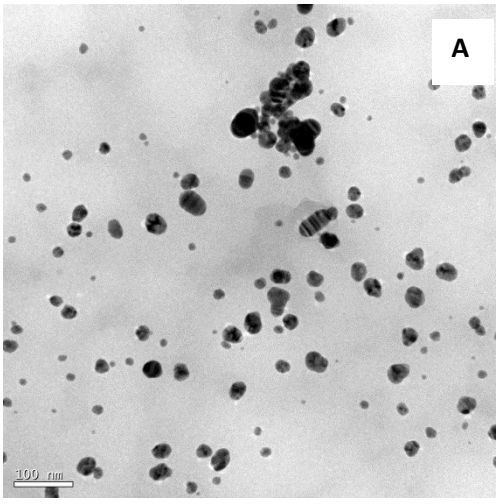
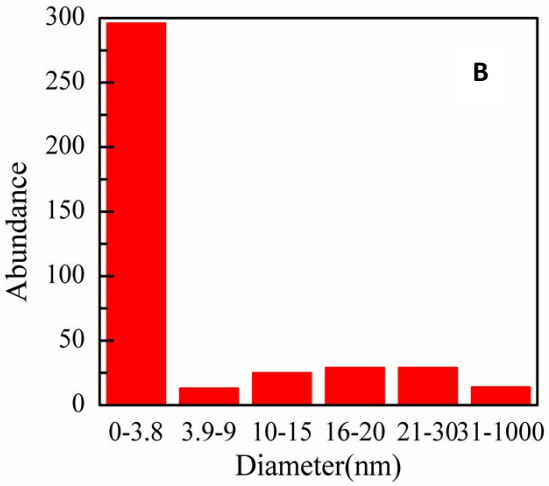
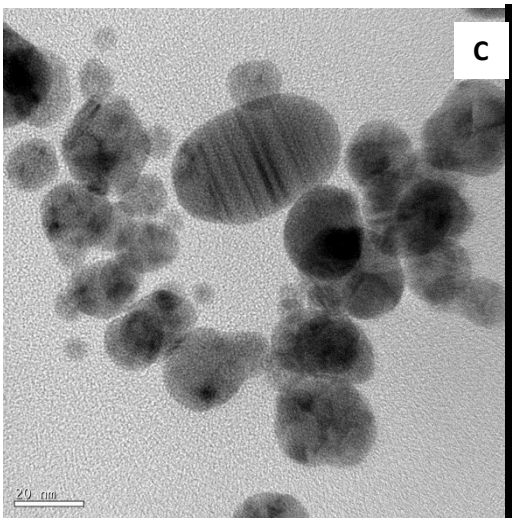
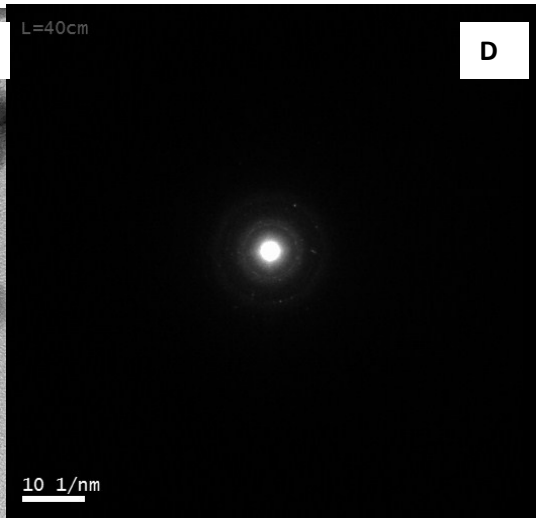


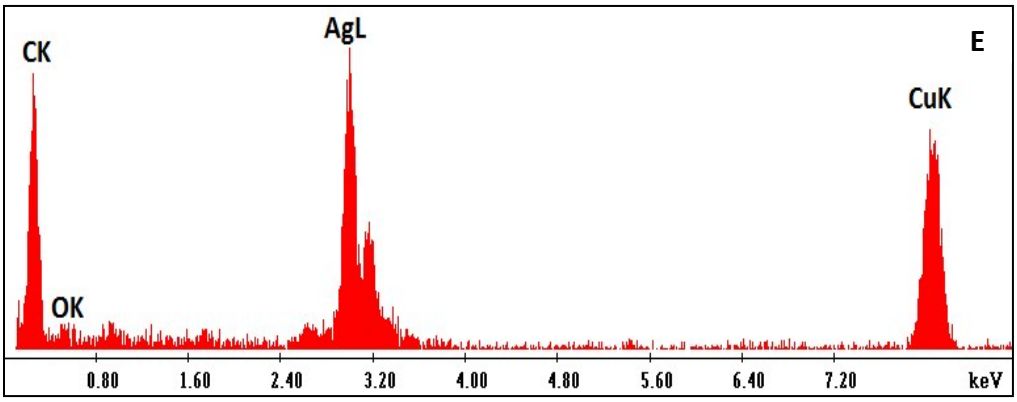
Figure S3: *UV-Vis spectra of PAA stabilized AgNPs at 70 C*


A
100 nm


B
300
250
200
150
100
50
0
Abundance
0-3.8
3.9-9
10-15
16-20
21-30
31-1000
Diameter(nm)


