Effect of Silver Nanoparticles on Zebrafish and Bacteria in a Constructed Wetland with Potential Remediation by Soil and Water Treatment Residuals

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Abstract

With recent advancements in technology, nanoparticles are becoming more prevalent in everyday use. There are many unanswered questions about the fate of nanoparticles, such as where they end up in the environment and the possibility of bioaccumulation in plants and animals. The specific aim of this study is three-fold; 1) to determine the environmental impacts of silver nanoparticles in zebrafish, 2) to investigate the impacts of silver nanoparticles and water treatment residuals on bacteria, and 3) to concurrently investigate the potential remediation of nanoparticles by a constructed wetland via soil and water treatment residual adsorption.

To accomplish these goals, the researchers exposed adult zebrafish (*D. rerio*) in a lab-scale constructed wetland to 15, 25, and 50 mg/L silver nanoparticles (<90 nm) and examined their structural characteristics after 7 days. The zebrafish were euthanized, sectioned and their histology compared to control fish with no silver exposure as well as zebrafish with silver nitrate exposure. Additionally, the effects of silver nanoparticles on the survival of *Escherichia coli* (an intestinal bacterium that enters wetlands from human waste), and various bacteria naturally found in wetlands (native) was examined. Finally, silver nanoparticles were measured in the soil of the constructed wetland after the 7 day exposure. Separately, a sorption experiment was carried out examining a wetland soil (high organic matter), a silt loam, and a water treatment residual (mostly aluminum sulfate) in their ability to sorb silver nanoparticles. Previous research has studied the effects of silver nanoparticles on various organisms in laboratory settings; however this type of study does not represent environmental conditions. The results of this study will shed light on silver nanoparticles effect on organisms in a simulated natural setting and suggest possible mechanisms for their removal from water.

Introduction

- Sufficient clean water is becoming more scarce
- Pollutants such as nutrients (N, P), particulates, and pathogens have been studied for a long time
- Other pollutants such as pharmaceuticals (hormones, anti-depressants) and metal-nanoparticles in wastewater are becoming more common
- Conventional and non-conventional wastewater treatment handle nutrients, particulates, and pathogens relatively well; but less is known about the removal of other pollutants, specifically nanoparticles
- Use of various mediums (soil, water treatment residuals) to filter or sorb pollutants is known to remove nutrients; it is possible they could be used to remove other types of pollutants as well
- Toxicity of metal nanoparticles is well known in laboratory settings, but the fate of nanoparticles in the environment is unclear

Research Questions

1. Do silver nanoparticles cause zebrafish mortality in a (simulated) natural setting such as a constructed wetland?
2. Do silver nanoparticles or aluminum-based water treatment residuals cause mortality in bacteria?
3. Can soil or water treatment residuals be used to sorb silver nanoparticles and remove them from wastewater?

Ag Nanoparticle effect on Zebrafish Survival in a Lab-scale Constructed Wetland

MATERIALS AND METHODS

- 6 buckets (treatments): ocean salt (negative control), ocean salt with 2mg/L Tide (negative control with dispersing agent), 15 mg/L of Ag nanoparticles (<90nm, mKnano), 25 mg/L of Ag nanoparticles, 50 mg/L of Ag nanoparticles, and 15 mg/L of silver nitrate (positive control). See Fig. 3.
- Each bucket contained a PVC column with 20 cm of ½ inch washed gravel and 8 cm packed with 800 g of wetland soil (soil characteristics in Table 1). Water was circulated through the column (Fig. 4) by a circulating pump.
- 5 adult zebrafish were placed in each bucket for one week.
- Buckets were monitored daily for mortality, temperature, and pH along with a daily water sample for AgNP concentration.
- At the end of the week remaining fish were euthanized and a soil sample taken from the column at 3 depths.
- The water and soil samples were digested with 6M nitric acid and analyzed with atomic absorption spectroscopy.

