

Toxicity effects of Cubic Cu₂O nanoparticles on defecation rate and length in *C. Elegans*

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ABSTRACT

Introduction: The increased presence of radiation and toxins in the atmosphere has given rise to fuel cells and nanoparticle technology with the ability to catalyze reactions at favorable energy levels. The purpose of this experiment was to determine the lasting effects of a synthesized catalyst on a model organism, *Caenorhabditis (C.) elegans*. **Methods:** Copper sulfate was tested alongside copper (I) oxide (*i.e.* Cu₂O) to dissociate the copper composition effects from those of the nanoparticles themselves. The prospect of testing both defecation rate and size differences allowed for *C. elegans* to be utilized due to their low maintenance costs, mapped neuronal pathways, and short-generation times. **Results:** The results indicated significant toxic effects in wild-type worms as witnessed by the decreases in nematode defecation rate and length by copper sulfate, with similar results in SMF-1 and PCS-1 mutants by Cu₂O catalysts in cubic synthesized form. **Conclusion:** These outcomes reinforce the known effects of metal oxides on pollutants and highlight the need for further testing with additional variables such as varying pH and temperature.

Key words: Neurodegeneration, *C. elegans*, Nanoparticles, Environmental Pollutants, Metal Oxide Catalyst, SEM

INTRODUCTION

The predicament created by the rise in manufacturing, attributed to industrial growth after the 1850s, has slowly eroded the once “pure” atmosphere that was present before the age of human emissions. Although billions of dollars of research ensue every year in search of catalytic converters, little attention is given regarding the effective application and installation of these converters to solve unfavorable energy processes.

A potential promising solution to this issue includes the use of complete oxidative catalyst agents, capable of absorbing organic pollutants while leaving a limited resulting environmental trace¹. This particular study employs cubic-shaped particles for testing nematodes under 48-h time intervals with copper(I) oxide (*i.e.* Cu₂O) nanoparticles and copper sulfate, and for quantifying each facet’s potential bodily effects if implemented in toxin remediation². Wild-type, as well as SMF-1 and phytochelatin synthase (PCS-1) gene-lacking mutant *Caenorhabditis (C.) elegans*, were used to quantify the toxicity effects on bodily size and defecation rate. It was predicted that all three genotypes would have insignificant differences in both assays when treated with water. It was also expected that SMF-1 and PCS-1 mutants would have

decreased body size and defecation rate due to the respective transport and metal tolerant roles assigned to each missing gene.

MATERIALS — METHODS

Synthesis

All reagents were used as received. In a typical procedure, all steps were carried out as referenced in Sui *et al.*³.

Characterization

The plugs were inserted into an FEI XL-30 Field Emission ESEM/SEM (scanning electron microscope) (Field Electron and Ion Company, Hillsboro, OR) with energy-dispersive X-ray spectroscopy (EDS) capability, as described by Murphy *et al.*⁴. The field emission gun tip then initiated a narrow electron beam, allowing for image analysis (as documented in **Figure 1**), as well as atomic mass percentage breakdown⁵.

C. elegans worm culture and treatment with nanoparticles

All *C. elegans* strains were cultured on a growth medium with *Escherichia (E.) coli* strain OP50 as the food source. SMF-1 and PCS-1 mutants were included in each sampling preparation, as described

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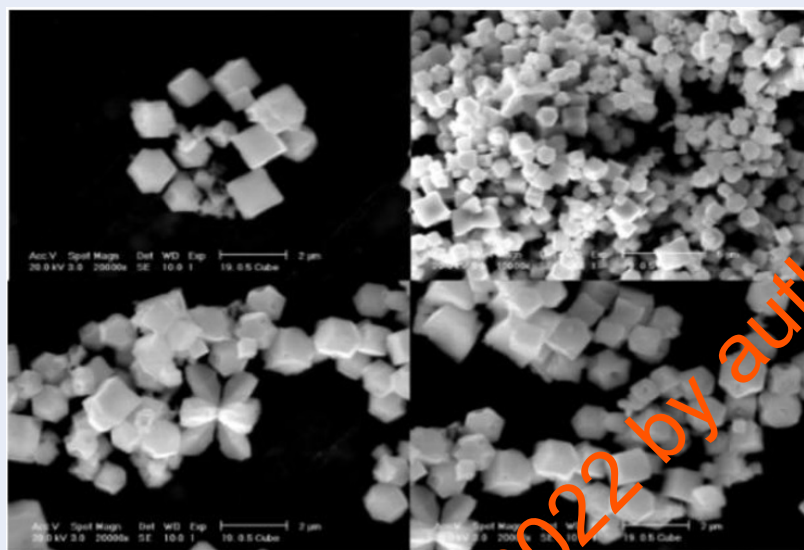