C
20 nm


L=40cm
D
10 1/nm


CK
AgL
E
CuK
OK
0.80
1.60
2.40
3.20
4.00
4.80
5.60
6.40
7.20
keV

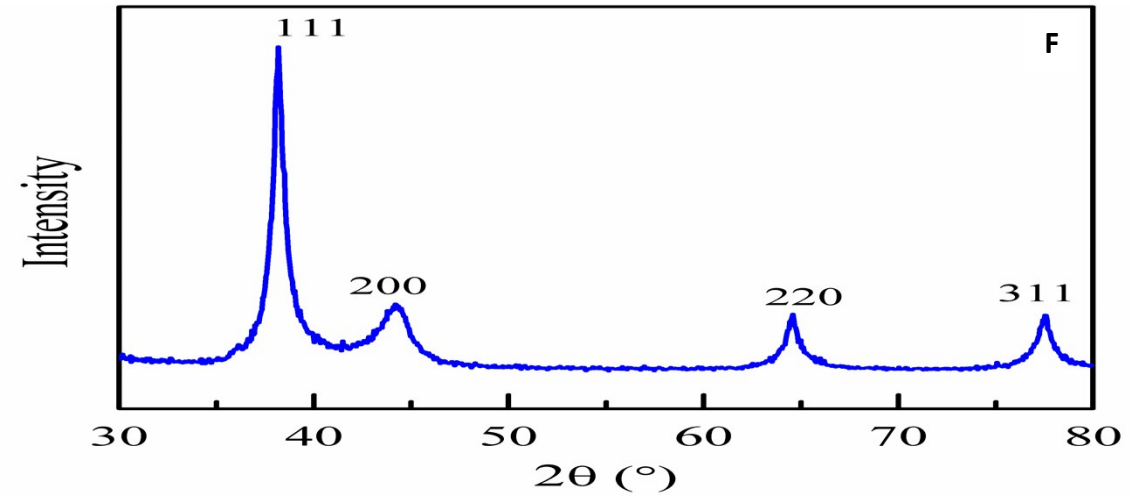


Figure S4: *TEM(A), size distribution histogram(B), High Resolution TEM(C), Selected area electron diffraction(D),Energy dispersive spectroscopy(E) and XRD(F)*

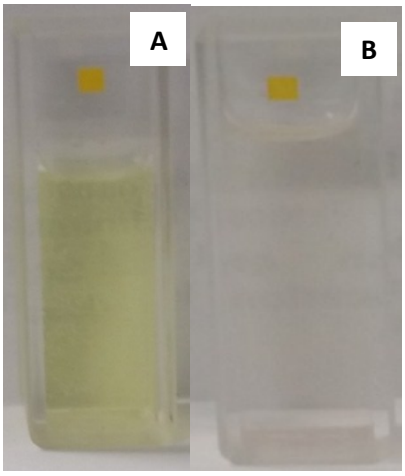


Figure S5: *Color changes for 4-nitrophenol solution (**A**) before and (**B**) after catalytic reaction*

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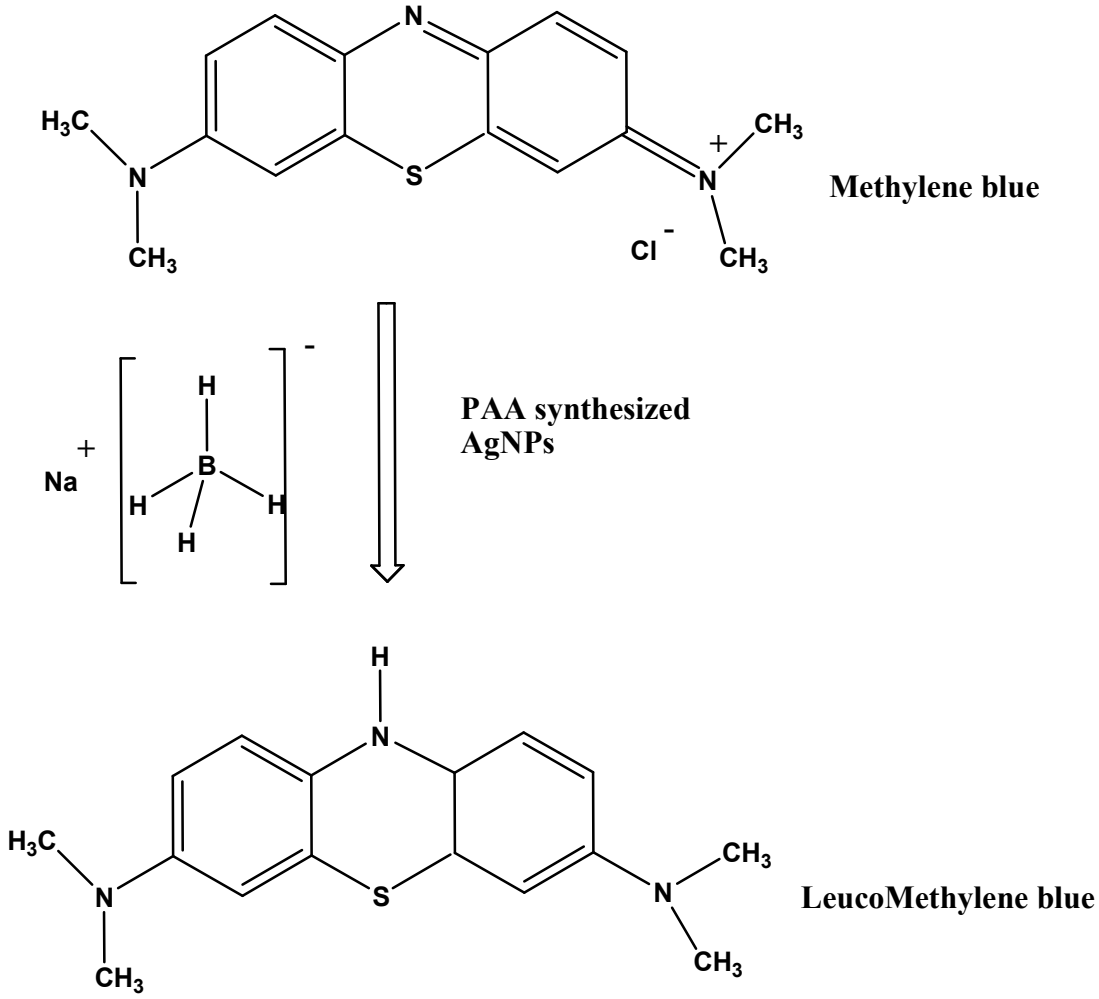


Figure S6: *Schematic for catalytic reduction of Methylene blue to LeucoMethylene blue*

