Invertebrate Models to Assess Environmental Exposures and Toxicity of Silver Nanoparticles

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Abstract

Over the past few decades, nanoparticles of noble metals such as silver and gold have been found to demonstrate significantly distinct physical, chemical, and biological properties from their bulk counterparts. Nano-sized particles of less than 100 nm in diameter are attracting the scientific community for the wide range of new applications in various fields including biophysics, material and medical sciences. As manufacturing of nano-sized particles is increasing, their dissemination in natural environments is also at an increase. Finding the adverse effects of such nano-sized particles found in the environment on different faunal species is thus of utmost importance. In the present paper, toxic effects of silver nanoparticles on different invertebrate models have been presented so as to have a view about the type of the damage posed by them and the efficacy of using invertebrate models for such assessments.

Keywords: Environment, Exposure, Genotoxicity, Invertebrate Models, Oxidative Stress, Silver Nanoparticles, Toxicity

1. Introduction

Nano-sized particles are near atomic scale size and have at least one dimension in the range of 1-100 nm. Relatively little is known about the behavior and toxicity of nanoparticles in the environment. Nano-sized particles are attracting the scientific community for their wide range of applications in various fields[1]. Nanoparticles can exhibit properties that differ markedly from those of bulk materials, due to their small particle dimension, high surface area and quantum confinements. Silver nanoparticles (SNPs) are likely to enter the aquatic ecosystems because of their multiple applications and pose a health concern for humans and aquatic species[2]. Although SNPs are increasingly used in various consumer products and produced at industrial scale[3], information on harmful effects of nanosilver to environmentally relevant organisms is still scarce. In this paper, a review of toxicological aspects of SNPs has been presented on different invertebrate models.

2. Invertebrate Models used for Assessing Toxicological Aspects of SNPs

2.1 *Daphnia magna*

Daphnia magna, a planktonic crustacean (Figure 1) has been used by different researchers as an invertebrate model for assessing toxic aspects of SNPs[1,3-6]. Study of the effects of filtration and capping agents and absence or presence of food was conducted on the toxicity of SNPs to *Daphnia magna*[7]. Study was done by exposing them with silver nitrate solution and SNP suspensions including both commercially available SNPs (coated & uncoated) and laboratory synthesized SNP's (coated with coffee or citrate). Toxicity of filtered and unfiltered suspensions was compared and effect on addition of food was also examined. Dose response curves were generated and median Lethal Concentration (LC50) values

calculated. The LC50 values for unfiltered particles were found to be 1.1 ± 0.1µg/L - AgNO3 ; 1.0 ± 0.1-coffee coated; 1.1 ± 0.2-citrate coated; 16.7 ± 2.4 Sigma Aldrich Ag-nanoparticles (SA) uncoated; 31.5 ± 8.1 SA coated. LC50 values for the filtered particles were 0.7 ± 0.1 - AgNO3; 1.4 ± 0.1 -SA uncoated; 4.4 ± 1.4 -SA coated. Similarly[8] studied the comparison of acute and chronic toxicity of SNPs and silver nitrate in *Daphnia magna*. When *D. magna* was exposed to SNPs for toxicity test (48 hrs.), they observed no mortality but when they exposed *D. magna* with SNPs for chronic exposure (21 days), it was observed that water-borne AgNO3 inhibited the growth and reproduction in *D. magna*.

(Source: www.tropicalfishkeeping.com)

Figure 1. Microphotograph of *Daphnia magna* in culture.

Studied[4] the toxicity of various SNP's compared to silver ion in *D. magna* and found that it caused swimming abnormality in *D. magna* and the toxicity of silver species is dose and composition dependent. The colloidal SNPs and AgNO$_3$ were almost similar in terms of mortality. On the same line studied[9] the importance of surface coatings on SNP's toxicity in *D. magna*. The study revealed the effect of 3 surface coatings and observed difference in mortality among three coating exposures for 48 hrs. LC50 was found dependent on the characteristics of nano-particles including particle size and surface coating. Studied[1] the toxicity of 2 types of silver nano-particles to *D. magna* and *Thamnocephalus platyurus*. In this study, the toxicity of collargal (protein-coated nanosilver) and AgNO$_3$ was analyzed and both were found to be highly toxic to both crustaceans. EC50 values in artificial fresh-