Figure 1: SEM Images of the copper (I) oxide (Cu_2O) cubic nanoparticles synthesized.

by Mashock⁶. Formulation of dried nanoparticle into viable solution involved transferring 20 mg into a 1.5 mL microcentrifuge tube and dissolving in 95% ethanol at 200 mg/ml. This was followed by bath sonication for 5 min to disperse particles with subsequent vortexing for 5 s. Next, 10 μL of particles was then immediately transferred into a microcentrifuge tube, followed by addition of 990 μL of sterile deionized (DI) water. This mixture was then vortexed for 30 s. The copper sulfate solution was prepared using a stock 0.1 M vial with a 1:4 stock to DI water ratio. Thus, 1 mL in total was maintained across all organized sample tubes. The water control consisted of 990 μL of DI water mixed with 10 μL of 95% ethanol. Finally, 200 μL of the nanoparticle, copper sulfate, and water solutions were pipetted on top of the OP50 bacteria growth medium in each condition's respective plates. These were then allowed to incubate for 48 hours at 20°C.

Defecation and size analysis

Defecation was assayed using a Nikon Light Microscope with an Amscope Cold-Light Source Lamp (Amscope, Irvine, CA). The worms were examined for 5-min time intervals using the Apple stopwatch app to calculate the time between defecations. Only adult *C. elegans* were chosen for defecation analysis to ensure the proper biological effect⁷. Three worms were observed for each condition (wild type, PCS-1, and SMF-1) during each experimental period. This study was performed over 6 observation periods. Conditions that were unable to be surveyed due

to widespread nematode death were noted for statistical reference. Size analysis was carried out after the equivalent incubation period via a Leica M165FC stereomicroscope (Leica Microsystems, Wetzlar, Germany) using a PLAN APO 1.0x/0.5 NA objective, as performed by Munro *et al.*⁸. Similarly, FIJI software (National Institutes of Health, USA) was utilized to measure worm lengths from image progressions. Approximately 18 individuals per treatment condition (wild-type, PCS-1, and SMF-1) were measured for defecation and size analysis. Worms were considered dead if no movement was observed after a period of 60 seconds or 15 seconds after applying mechanical stimulation.

Statistical Analysis

Data analysis from the image analysis and defecation rate observations was conducted using JASP statistical software (The JASP Team, Amsterdam, The Netherlands). A p-value of ≤ 0.05 was considered significant. Conditions with significant p-values are indicated in their respective figure legends. All statistical tests performed were corrected to account for multiple comparisons.

RESULTS

Defecation Results

Statistical data taken from the four defecation analysis trial periods yielded multiple significant p-value comparisons across both treatments and *C. elegans* conditions. SMF-1 mutants showed decreased defecation

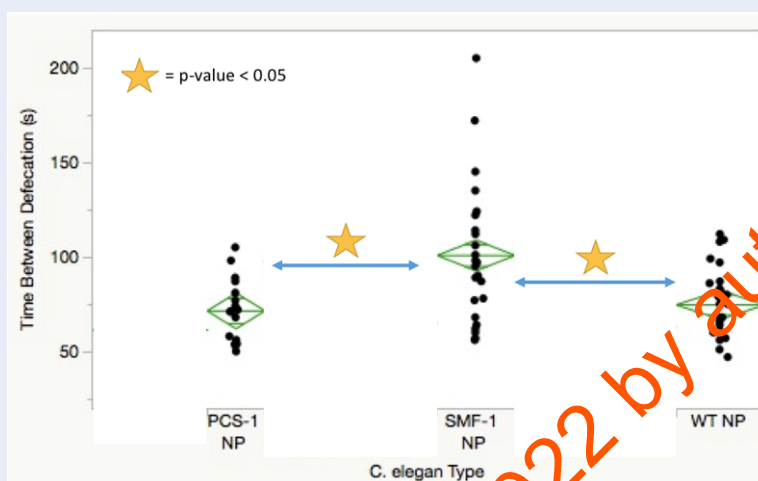


Figure 2: Graphical representation of defecation results in wild type, SMF-1, and PCS-1 mutant *C. elegans* treated with copper (I) oxide nanoparticles. The key included represents the p-value used and symbol representation in the graph. "WT" is used to represent wild type worms. "NP" is used to represent nanoparticle treatment.

