

Supplementary Materials

Morphologic Alterations Across Three Levels of Biological Organization Following Oral Exposure to Silver-Polymer Nanocomposites in Japanese Medaka (*Oryzias latipes*)

Melissa Chernick^{a,*}, Alan J. Kennedy^c, Treye Thomas^d, Keana C.K. Scott^f, Joana Marie Sipe^b, Christine Ogilvie Hendren^{b,e}, Mark R. Wiesner^b, and David E. Hinton^a

^a *Nicholas School of the Environment, Duke University, Durham, NC, 27708 USA*

^b *Civil and Environmental Engineering, Duke University, Durham, NC, 27708 USA*

^c *U.S. Army Engineer Research and Development Center, Environmental Laboratory, 3909 Halls Ferry Rd. Vicksburg, MS, USA*

^d *United States Consumer Product Safety Commission, 4330 East-West Highway, Bethesda, Maryland 20814, USA*

^e *Department of Geological and Environmental Sciences, Appalachian State University, Boone, NC 28608, USA*

^f *National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899, USA*

* Corresponding author:

Duke University, Nicholas School of the Environment

308 Research Drive

LSRC A341, Box 90328

Durham, NC 27708

mc131@duke.edu

+1 (919) 613-8045

Settings for 3D printing of pucks:

Layer height: 0.5 mm
Shell thickness: 0.5 mm
Bottom/top thickness: 0.4 mm
Fill density: 100 %
Perimeters before infill
Print speed: 40 mm/s
Printing temperature: 230 °C
Bed temperature: 60 °C
Raft OFF
Number of skirt lines: 1
Filament diameter: Adjusted to measured filament diameter (2.45 mm to 2.95 mm)
Flow: 100 %
Nozzle size: 0.5 mm
Retraction ON
Retraction speed: 10 mm/s
Retraction amount: 1 mm
Initial layer thickness: 0.4 mm
Initial layer line width: 100 %
Travel speed: 150 mm/s
Bottom layer speed: 30 mm/s
Cooling fan: OFF

Table S1. Dissolution of silver (Ag) in ddH₂O at 1 hour and 24 hours for each of the materials tested. Quantities of each material were equal to what was used for dosing in fish (section 2.3). Values are mean silver concentrations \pm SEM. Values of <0.125 are representative of concentrations below the minimum detection limit. n=3 per material per time point.

	Ag Concentration ($\mu\text{g/L}$)	
	1 hour	24 hours
Control	<0.125	<0.125
PETG	<0.125	<0.125
AgNO ₃	317.74	
AgNPs	12.2 \pm 0.25	15.02 \pm 1.33
2 % composite	1.42 \pm 0.51	2.46 \pm 0.88
0.5 % composite	2.81 \pm 0.18	5.92 \pm 0.15