water were 15-17 ppb for *D. magna* and 20-27 ppb for *T. platyurus* whereas natural water mitigated toxic effect up to 8 folds as compared to artificial fresh water. The toxicity of SNPs in all test media was up to 10-fold lower than that of soluble silver salt, AgNO$_3$. Studied[10] the SNP's and AgNO$_3$ induced high toxicity to *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*. In this study, the SNPs were characterised to investigate the aggregation and agglomeration behaviour of SNPs under different media, chemical composition and test period. The results revealed that the toxicity of both AgNO$_3$ and SNPs differ significantly based on the test species. They found no difference in toxicity for algae, a small difference for zebra fish and a major difference in toxicity for *D. magna*.

2.2 Caenorhabditis elegans

Apart from *D. magna*, various other invertebrate models have also been explored for assessing toxicity of SNPs including *Caenorhabditis elegans* (Figure 2)[11-13]. Studied[11] SNP and silver ion toxicity in the nematode, *C. elegans* and reported that Ag nanotoxicity is dependent not only on dissolution of Ag ion but also on particle specific effects. Similarly[13] studied the intracellular uptake and associated toxicity of SNPs in *C. elegans*. In this study, they used wild type of *C. elegans* and the strain was expected to be sensitive to oxidative stress, genotoxins and metals. They observed growth inhibition by all tested SNPs at low concentration. They also describe a modified growth assay that permits differentiation between direct growth inhibitory effects and indirect inhibition mediated by toxicity to the food source. Studied[12] the oxidative stress related PMK-1 p38 MAPK activation as a mechanism for toxicity of SNPs to reproduction in the *C. elegans* and they concluded that oxidative stress is an important mechanism of SNP-induced reproduction toxicity in *C. elegans*, and that PMK-1 p38 MAPK plays an important role in it.

(Source: www.wp.wpi.edu)

Figure 2. Microphotograph of *C. elegans*.

2.3 *Drosophila melanogaster*

Studied[14] the acute and chronic toxicity effect of SNPs on *Drosophila melanogaster*. SNPs were prepared in the form of solid dispersion using micro-encapsulation method. The acute toxic effect of SNPs was observed for the silver concentration of 20 mg/L. At this silver concentration, 50% of the tested flies were unable to leave the pupae, and they did not finish their developmental cycle. Chronic toxicity of SNPs was assessed by a long-term exposure of overall eight filial generations of *Drosophila melanogaster* to SNPs. The long-term exposure to silver NPs influenced the fertility of *Drosophila* during the first three filial generations, nevertheless the fecundity of flies in subsequent generations consequently increased up to the level of the flies from the control sample due to the adaptability of flies to the SNPs exposure (Figure 3).

2.4 *Hydra*

Hydra has been used in different studies as a successful invertebrate model. Activated Carbon from Pyrolysed sugarcane Bagasse (ACPB) presented pore size ranges from 1.0 to 3.5nm which was successfully loaded with silver nanoparticles of diameter around 35nm. These loaded nanoparticles (ACPB-AgNP) showed environmental risks, with toxic effect to the aquatic organism *Hydra attenuata* (LC50 value of 1.94mg/L)[15] (Figure 4).

(Source: www.gbiosciences.com)
Figure 3. *Drosophila melanogaster*.

(Source: www.scinews.com)
Figure 4. *Hydra vulgaris*.

2.5 Snails

Silver is one of the most toxic metals to freshwater aquatic organisms[16]. Molluscs have always been used for assessing environmental stresses and toxicity parameters. Snails of different genera have been found to be employed to assess the toxic effects of SNPs also[17-22]. Study[16] the induction of apoptosis and DNA damage by SNPs in snail *Lymnea luteola*. Three concentrations of SNPs were selected, the concentration I (4 mug/l), concentration II (12 mug/l), and the concentration III (24 mug/l). Induction of oxidative stress in snail hemolymph was observed by a decrease in reduced glutathione (GSH) and Glutathione S-Transferase (GST) levels at different concentration of SNPs, and on the other hand, malondialdehyde (MDA) levels increased at lower concentrations but decreased in higher concentration of SNPs. DNA damage scores increased with the exposure levels of SNPs, and dose- and time-dependent effects were observed[16]. In another study[2], *L. luteola* was used to investigate the acute toxicity and genotoxicity of SNPs in a static-renewal system for 96 h. SNPs caused molluscicidal activity in *L. luteola*, with 96-h median lethal concentrations (LC50: 48.10 μg L^{-1}). It was observed that SNPs (36 μg L^{-1}) elicited a significant (p<0.01) reduction in glutathione, glutathione-s-transferase and glutathione

peroxidase with a concomitant increase in malondialdehyde level and catalase in digestive gland of *L. luteola*.