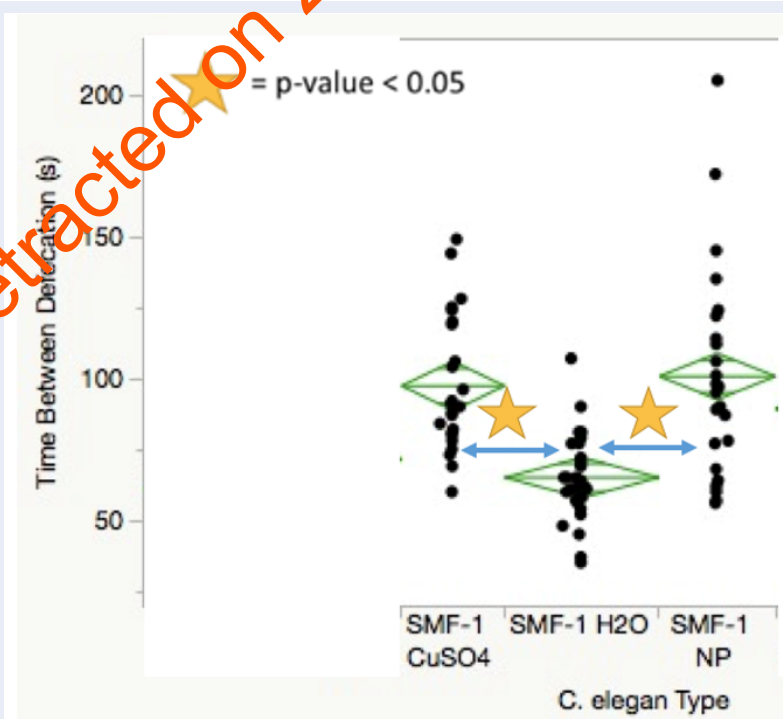
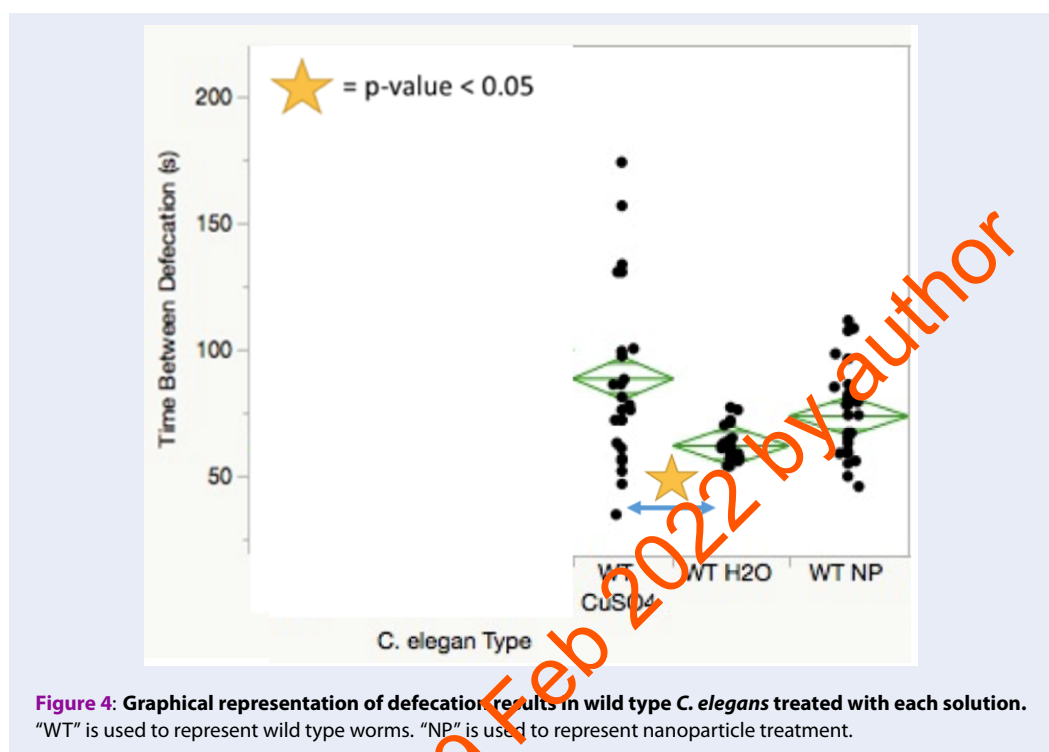


Figure 3: Graphical representation of defecation results in SMF-1 mutants treated with each solution. The key included represents the p-value used and symbol representation in the graph. "NP" is used to represent nanoparticle treatment.

