

DOI: https://doi.org/10.52756/boesd.2023.e02.015

Cytotoxic Effects of Silver Nanoparticles on Plants: A Potential Threat to the Environment and Its Management

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Keywords: Nanoparticles, nanomaterials, nano waste management.

Abstract:

Nanomaterials are nowadays very common in our daily used products. The most prevalent nanoparticles that we encounter are silver nanoparticles. Almost all electronic appliances, including mobile phones, contain a certain amount of silver nanoparticles. Due to the unmanaged and unforeseen disposal of products containing nanomaterials over the years, silver nanoparticles have become almost omnipresent in the environment in different forms and concentrations. Research has shown that silver nanoparticles, in their lower size range with higher concentration and longer exposure time, can cause severe toxic effects on the plant cell cycle, growth, and development. Therefore, to restrict the encroachment of nanoparticle-containing waste or nano-waste into the environment, there should be a specialized management system that can assess, categorize, and formulate suitable strategies for the safe disposal of those nanowastes.

Introduction:

Particles falling within the size range of 1 to 100 nm in at least one dimension maintain their individuality, despite their minuscule size, and are categorized as Nano Particles (Albrecht et al., 2006). The extremely small size of these particles leads to a change in the physical and chemical properties compared to the bulk samples of the original compound from which these Nano Particles are derived (Auffan et al., 2009). Recent advancements in technology have facilitated the design and engineering of nanoparticles for diverse applications in medicine, biology, material science, physics, and chemistry (Rastogi et al., 2017; Sadhu et al., 2022; Dianová et al., 2023). Unfortunately, the introduction of nanoparticles into human life and the environment on Earth has occurred without due consideration of associated risks (Roco, 2003). Notably, in recent years, the synthesis of nanoparticles has shifted towards utilizing biological materials, particularly plant-based materials in a process known as green synthesis (Mukherjee et al., 2001; Pasupuleti et al., 2013; Paul & Yadav, 2015).

Silver nanoparticles (AgNPs) hold significant importance, being recognized as a broadspectrum biocide effective against a wide range of bacteria (Sengottaiyan et al., 2016), fungi (Lamsal et al., 2011; Tripathi et al., 2017) and exhibiting antiviral properties (Sun et al., 2005)

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Shubhadeep Roychoudhury, Tanmay Sanyal, Koushik Sen & Sudipa Mukherjee Sanyal (eds.), A Basic Overview of Environment and Sustainable Development [Volume: 2]. ISBN: 978-81-962683-8-1; pp. 231-243; Published online: 17th December, 2023

along with anticancer potential (Rajeshkumar et al., 2016). The adoption of a green process for metal nanoparticle synthesis has resulted in a manifold increase in the application of silver nanoparticles across various domains, including biosensors (Nam et al., 2003), cell labeling (Wu et al., 2003), DNA detection (Taylor et al., 2000), oxidative catalysis (Shiraishi & Toshima, 2000), and antibiotics (Kreuter & Gelperina, 2008).

An alarming consequence of widespread Nano Particle use is the release of nanomaterials into the environment. With industries employing nanotechnology in diverse forms, the generation of waste containing residual nanomaterials and the introduction of nano-waste into the environment have become unavoidable. Interestingly, nanoparticles were present in the environment even before the advent of nanotechnology. Depending on their type, nanoparticles may be released into the atmosphere as aerosols, into the soil and surface water. Nanoparticles can be released as bare particles, functionalized particles, aggregates, or embedded in a matrix (Nowack & Bucheli, 2007). They can pose ecotoxicological hazards, undergo biodegradation, or bioaccumulate in the food chain (Scenihr, 2006; Rajalakshmi & Paari, 2023).

Common sources of natural nanoparticles in the environment include by-products generated from the combustion of fuels such as coal, petroleum, and wood burning, as well as automobile exhaust. Additionally, aerosols from atmospheric phytochemistry and volcanic activity contribute to the presence of natural nanoparticles. Given the rapid proliferation of nano products in the market, there is an urgent need for comprehensive research in the expansive fields of nanotoxicology and nanowaste management (Bystrzejewska-Piotrowska et al., 2009).

Cytotoxic effects of Silver Nanoparticles:

In their study, Babu et al. (2008) observed a decrease in the mitotic index (MI) of the *Allium cepa* root tip meristem when treated with silver nanoparticles (AgNPs). Notably, the decrease in MI was more pronounced with longer exposure times for each concentration of AgNPs. The data revealed a higher decrease in MI in a 20 PPM solution compared to 10 PPM, while 40 PPM and 50 PPM showed almost the same and higher MI than 20 PPM (Fig. 1). Additionally, an increase in the frequency of various chromosomal aberrations (CA) was noted with escalating concentrations of AgNPs and prolonged exposure times. Unlike MI, the change in CA frequency was found to be more significant with variations in AgNP concentration than exposure time (Fig. 2) (Babu et al., 2008).