Another snail *Physa acuta* was chosen to evaluate the potential deleterious effects of SNPs and their counterpart $AgNO_3$, through water-only exposures[23]. 2 media were tested: Artificial Pond Water (APW) and modified APW (adapted by removing calcium chloride).Chloride was found playing an important role in toxicity, most likely by complexation with silver ions, which would reduce the bioavailability, uptake, and toxicity of silver. Chronic tests showed that hatching success was more sensitive to silver in the ionic form than in the particulate form[23].

Peringia ulvae were exposed to 150 µg Ag L^{-1} added as citrate capped SNPs or aqueous Ag ($AgNO_3$) in combination with inhibitor treatment[24]. No inhibitor treatment completely eliminated the uptake of Ag in either aqueous or NP form, but all inhibitor treatments, except phenamil, significantly reduced the uptake of Ag presented as Ag NPs. Clathrin- and caveolae-mediated endocytosis appear to be mechanisms exploited by SNPs, with the latter pathway only active at 24 h[24]. Another study evaluated whether water chemistry affects the dietary uptake and toxicity of SNPs by the freshwater snail *Lymnaea stagnalis*[25]. The study characterized the effects of water hardness and humic acids on the bioaccumulation and toxicity of SNPs coated with PolyVinyl Pyrrolidone (PVP) to the freshwater snail *L. stagnalis* after dietary exposures. Results indicated that bioaccumulation and toxicity of Ag from PVP-SNPs ingested with food are not affected by water hardness and by humic acids, although both could affect interactions with the biological membrane and trigger nanoparticle transformations[25]. Similarly, another

study characterized the bioavailability of Ag from $AgNO_3$ and from SNPs capped with polyvinylpyrrolidone (PVP SNP) and thiolated polyethylene glycol (PEG SNP) in the freshwater snail, *L. stagnalis*, after short waterborne exposures. But in this study, results showed that water hardness, SNP capping agents, and metal speciation affected the uptake rate of Ag from SNPs[26] (Figure 5).

3. Conclusion

Silver nanoparticles are important because of their multiple applications in different fields of science. These nanoparticles are likely to enter the aquatic ecosystems and pose a health concern for humans and aquatic species. This paper emphasized on the use of different invertebrate models in various researches so as to put forward the direct or indirect impact of silver nanoparticles. We can see that a variety of invertebrates including coelenterates, roundworms, crustaceans and molluscs have been used. Further studies are recommended to compile the full toxic profile of silver nanoparticles present in the ecosystem.

4. References

1. Blinova I, Niskanen J, Kajankari P, Kanarbik L, Kakinen A, Tenhu H, Penttinen OP, Kahru A. Toxicity of two types of silver nanoparticles to aquatic crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Environ. Sci. Pollut. Res. Int. 2013 May; 20(5):3456-63. https://doi.org/10.1007/s11356-012-1290-5. PMid: 23143296.

2. Ali D, Yadav PG, Kumar S, Ali H, Alarifi S, Harrath AH. Sensitivity of freshwater pulmonate snail *Lymnaea luteola*, to silver nanoparticles. Chemosphere. 2014 June; 104:134-40. https://doi.org/10.1016/j.chemosphere.2013.10.081. PMid: 24309155.

3. Romer I, White TA, Baalousha M, Chipman K, Viant MR, Lead JR. Aggregation and dispersion of silver nanoparticles in exposure media for aquatic toxicity tests. J. Chromatogr. A. 2011 July; 1218(27):4226-33. https://doi.org/10.1016/j.chroma.2011.03.034. PMid: 21529813.