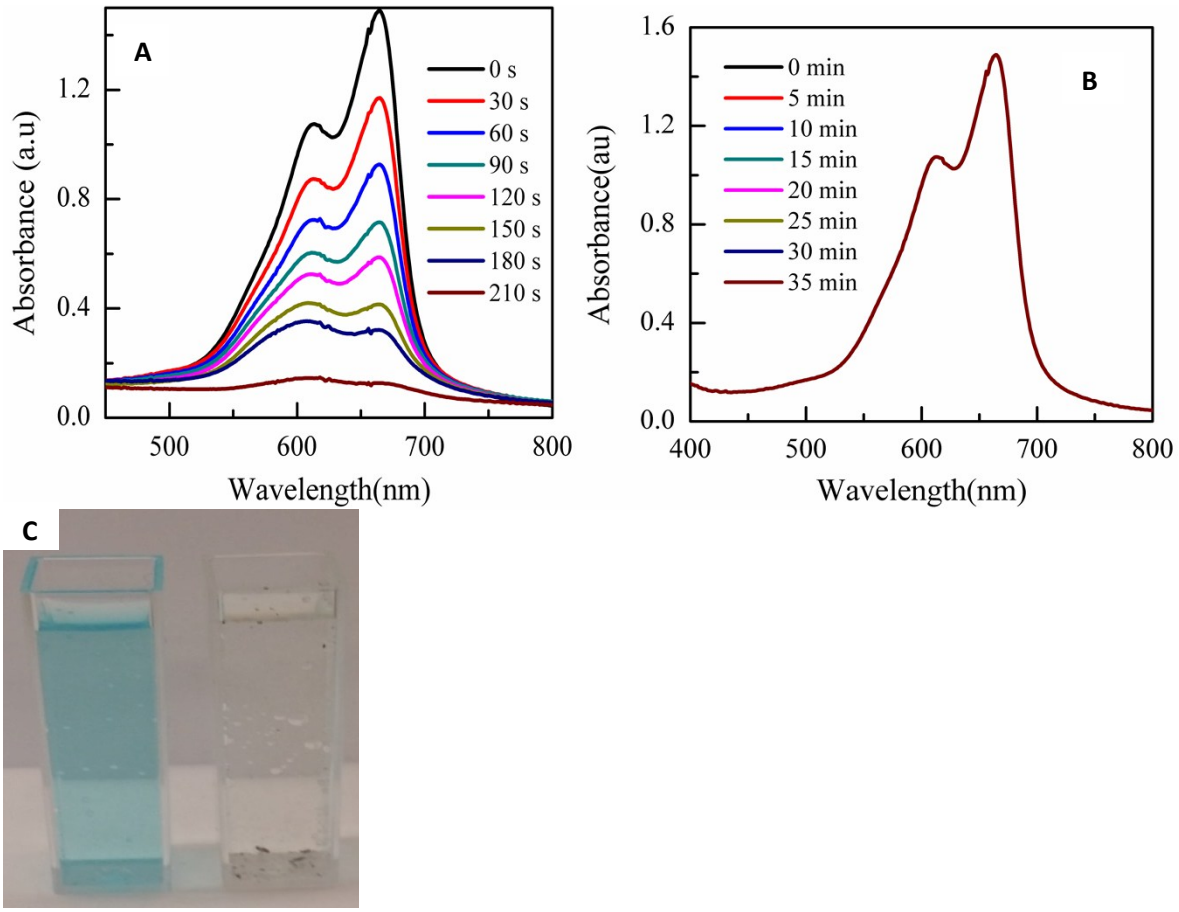


Figure S7: *(A) Time dependent UV-vis absorption spectra of the reduction of methylene blue by $NaBH_4$ in the presence of PAA synthesized AgNPs. (B) Time dependent UV-vis absorption spectra of the reduction of methylene blue using $NaBH_4$ in the absence of PAA-synthesized AgNPs; (C) Visual image of the conversion of methylene blue to leucomethylene blue*.

Toxicity of PAA-synthesized AgNPs against microbes in Muller Hinton Broth

In tube **[A]**, *Trichaptum biforme* was grown in 10 µg/mL AgNPs containing Muller Hinton Broth, and within 3 days excessive *Trichaptum biforme* development were observed. 10 µg/mL AgNPs were dissolved in Muller Hinton Broth (**Figure S8A** and **C**), followed by autoclave which helped to increase the even distribution of the nanoparticles in the broth. 50 µL inoculum from 10^5 cfu/mL was introduced as shown in tubes b and c. After 24 h incubation, yellow color of Tube b turned into milky-white due to excessive growth of *E.coli*. However, Tube **C** did not show any color changes related to bacterial growth. This is not an indication that it did kill all the

bacteria because 3 consecutive plate counting showed that nearly 90% protected their viability within 5 h. The protection of viability was also confirmed by electrochemistry via monitoring decreases in oxygen peak at -0.75V vs AgCl. Electrochemical monitoring of the decrease in oxygen peak revealed that the silver nanoparticles caused *E.coli* to use oxygen much faster than E.coli in control set up, which can be a sign that E.coli used up oxygen at faster rate to protect its stability even though the number of *E.coli* did not show any increase. Three-day incubation did not cause any recognizable changes in turbidity. After 2 weeks incubation [**D**], the medium showed some color changes from yellow to milky white, which means that excessive *E.coli* growth occurred within two weeks. This result was also confirmed with turbidity test.

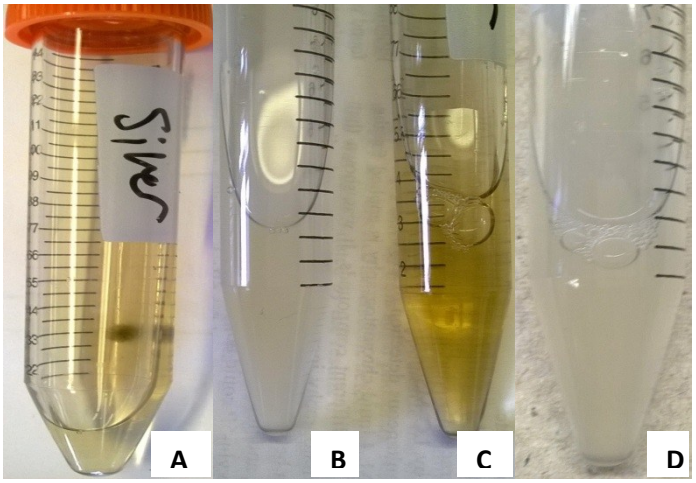


Figure S8: *Toxicity of PAA-synthesized AgNPs against microbes in Muller Hinton Broth*