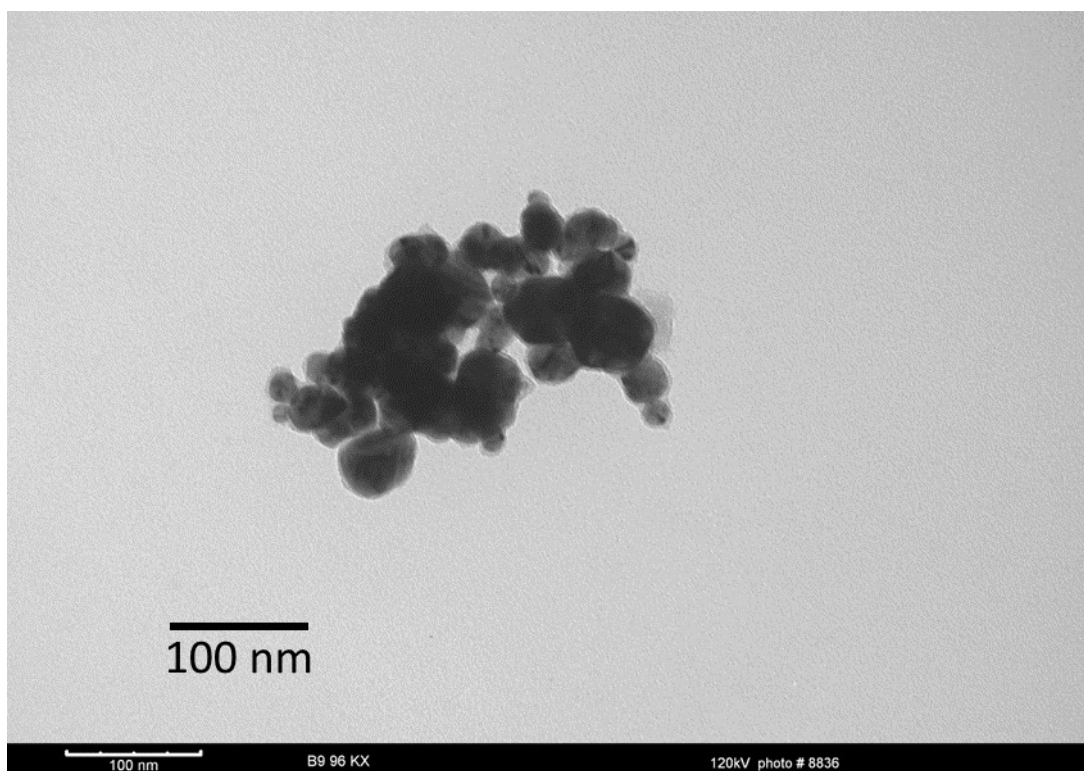


Figure S1. TEM micrograph of AgNPs in water prior to sonication. AgNPs diameters range from 20 nm to 50 nm.

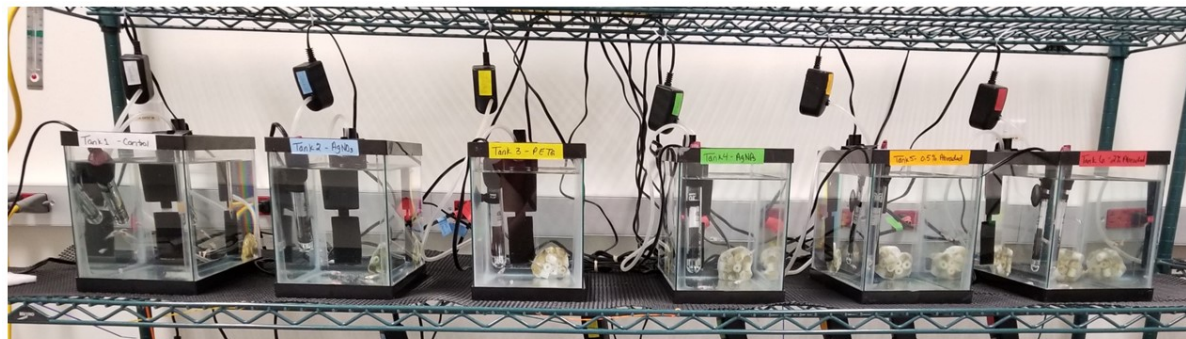


Figure S2. Setup of experimental tanks. Each tank had individual mechanical filtration, heaters, and biological filtration. Tanks were located in a dedicated room at 23 °C with a 14:10 light dark cycle. Treatment groups had dedicated instruments (e.g., siphons, nets) to prevent cross-contamination.

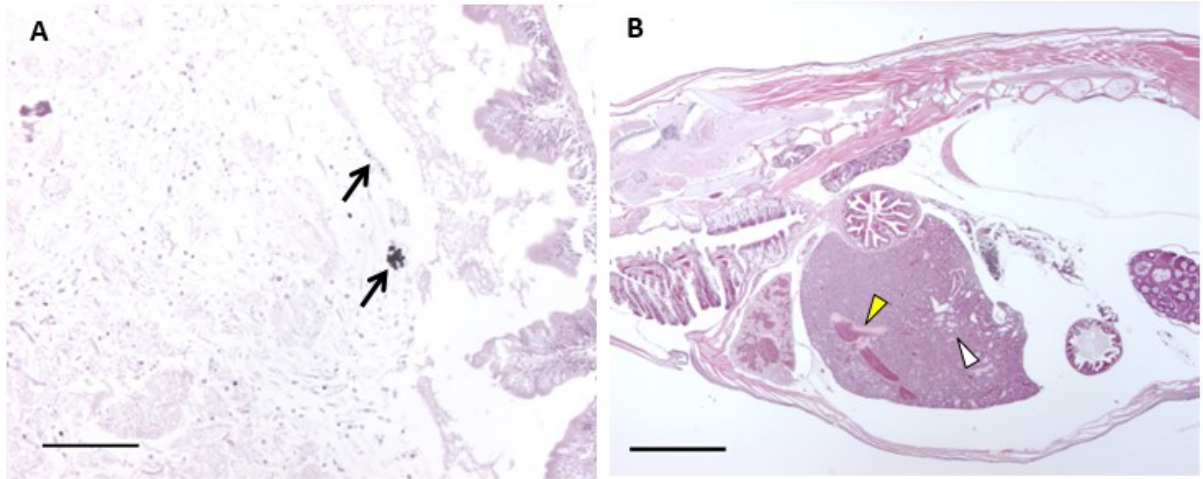


Figure S3. Histological sections of exposed fish. A) Section of midgut showing aggregations of AgNPs within the lumen (arrows); B) parasagittal section of fish exposed to 2% composite material showing enlarged liver with perivascular (yellow arrowhead) and peribiliary (white arrowhead) fluid accumulation. Scale bars are 100 μm (A) and 500 μm (B).