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rates compared to wild-type worms when treated with nanoparticles (Figure 2). SMF-1 mutants treated with both copper sulfate and nanoparticles showed significantly more time between defecations in comparison to those treated with water (Figure 3). Figure 4 indicates that wild-type nematodes treated with copper sulfate exhibited decreased defecation rates compared to those treated with water.

Length Results

Wild type nematodes treated with copper sulfate were found to be smaller than their water-treated counterparts with notably precise distribution points (Figure 5). PCS-1 mutants treated with both copper sulfate and nanoparticles were significantly shorter in length than those treated with water (Figure 6). Of those treated with nanoparticles, SMF-1 mutants were significantly larger than their PCS-1 and wild-type counterparts. Concurrently, PCS-1 mutants were significantly shorter compared to wild-type nematodes, averaging around 0.3 mm in length less than wild type *C. elegans*. The same relationships were observed in the copper sulfate-induced group; PCS-1 mutants were remarkably smaller than both the wild-type and SMF-1 mutants, with SMF-1 mutants being the largest of the three conditions treated with copper sulfate (Figure 8). In contrast, the SMF-1 mutant nematodes

had no significant size differences in comparison to the wild-type treatments.

DISCUSSION

For the defecation analysis trial periods, insufficient data was generated due to the broad worm death observed in worms exposed to copper sulfate for the 48-h period. Perhaps the most significant finding of the defecation section is that wild-type worms treated with nanoparticles showed no significant difference in defecation rate when compared to those treated with water. While wild-type nematodes treated with copper sulfate demonstrated lower defecation rates compared to those treated with water, this may be explained by the wide distribution of data points seen in Figure 4. The sample size disparity shown between the two conditions was due, in part, to worm death in wild type *C. elegans* treated with copper sulfate on multiple trial sessions.

Length assays were carried out on fewer occasions than defecation analyses due to the premature cell death mentioned above. However, this allowed for equivalent sample sizes across each condition for statistical representation. A very dispersed sample distribution can be seen in the wild type nematodes in Figure 7 treated with nanoparticles, possibly explained by the relatively large size differences. It can be concluded from Figure 6 that the PCS-1 mutants