However, 1 µg/mL silver nanoparticles did not produce similar effect because color changes were seen in 8 days while the use of 10 µg/mL nanoparticles containing medium showed similar effect at the 13th day. After diluting 10 µg/mL silver nanoparticle-containing broth with sterile silver-nanoparticle-free broth to 5 and 1 µg/mL nanoparticles concentrations did not produce any instant antibacterial activity. This is attributed to that of nutrients consumed by *E.coli* not interacting with the nanoparticle during the autoclaving process; the silver nanoparticle cannot inhibit bacterial growth. Free silver nanoparticles dispersed in medium did not show more than 1 log decreases for 0.1 µg/mL concentration. However, *E.coli* growth for the same volume of silver nanoparticles-free broth and mixture of the silver nanoparticle containing broth and nanoparticle-free broth showed differences at 3rd day of incubation. This is because when *E.coli*

run out with nanoparticle free nutrient, then they needed to consume the nanoparticle containing ones.

Antibacterial activity of PAA synthesized AgNPs in Muller Hinton Agar

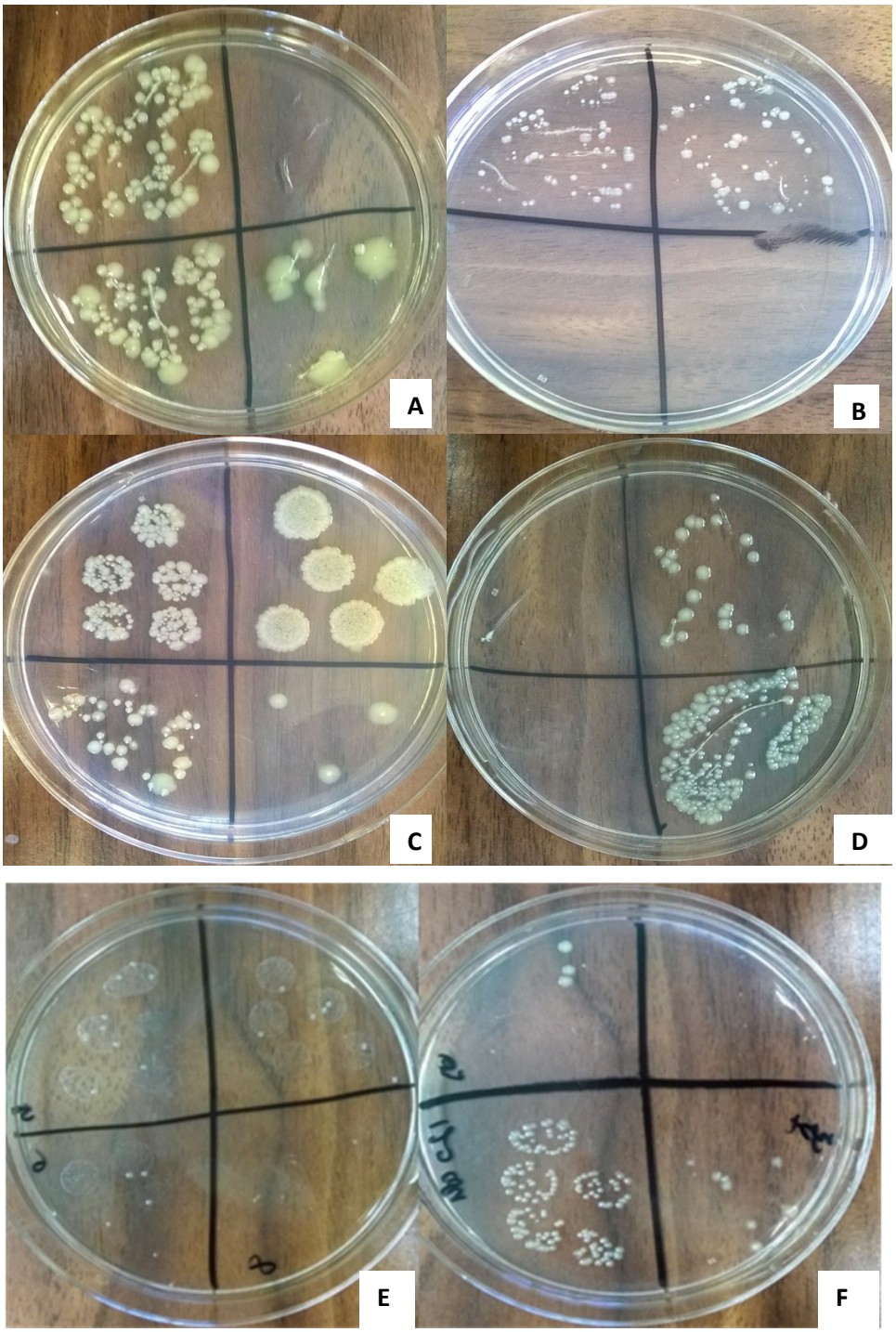


Figure S9: *Antibacterial activity of PAA synthesized AgNPs in Muller Hinton Broth. E.coli growth at 24h using silver nanoparticles dissolved in agar (**B**) similar experiment using 3.7mm control agar. S. epidermidis MH broth treated with silver nanoparticles (**D**) vs control at 24 hours incubation (**C**). S. epidermidis treated with silver nanoparticles at 48hr at low concentration (**E**) vs control (**F**).*