RESULTS

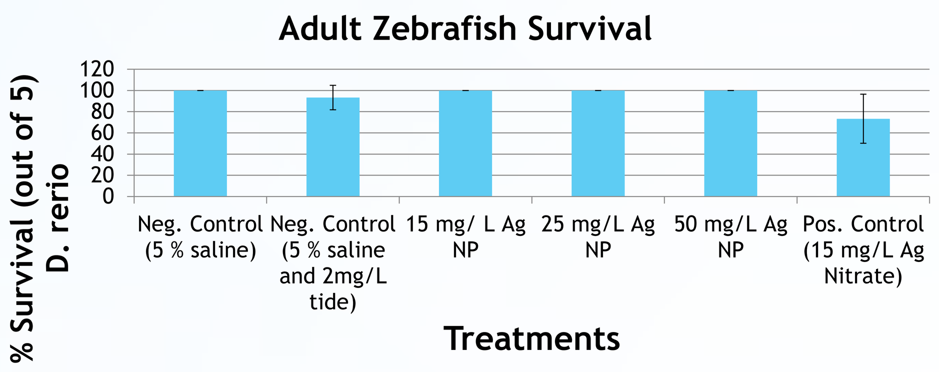

Figure 1: Adult zebrafish survival expressed as a % after a 7 day exposure. Each bar is the average of three trials of 5 fish (2 male, 3 female) per lab-scale constructed wetland bucket. Error bars indicate standard deviation; Kruskal-Wallis test gave a p-value of 0.134 between treatments.

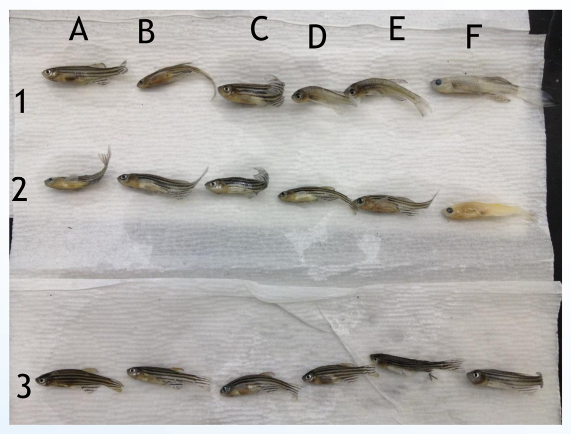

Figure 2: Sample photo of zebrafish from each treatment. 1-3 represent the trial number. A) Negative control-ocean salt water; B) Negative control2- ocean salt water with 2mg/L Tide; C:15 mg/L of Ag nanoparticles; D: 25 mg/L of Ag nanoparticles; E: 50 mg/L of Ag nanoparticles F: Positive control: 15mg/L silver nitrate.

Figure 3: Constructed Wetland Setup: five-gallon bucket with soil column.

Figure 4: Constructed Wetland Soil Column: 4 inch PVC pipe with drainage holes and mesh covering.

CONCLUSIONS

The silver nanoparticles did not cause mortality to the zebrafish (Figs. 1, 2), most likely because the Ag NP did not fully suspend in the solution (zebrafish live in saltwater and aggregation of nanoparticles is possible). We believe this is the likely scenario since no silver nanoparticles were measured in the daily water samples and soil column depth samples.

Ag Nanoparticle and Water Treatment Residual effect on Bacteria

MATERIALS AND METHODS

- Tubes set up containing: 0.5g soil, saline, and PVP dispersing 0, 25, 50, 75, 100 μg/mL Ag nanoparticles (<90 nm, mKnano) or 0.5 g WTR (Table 1) with *E. coli* or native soil bacteria.
- Tubes shook overnight in a warm room and then plated on agar.
- Colony forming units were counted.