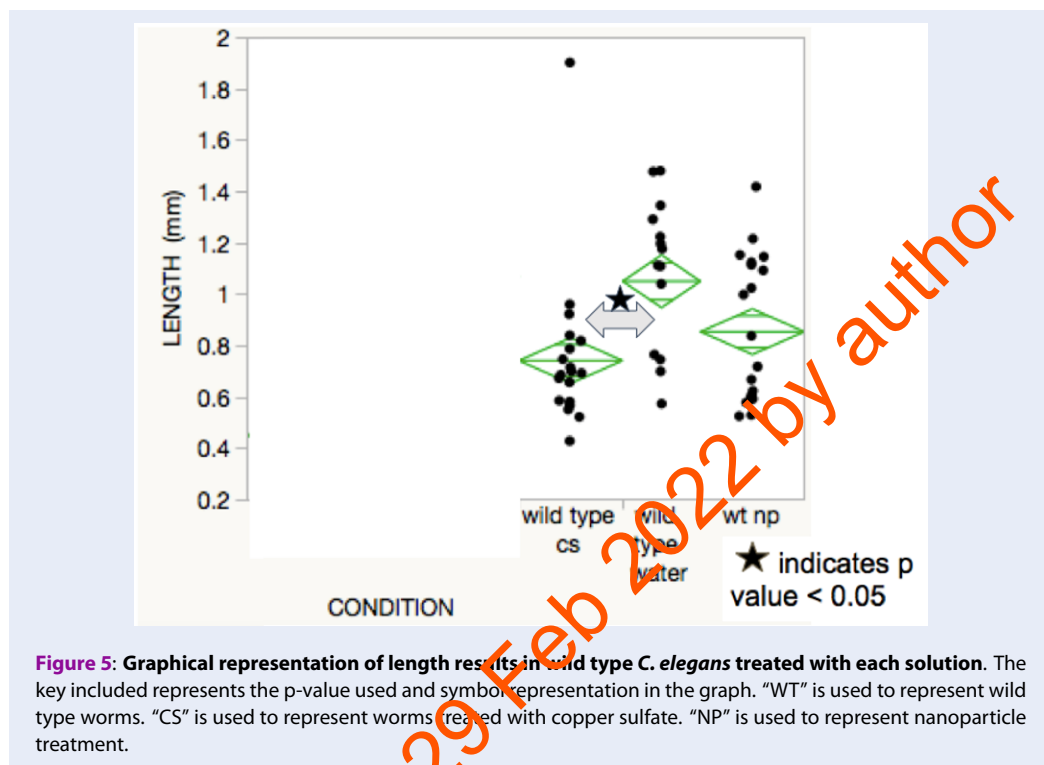


Figure 5: Graphical representation of length results in wild type *C. elegans* treated with each solution. The key included represents the p-value used and symbol representation in the graph. "WT" is used to represent wild type worms. "CS" is used to represent worms treated with copper sulfate. "NP" is used to represent nanoparticle treatment.

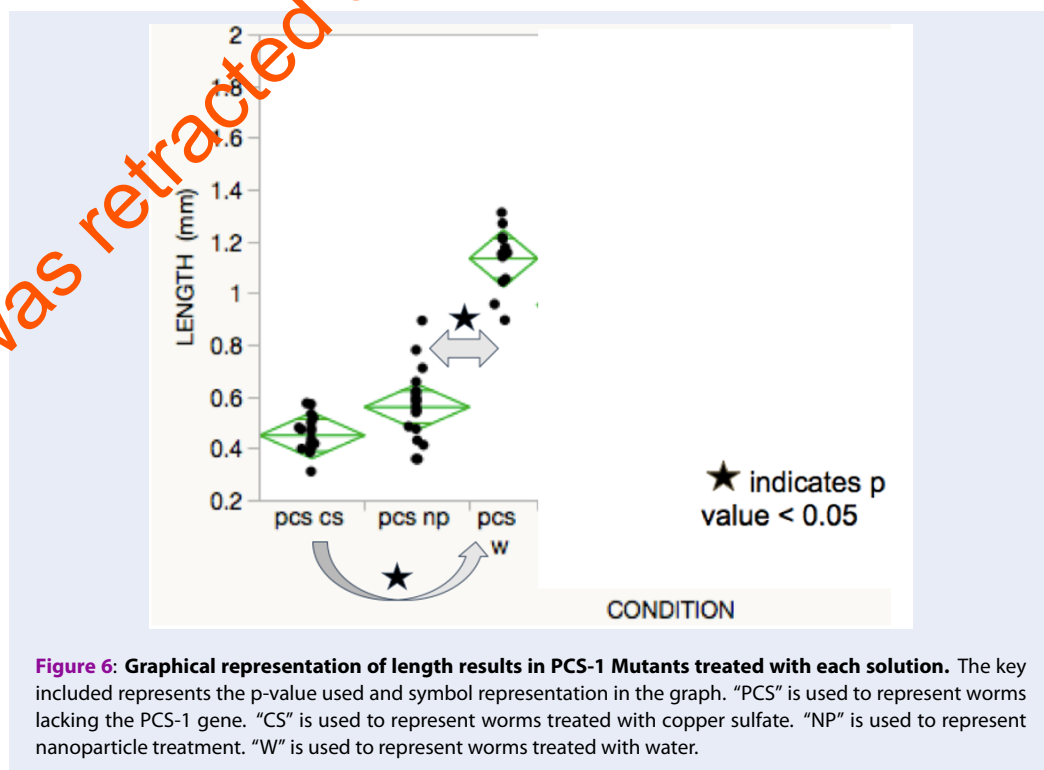


Figure 6: Graphical representation of length results in PCS-1 Mutants treated with each solution. The key included represents the p-value used and symbol representation in the graph. "PCS" is used to represent worms lacking the PCS-1 gene. "CS" is used to represent worms treated with copper sulfate. "NP" is used to represent nanoparticle treatment. "W" is used to represent worms treated with water.