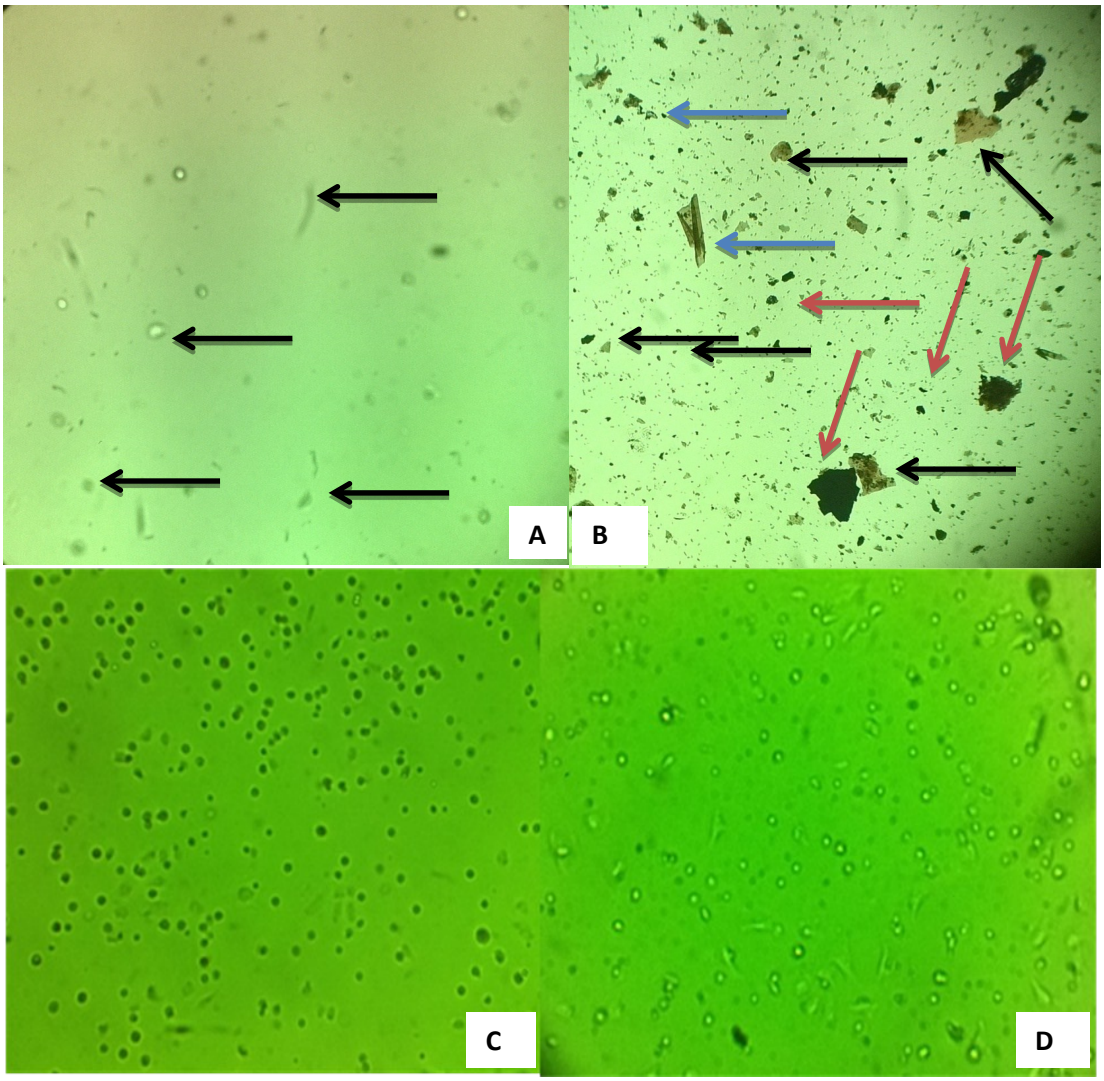
1 µg/mL silver-nanoparticles were dissolved in Agar before it underwent autoclave. Incubating 5 drops of 20 µL from 150 cfu /mL did not show any *E.coli* growth in 24 h while incubating 5 drops of 20 µL from 10×10^2 and 15×10^2 cfu/mL showed *E.coli* colony formations using silver-nanoparticle dissolved agar [b]. Decreases in number were not clearly counted because some colonies were merged into bigger ones. Decreases in the total area were calculated to make better evaluation of the anti-bacterial activity of AgNPs. The average colony size recorded was 1.6 mm using the nanoparticle treated agar while similar measurements were 3.7 mm on control agar **[a]**.

10 colonies were randomly taken into 1 mL of 50 mM pH 7.4 PBS buffer: Muller Hinton Broth mixture (50:50) to perform turbidity test. These tests provided better comparison for bacterial growth difference between silver nanoparticles treated and control agar. Turbidity test showed that silver nanoparticles suppressed more than 99.9% of E.coli grow inoculated on the agar in comparison to control. Incubating 5 drops of 20 µL from 150 and 750 cfu /mL did not show any *S. epidermidis* growth in 24 h while incubating 5 drops of 20 µL from 2×10^3 and 6×10^3 cfu/mL gave *S. epidermidis* colony formations were seen in silver nanoparticles dissolved agar. Unlike *E.coli*, *S. epidermidis* colony number was strongly decreased (8 times) with silver treatment **[d**] in comparison to control, and turbidity test showed that more than 99.9% *S. epidermidis* growth was suppressed. Even though, the average colony size was bigger in silver nanoparticle treated agar [actually inoculating less *S. epidermidis* for longer incubation times such as 200 cfu/mL for 72 h gave *S. epidermidis* colony formation with 1/3 colony size in comparison to control].

As seen in **Figure S9E** and **S9F**, 48 h incubation was required for *S. epidermidis* to give visible colony formation for low *S. epidermidis* inoculation in AgNPs treated agar [**Figure S9E**] in comparison to control [**Figure S9F**]. Turbidity test provided better understanding how the nanomaterials are effective because of that colony size and number do not show how the colonies are dense; while the colonies cover the surface, they also get into the agar, which was

tested with light microscopy and plate counting. While *Staphylococcus epidermidis* is a gram positive bacteria, *E.coli* is gram negative enterobacter.