4. Asghari S, Johari SA, Lee JH, Kim YS, Jeon YB, Choi HJ, Moon MC, Yu J. Toxicity of various silver nanoparticles compared to silver ions in *Daphnia magna*. J. Nanobiotechnology. 2012; 10:14. https://doi.org/10.1186/1477-3155-10-14. PMid: 22472056, PMCid: PMC3344683

5. Gao J, Powers K, Wang Y, Zhou H, Roberts SM, Moudgil BM, Koopman B, Barber DS. Influence of Suwannee River humic acid on particle properties and toxicity of silver

(Source: www.natureloveyou.org)
Figure 5. *Physaacuta.*

nanoparticles. Chemosphere. 2012 Sept; 89(1):96-101. https://doi.org/10.1016/j.chemosphere.2012.04.024. PMid: 22583785.

6. Kennedy AJ, Chappell MA, Bednar AJ, Ryan AC, Laird JG, Stanley JK, Steevens JA. Impact of organic carbon on the stability and toxicity of fresh and stored silver nanoparticles. Environ. Sci. Technol. 2012 Oct. 46(19):10772-80. https://doi.org/10.1021/es302322y. PMid: 22950762.

7. Allen HJ, Impellitteri CA, Macke DA, Heckman JL, Poynton HC, Lazorchak JM, Govindaswamy S, Roose DL, Nadagouda MN. Effects from filtration, capping agents, and presence/absence of food on the toxicity of silver nanoparticles to *Daphnia magna*. Environ. Toxicol. Chem. 2010 Dec; 29(12):2742-50. https://doi.org/10.1002/etc.329. PMid: 20890913.

8. Zhao CM, Wang WX. Comparison of acute and chronic toxicity of silver nanoparticles and silver nitrate to *Daphnia magna*. Environ. Toxicol. Chem. 2011 Apr; 30(4):885-92. https://doi.org/10.1002/etc.451. PMid: 21191880.

9. Zhao CM, Wang WX. Importance of surface coatings and soluble silver in silver nanoparticles toxicity to *Daphnia magna*. Nanotoxicology. 2012 June; 6(4):361-70. https://doi.org/10.3109/17435390.2011.579632. PMid: 21591875.

10. Ribeiro F, Gallego-Urrea JA, Jurkschat K, Crossley A, Hassellov M, Taylor C, Soares AM, Loureiro S. Silver nanoparticles and silver nitrate induce high toxicity to *Pseudokirchneriella subcapitata, Daphnia magna* and *Danio rerio*. Sci. Total Environ. 2013 July; 466-467C:232-41. https://doi.org/10.1016/j.scitotenv.2013.06.101. PMid: 23895786.

11. Eom HJ, Ahn JM, Kim Y, Choi J. Hypoxia inducible factor-1 (HIF-1)-flavin containing monooxygenase-2 (FMO-2) signaling acts in silver nanoparticles and silver ion toxicity in the nematode, *Caenorhabditis elegans*. Toxicol. Appl. Pharmacol. 2013 July; 270(2):106-13. https://doi.org/10.1016/j.taap.2013.03.028. PMid: 23583631.

12. Lim D, Roh JY, Eom HJ, Choi JY, Hyun J, Choi J. Oxidative stress-related PMK-1 P38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode *Caenorhabditis elegans*. Environ. Toxicol. Chem. 2012 Mar; 31(3):585-92. https://doi.org/10.1002/etc.1706. PMid: 22128035.

13. Meyer JN, Lord CA, Yang XY, Turner E.A, Badireddy, Marinakos SM, Chilkoti A, Wiesner MR, Auffan M. Intracellular uptake and associated toxicity of silver nanoparticles in *Caenorhabditis elegans*. Aquat. Toxicol. 2010 Oct; 100(2):140-50. https://doi.org/10.1016/j.aquatox.2010.07.016. PMid: 20708279.

14. Panacek A, Prucek R, Safarova D, Dittrich M, Richtrova J, Benickova K, Zboril R, Kvitek L. Acute and chronic toxicity effects of silver NanoParticles (NPs) on *Drosophila melanogaster*, Environ. Sci. Technol. 2011 June; 45(11):4974-79. https://doi.org/10.1021/es104216b. PMid: 21553866.

15. Goncalves SPC, Strauss M, Delite FS, Clemente Z, Castro VL, Martinez DST. Activated carbon from pyrolysed sugarcane bagasse: Silver nanoparticle modification and ecotoxicity assessment. Sci. Total Environ. 2016 Sept; 565:833-40. https://doi.org/10.1016/j.scitotenv.2016.03.041. PMid: 27039274.