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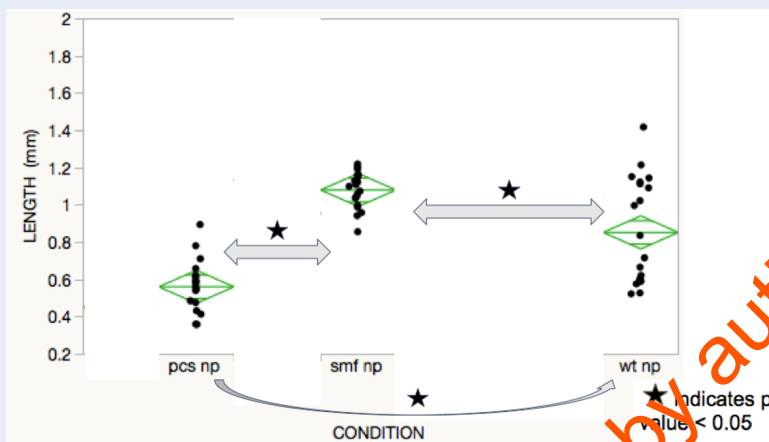


Figure 7: Graphical representation of length results in wild type, SMF-1, and PCS-1 mutant *C. elegans* treated with copper (II) oxide nanoparticles. The key included represents the p-value used and symbol representation in the graph. "SMF" is used to represent worms lacking the SMF-1 gene. "PCS" is used to represent worms lacking the PCS-1 gene. "WT" is used to represent wild type worms. "NP" is used to represent nanoparticle treatment.

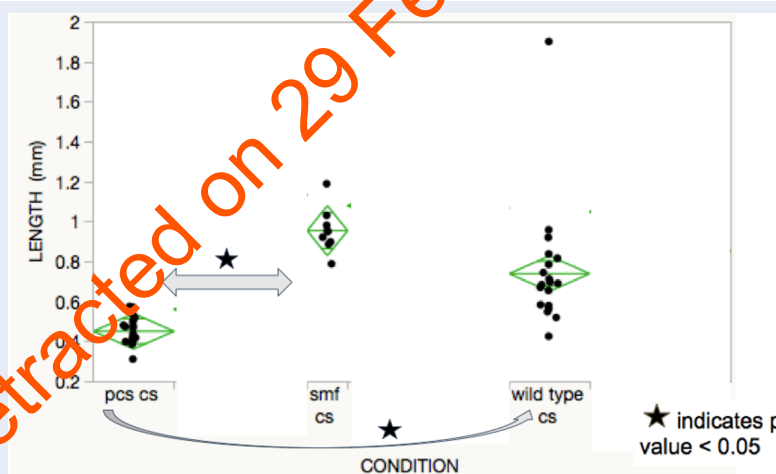


Figure 8: Graphical representation of length results in wild type, SMF-1, and PCS-1 mutant *C. elegans* treated with copper sulfate. The key included represents the p-value used and symbol representation in the graph. "CS" is used to represent worms treated with copper sulfate. "SMF" is used to represent worms lacking the SMF-1 gene. "PCS" is used to represent worms lacking the PCS-1 gene.

were hypersensitive to nanoparticles and even more sensitive to copper sulfate in terms of length. These results can be interpreted reasonably due to the equivalent control group sample sizes for each worm type.

CONCLUSIONS

The potential effects of implementing copper oxide nanoparticles in toxin remediation were examined through defecation rate and size analysis of a soil-inhabiting model organism, *C. elegans*. A very insightful finding of this study was the decreased defe-

cation rate and length of wild-type nematodes when treated with copper sulfate, indicative of copper ion-induced toxicity. Similarly, the hypersensitive shrinking observed in PCS-1 mutants exposed to copper sulfate and nanoparticles reflects the metal-tolerant functional role of PCS-1 gene expression⁹. The steady length of SMF-1 mutants in the presence of both copper sulfate and nanoparticles relative to the wild-type levels can be explained by the metal transport dysfunction observed when the SMF gene is not expressed. This causes reduced metal transport to neu-

ron pathways and interior organs, lessening the degenerative effect of metal ions on size for short time intervals. Perhaps most central to this study was the lack of significant nanoparticle-induced effects on defecation rate and size of wild-type worms. While these are positive result in the pursuit of environmental pollutant catalysts, these findings also highlight the need for additional testing under longer incubation time intervals, other metal types, and ulterior application-affected organisms¹⁰.

ABBREVIATIONS

APO: Apochromatic

ESEM: Environmental scanning electron microscope

NA: numerical aperture

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AUTHOR'S CONTRIBUTIONS

Author read and approved the final manuscript.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The author declares that they have no competing interests.

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