Cytotoxicity study



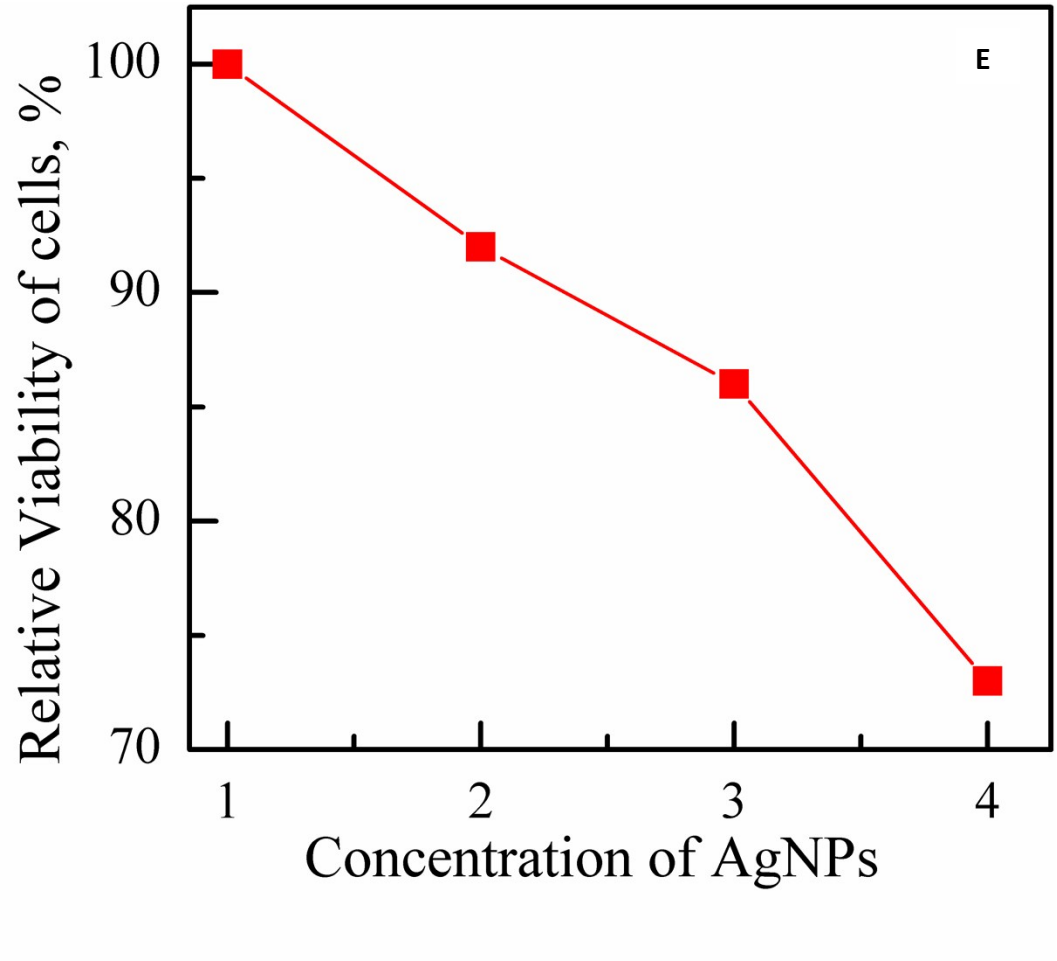


Figure S10: Cytotoxicity of nanoparticles using. Caco-2 cell line [DMEM medium with 10% FBS and 1% penicillin & streptomycin mixture (**A)** control nanoparticles treated cells(**B**). Growth pattern for IEC-6 cell line [DMEM medium with 10% FBS,1% penicillin & streptomycin mixture and 1 mg/L insulin. using (**C**) control and (**D**) nanoparticle treated cells. Cytotoxicity of non-cancerous immortalized 10^4 IEC6 cells at 24hr incubation using (**E**) Prestol Blue fluorescence dye showing mild toxicity at low concentrations (1(0µg/mL); 2(5µg/mL), 3(15ug/mL) and 4(30 µg/mL).

Caco-2 cell line [DMEM medium with 10% FBS and 1% penicillin & streptomycin mixture] and IEC-6 cell line [DMEM medium with 10% FBS, 1% penicillin & streptomycin mixture and 1 mg/L insulin] were used to test whether the silver nanoparticles have any impact on growth pattern. Black arrows show different type of cells in both control [a] and nanoparticle treated

cells [b]. Red arrows show the types of silver nanoparticles while blue arrow shows floating cells. As it is seen in **Figure S10A**, the growth pattern is nearly standard for control while it showed at least 5 different growth patterns. It seems that the alteration in growth pattern is mostly from the heterogeneity of silver nanoparticles in the medium. Some cells, in **Figure S10B**, formed tissue-like formations which could be attributed to the cells grown on silver nanoparticles, which showed similar growth to the cells grow in 3D systems. Figures S10c and S10d show growth pattern of IEC6 cells; silver toxicity did not affect the growth pattern of IEC6 cells. As seen, silver nanoparticles did not cause more than 5 % confluence decrease in the case of 3e4 cells/mL inoculation. This was even not visible when the inoculation was 3e5 cells/mL.