Daphedar & Taranath (2017, 2018) investigated *Drimia indica* and *Drimia polyantha*, respectively, and found similar increasing trends in chromosomal aberrations, such as anaphase bridges, sticky chromosomes, laggards, diagonal chromosomes, C-metaphase, multipolar anaphase, and disturbed metaphase. The overall mitotic activity decreased with higher concentrations and longer exposure times to silver nanoparticles. *D. polyantha* root tips treated with 16 µg/ml of AgNPs for 24 hours exhibited dead cells (Fig. 3). The highest frequencies of chromosomal bridges and stickiness were observed after treatment with 12 µg/ml AgNPs for 24





Figure 1. The graph has been developed from the secondary data obtained from Babu *et. al.* (2008) showing the decreasing trend of the Mitotic Index with increasing AgNP concentration and exposure time in *Allium Cepa*.







Figure 3. The graph has been developed from the secondary data obtained from Daphedar & Taranath (2018), showing the increasing trend of the chromosomal aberrations with increasing AgNP concentration and exposure time in *Drimia polyantha*.





Abdel-Azeem & Elsayed (2013) demonstrated that different sizes of AgNPs can result in varying degrees of cytotoxicity in *Vicia faba* root tip meristem. They observed a decrease in the

mitotic index when treated with smaller nanoparticles, and this decrease further intensified with longer treatment times (Fig. 5). The concentration of AgNPs for all treatments was fixed at 50 PPM. The frequencies of chromosomal aberrations significantly increased with decreasing NP size (65 nm to 20 nm) and increasing exposure time (6 to 24 hours). Treatment with 20 nm NPs for 12 hours resulted in 80.72% mitotic abnormalities, leading to cell death with prolonged exposure (Fig. 6). The inhibitory effect of AgNPs on DNA synthesis at S-phase and the interaction of AgNPs with tubulin SH group were proposed as potential causes for the observed effects (Abdel-Azeem & Elsayed, 2013; Sudhakar et al., 2001; Kuriyama & Sakai, 1974).



Figure 5. The graph has been developed from the secondary data obtained from Abdel-Azeem & Elsayed (2013), showing the decreasing trend of the Mitotic Index with increasing AgNP concentration and exposure time in *Vicia faba*.

Fouad & Hafez (2018) investigated the cdc2 gene expression along with cytological studies of the root tip meristem of *Allium cepa*. They observed a decreased mitotic index and increased frequencies of chromosomal abnormalities with the treatment of silver nanoparticles. The expression of the cdc2 gene was found to be reduced by 28-61.8% with increasing concentrations and exposure times of AgNPs. The decrease in CDK gene expression in response to stress leading to cell cycle arrest or delayed entry into mitosis was suggested as an explanation (Kitsios & Doonan, 2011). In contrast, Syu et al. (2014) recorded an increase in protein cell-division-cycle kinase 2 in Arabidopsis when treated with AgNPs, highlighting the differential characteristics of nanoparticles of the same compound (Remédios et al., 2012). The interaction between CDC2 kinase and spindle formation during mitosis was linked to various types of chromosome aberrations, and genotoxic effects of AgNPs appeared even in the absence of cytotoxic symptoms, suggesting a more genotoxic nature (Fouad & Hafez, 2018).

The eukaryotic cell cycle is regulated by various mechanisms, including the reversible phosphorylation of cyclins (CYC) by cyclin-dependent protein kinases (CDKs). CDKs, such as CDKA, play a crucial role in mitosis by interacting with chromosomes and localizing to mitotic structures. CDKA has important functions in both G1/S and G2/M transitions (John et al., 2001; Tank & Thaker, 2011; Francis, 2009; Hirayama et al., 1991; Stals et al., 1997; Boruc et al., 2010; Hemerly et al., 1995).



Figure 6. The graph has been developed from the secondary data obtained from Abdel-Azeem & Elsayed (2013), showing the increasing trend of the chromosomal aberrations with increasing AgNP concentration and exposure time in *Vicia faba*.

Some related toxic effects of silver nanoparticles:

Labeeb et al. (2020) investigated the impact of AgNPs on seed germination in *Pisum* sativum, along with the assessment of the mitotic index. Significant reductions in germinability and the occurrence of root deformities were observed upon treatment with AgNPs. Abdel-Azeem & Elsayed (2013) noted a significant decrease in germinability and root growth of *Vicia* faba seeds exposed to a fixed concentration (50 PPM) and duration (9 hours) of AgNPs, with a correlation to the smaller size of the nanoparticles.