16. Ali D. Oxidative stress-mediated apoptosis and genotoxicity induced by silver nanoparticles in freshwater snail *Lymnaea luteola*. Biol. Trace Elem. Res. 2014 Dec; 162(1-3):333-41. https://doi.org/10.1007/s12011-014-0158-6. PMid: 25351851.

17. Goncalves F, Pavlaki D, Lopes R, Hammes J, Gallego-Urrea JA, Hassellov M, Jurkschat K, Crossley A, Loureiro S. Effects of silver nanoparticles on the freshwater snail *Physaacuta*: The role of test media and snails' life cycle stage. Environ. Toxicol. Chem. 36(1):243-53, 2017 Jan. https://doi.org/10.1002/etc.3532. PMid: 27312215.

18. Stoiber T, Croteau MN, Romer I, Tejamaya M, Lead JR, Luoma SN. Influence of hardness on the bioavailability of silver to a freshwater snail after waterborne exposure to silver nitrate and silver nanoparticles. Nanotoxicology. 2015; 9(7):918-27. https://doi.org/10.3109/17435390.2014.991772. PMid: 25676617.

19. Ali D. Oxidative stress-mediated apoptosis and genotoxicity induced by silver nanoparticles in freshwater snail *Lymnaea luteola*. Biol. Trace Elem. Res. 2014 Dec; 162(1-3):333-41. https://doi.org/10.1007/s12011-014-0158-6. PMid: 25351851.

20. Khan FR, Misra SK, Bury NR, Smith BD, Rainbow PS, Luoma SN, Valsami-Jones E. Inhibition of potential uptake pathways for silver nanoparticles in the estuarine snail Peringiaulvae. Nanotoxicology. 2015 May; 9(4):493-501. https://doi.org/10.3109/17435390.2014.948519. PMid: 25137295.

21. Oliver AL, Croteau MN, Stoiber TL, Tejamaya M, Romer I, Lead JR, Luoma SN. Does water chemistry affect the dietary uptake and toxicity of silver nanoparticles by the freshwater snail *Lymnaea stagnalis.?*. Environ. Pollut. 2014 June; 189:87-91. https://doi.org/10.1016/j.envpol.2014.02.010. PMid: 24641838.

22. Ali D, Yadav PG, Kumar S, Ali H, Alarifi S, Harrath AH. Sensitivity of freshwater pulmonate snail *Lymnaea luteola*, to silver nanoparticles. Chemosphere, 104:134-40, June2014. https://doi.org/10.1016/j.chemosphere.2013.10.081. PMid: 24309155.

23. Goncalves F, Pavlaki D, Lopes R, Hammes J, Gallego-Urrea JA, Hassellov M, Jurkschat K, Crossley A, Loureiro S. Effects of silver nanoparticles on the freshwater snail *Physaacuta*: The role of test media and snails' life cycle stage. Environ. Toxicol. Chem. 2017 Jan; 36(1):243-53. https://doi.org/10.1002/etc.3532. PMid: 27312215.

24. Khan FR, Misra SK, Bury NR, Smith BD, Rainbow PS, Luoma SN, Valsami-Jones E. Inhibition of potential uptake

pathways for silver nanoparticles in the estuarine snail *Peringi aulvae* Nanotoxicology. 2015 May; 9(4):493-501. https://doi.org/10.3109/17435390.2014.948519. PMid: 25137295.

25. Oliver AL, Croteau MN, Stoiber TL, Tejamaya M, Romer I, Lead JR, Luoma SN. Does water chemistry affect the dietary uptake and toxicity of silver nanoparticles by the freshwater snail *Lymnaea stagnalis.?* Environ. Pollut. June 2014; 189:87-91. https://doi.org/10.1016/j.envpol.2014.02.010. PMid: 24641838.

26. Stoiber T, Croteau MN, Romer I, Tejamaya M, Lead JR, Luoma SN. Influence of hardness on the bioavailability of silver to a freshwater snail after waterborne exposure to silver nitrate and silver nanoparticles. Nanotoxicology. 2015; 9(7):918-27. https://doi.org/10.3109/17435390.2014.991772. PMid: 25676617.