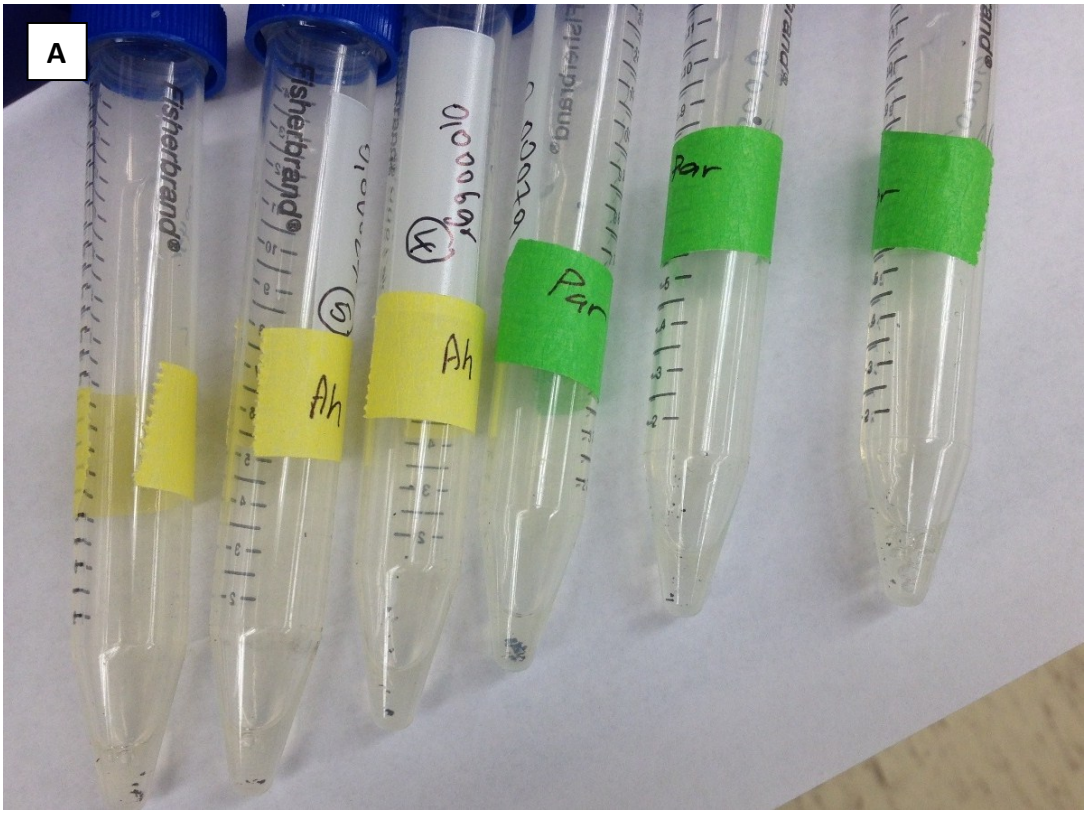
Cytotoxicity of Nanoparticles was tested via non-cancerous immortalized rabbit intestinal cell line, IEC6 cells. Different concentrations of nanoparticles were tested for 10^4 cells /mL incubated for 24 h at 37 °C under 5 % CO_2 concentration. **Figure S10 E** shows the results for 1 mL of 10^4 cells/mL IEC-6 cells were seeded in 24-well plate. Autoclave sterilized nanoparticles were introduced to the cells in 5 µL 18.2 MΩ pure water. Right after 24 h incubation, 25 µL Prestol Blue® fluorescence dye was added into each well. The cells were then incubated at 37 °C under 5 % CO_2 concentration for 20 min. The results were recorded using Biotek Microplate Reader at excitation/Emission of 530nm/25 and 590nm/35, and the plates were read from bottom. As evident from the graphics, AgNPs possessed mild toxicity against IEC6 cells at low concentrations. However, for higher concentrations AgNPs showed strong cytotoxicity.

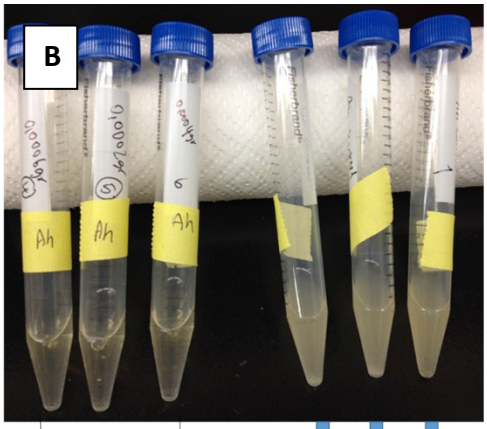
S11: **Additional information of antibacterial activity setup Figure 9**

Bacteria samples were obtained from the culture collection in the Department of Basic Science, College of Veterinary Medicine, Mississippi State University Mississippi-USA. The samples were kept at unit -80ºC freezer. 1 and 10 µg/ml of silver nanoparticles were added to LB agar media, and 100 µl of overnight culture bacteria were spread after serial dilution on treatment and non-treated control plates. Tested *E.coli* grown overnight at 37 º C incubator and *L. monocytogenes* were incubated in 30 ºC more for than 48 hours. All tests were repeated 2 times at 3 replicate of plates. The bacterial growth of *E. coli* on the treatment of 10 µg/ml of silver-nanoparticle agar [b], and control non-treatment LB plate [a] while the lower images for the bacterial growth of two strains of *L. monocytogenes* on treatment 10 µg/ml of nanoparticle agar [d and f], and control non treatment LB plate [c and e]. 1 µg/ml was also tested to see if there

was any antimicrobial activity, and the antibacterial activity was similar. This implies that high silver concentration did not provide even distribution in Agar, which decreased the exact number of silver nanoparticle that bacteria were exposed to.

S12: Toxicity of PAA-synthesized AgNPs against *Pseudomonas aeruginosa* and *Aeromonas hydrophyla*