RESULTS

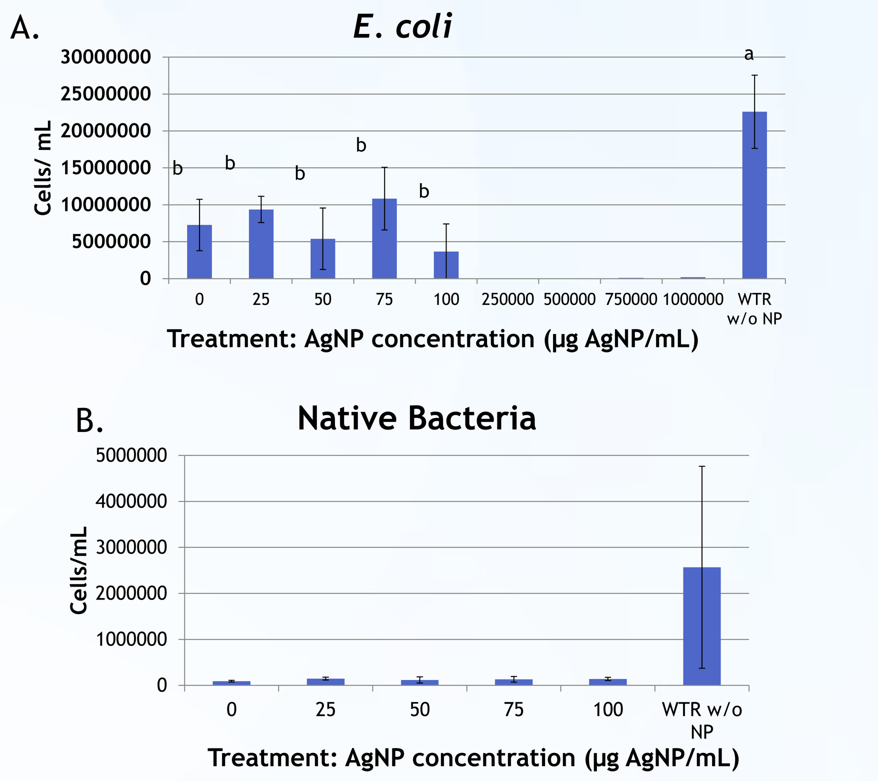

Figure 5: Silver nanoparticle effect on bacterial growth. Bars are an average of three replicates; error bars indicating the standard deviation. Bars with the same letter (or no letters) are not statistically different at α=0.05.

A) *E.coli* colonization after treatment with various concentrations of AgNP (with 0.5g soil, 10 mL saline). Kruskal-Wallis rank sum test gave a p-value of 0.04578 between treatments (AgNP treatments higher than 100 μg/mL did not have replicates and are not included in the statistical analysis).

B) Native bacteria (bacteria living naturally in soil) colonization after treatment with various concentrations of AgNP (with 0.5g soil, 10 mL saline). Kruskal-Wallis rank sum test gave a p-value of 0.1031.