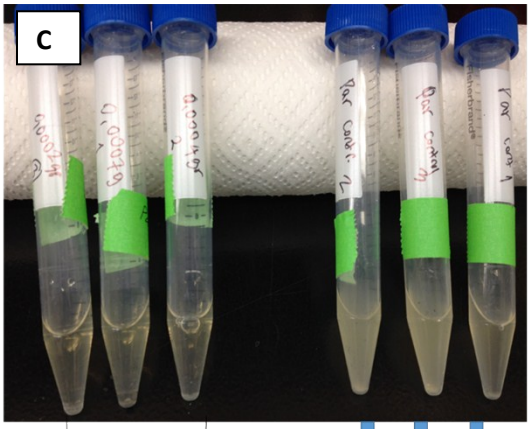


Nanoparticles + Aeromonas

NO GROWTH

OD 1.505 1.525 1.510

CONTROLS
Aeromonas

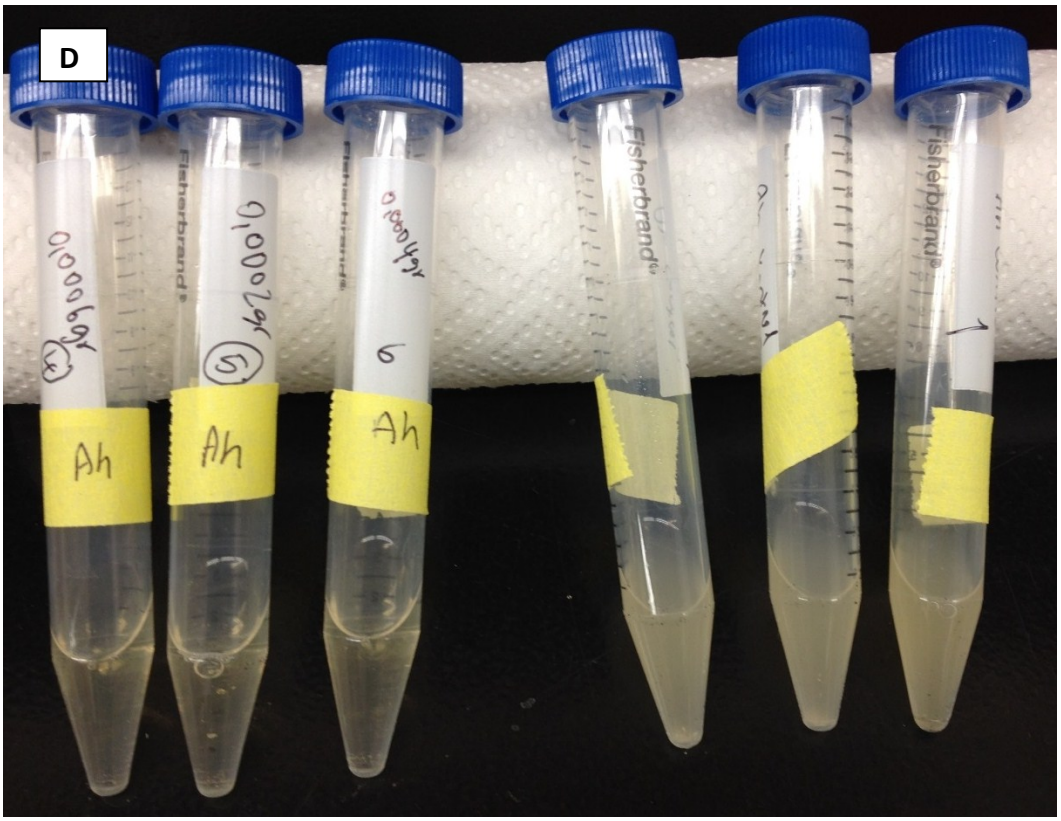


Nanoparticles + Pseudomonas

NO GROWTH

OD 1.39 1.422 0.772

CONTROLS
Pseudomonas



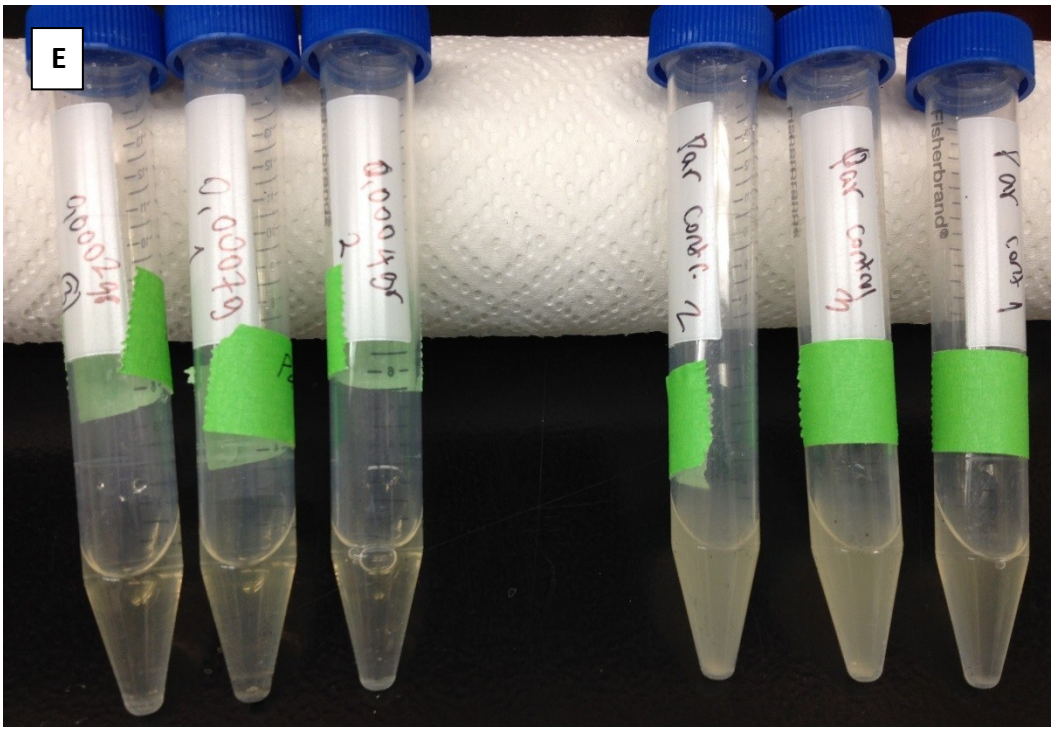


Figure S11: (A) Image of nanoparticle inside the medium, (B, C, D & E) after overnight growth with the pictures showing non-growth treatment groups and growth control for *Aeromonas hydrophilia (Ah) and Pseudomonas aeruginosa (Ae).*

Between 0.2 mg-0.7 mg of nanoparticle were used in the experiment [**Figure S11A**]. The growth in 2ml LB broth, and 5 ul of *Pseudomonas aeruginosa* and *Aeromonas hydrophyla* were added on the culture. 3 Tubes of non-treated cultures were used as a control for each bacterial group, and 3 different concentration of nanoparticle used in the treatment with bacteria growth overnight at 16h in 30 shaker incubator. The result showed that there is no growth in nanoparticle group. On the other hand *Aeromonas* and *Pseudomonas* control group exhibit significant growth. However, this does not imply that the AgNPs killed the bacteria in the medium as shown for *E.coli* 25922 [**S8**]. OD values were between 0.8 and 1.5 [**Fig S11B**]. It should be observed that using extremely high concentrations did not enhance even distribution of AgNPs. When the AgNPs precipitated in bottom of the medium was calculated, it was seen that the silver nanoparticles dispersed in medium (20 µg/mL).