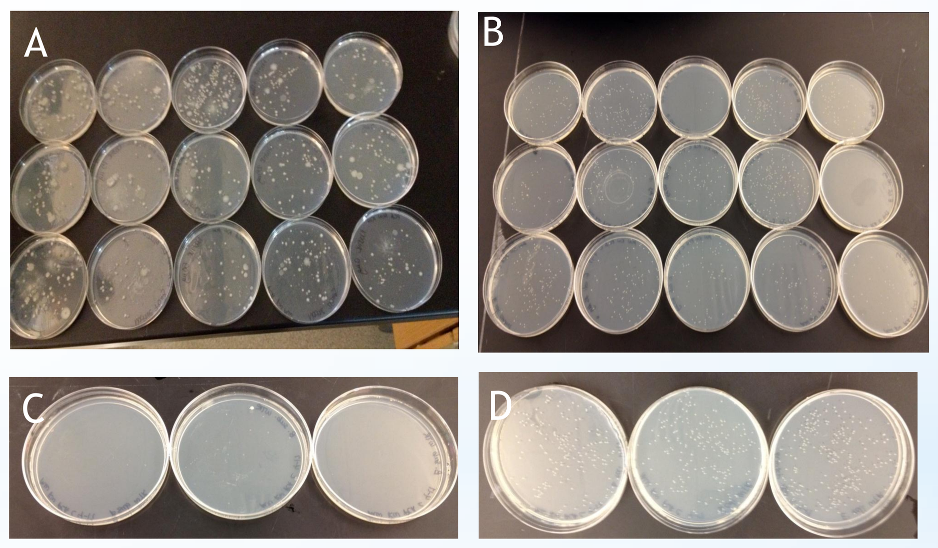

Figure 6: Photographs of Agar Plates A: Native bacteria 0, 15, 25, 50, 75 100 μg AgNP/mL. (left to right) B: *E. coli* Plates 0 0, 15, 25, 50, 75 100 μg AgNP/mL. (left to right) C: Native bacteria WTR plates. D: *E. coli* WTR plates.

CONCLUSIONS

The silver nanoparticles did cause mortality on *E. coli* and native wetland bacteria. The aluminum-based water treatment residuals did not cause mortality within the bacterial colonies (Fig 5 A and B). This supports other research suggesting AgNP negatively impact bacteria. Additionally, no evidence is shown that WTRs negatively impact bacteria (which is a important if they are to be used in remediation-see final research question).

Table 1: Characteristics of the three mediums (silt loam soil, wetland soil, and water treatment residual (WTR) used in the zebrafish constructed wetland study, the bacteria study, and the remediation/sorption experiment. Analyses completed at the Soil and Plant Analysis Lab, Madison, WI.

Sample	C	N	P	Ca	Mg	S	Fe	Al	Sand	Silt	Clay	Soil Texture
	%	%	mg/kg	%	%	%	mg/kg	mg/kg	%	%	%	
Silt Loam	1.99	0.18	389	0.35	0.43	0.02	18,830	15,480	19	63	18	Silt Loam
Wetland Soil	33.26	2.43	967	2.40	0.27	1.17	14,504	4,035	71	23	6	Sandy Loam
WTR	7.68	0.41	788	3.10	1.29	0.17	6,614	110,532	75	23	2	Loamy Sand

Potential Remediation by Soil and Water Treatment Residuals

MATERIALS AND METHODS

- 0.5 grams of the media (characteristics in Table 1) were placed into 15mL conical tubes.
- 10mL concentrations of 0, 2, 5, 10, and 20 mg/L monodispersed silver nanoparticles (90 nm Nano Composix) in 2mM sodium citrate buffer were added to each media.
- Tubes were shaken for 18 hours and 2mL samples were taken 30 seconds and 2 hours after settling began.
- Shaken tubes were centrifuged at 2500rpm for 10 minutes and an additional 2mL sample was taken.
- 1mL of 6M nitric acid was added to the samples which were then heated in a water bath at 90°C for 1 hour.
- Each sample was run on an AA with $AgNO_3$ standards.

RESULTS

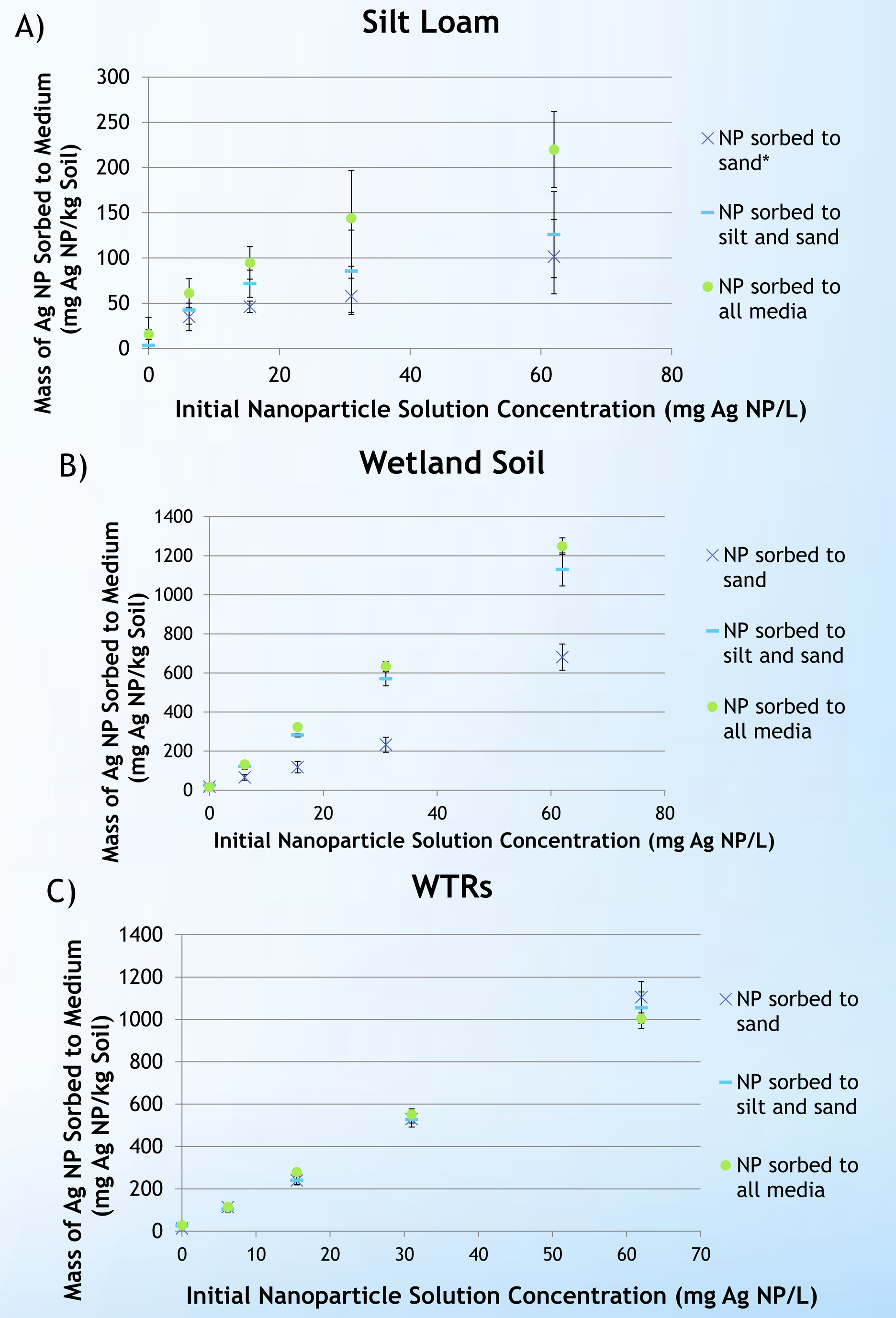

Figure 7: Silver nanoparticle (monodispersed) sorption to three mediums A) silt loam soil, B) wetland soil, and C) water treatment residual (WTR). Each data point is the average of three trials, with vertical error bars indicating standard deviation. The three curves on each graph indicate nanoparticles sorbed to different size classes of soil. "NP sorbed to sand" was measured from the solution concentration after 30s (approx. time for sand to settle), "NP sorbed to silt and clay" was measured from the solution concentration after 2 hours (approx. time for silt to settle, and "NP sorbed to all media" was measured from the solution concentration after centrifugation (only dissolved nanoparticles in solution).

CONCLUSIONS

The silt loam reached its maximum absorption much sooner than the wetland soil and the WTRs, both of which could hold more silver nanoparticles (Fig. 7). Based on the samples taken at different time intervals, in WTRs there is no difference in sorption as time passes and particles fall out. In wetland soil and silt loam, approximately half of the maximum nanoparticles sorbed are on the sand particles, the other half sorb to the clay and silt particles (Fig. 7). **Experiments are planned that will investigate desorption of AgNP from the three mediums and sorption isotherm parameters (such as Smax) will be calculated.

Acknowledgements

This work was done as a collaboration between the biology, chemistry, biochemistry, and environmental science departments at Wisconsin Lutheran College and was funded by a minigrant for "Development of a Scaled Constructed Wetland." Undergraduate researchers worked with Professors Erbe, Balza, Ebeling, and Ebeling.