In line with these findings, Lee et al. (2014) reported a reduction in the germination rate of *Arabidopsis thaliana* across three generations when subjected to AgNP treatment. Stampoulis et al. (2009) demonstrated the inhibitory effect of 40 nm AgNPs on the growth of *Cucurbita pepo*. Salama (2012) observed increased shoot and root elongation in *Phaseolus vulgaris* and *Zea mays* at AgNP concentrations of 20, 40, and 60 ppm, while concentrations of 80 and 100 ppm were found to inhibit shoot and root elongation.

Furthermore, Stampoulis et al. (2009) indicated that 100 nm AgNPs at concentrations of 100 and 500 mg/L led to significant decreases of 41% and 57% in biomass and respiration rates.

Gubbins et al. (2011) demonstrated the growth-inhibiting effects of AgNPs on *Lemna minor*. It is noteworthy that nanometer-sized particles exhibit special toxicity and are generally more toxic than their larger counterparts (Donaldson et al., 1999). Additionally, particles with a diameter of less than 50 nm have been identified as highly toxic (Oberdoster, 1996).

Sources of Silver nanoparticle wastes and their sustainable management:

Metallic nanoparticles, such as silver nanoparticles, may be released through leaching from structural components, including inner elements of appliances like fridges, vacuum cleaners, washing machines, air conditioning systems, and other electronic devices, as well as nanoparticle-coated wall paints. This release can occur when these items are casually and unsupervisedly disposed of in waste disposal sites or processed in waste treatment/recycling systems. Given the prevalence of silver nanoparticles as engineered nanomaterials (Rejeski & Lekas, 2008), questions arise regarding the use of AgNPs in domestic appliances. It has become evident that strategies for nanowaste management need to be developed before the disposal of nanoproducts begins. From a toxicological perspective, nanomaterials significantly differ from normal waste, making conventional tests and waste management systems such as incineration or basic landfills potentially unsuitable for them (Bystrzejewska-Piotrowska et al., 2009).

As per Leppard et al. (2003), conventional methods for wastewater treatment prove ineffective in the comprehensive removal of nanoparticles (NPs) from effluents (Westerhoff et al., 2008). Existing wastewater treatment systems demonstrate NP removal efficiencies ranging from 0% to 40%, underscoring a substantial likelihood of NP presence in drinking water, thereby creating a potential avenue for human exposure (Westerhoff et al., 2008). An investigation by Zhang et al. (2008) revealed that, despite rapid aggregation during potable water treatment processes, 20–60% of NPs persisted in settled water even after 24 hours. Consequently, NPs may endure for a relatively prolonged period, albeit in an aggregated state, within an aqueous environment characterized by low concentrations of electrolytes (Zhang et al., 2008). Nevertheless, traditional water treatment methods such as alum coagulation can eliminate 80% of NPs, and the incorporation of a 0.45 mm membrane filtration in the final stage enhances the removal efficiency to over 90% (Limbach et al., 2008).

The cytotoxic effects of certain nanoparticle types can be mitigated through the application of organic coatings (SCENIHR, 2006). These coatings serve to diminish the efficacy of nanoparticles, rendering them inert in the environment. However, this inert state is contingent upon the durability of the coating. Consequently, the future design and development of nanomaterials must prioritize the establishment of robust coatings to ensure sustained inactivity (Scenihr, 2006). Phytoremediation, a sustainable approach to environmental cleanup, has gained prominence in mitigating soil and water pollution. As silver nanoparticles become prevalent, understanding their impact is crucial for effective management strategies to ensure the long-term health of ecosystems and sustainable coexistence (Saha et al., 2022).

To address potential risks associated with synthetic nanomaterials, it is imperative to adopt a life cycle approach that encompasses production, utilization, and disposal (Bystrzejewska-Piotrowska et al., 2009). Implementing precautionary measures is essential, including:

- Mandating companies engaged in engineered nanomaterial production to label products containing nanoparticles. Displaying basic information about the levels and nature of emitted nanomaterials facilitates easy separation and recovery (Powell et al., 2008). Additionally, it is crucial to specify the expected lifetime of products containing nanoparticles, such as refrigerators with nanosilver compartments.
- 2) Conducting thorough investigations into the water and moisture-mediated leaching of nanoparticles from waste into the environment. The disposal of nano-waste should be managed to prevent water interaction (Bystrzejewska-Piotrowska et al., 2009).
- 3) Undertaking comprehensive research on the potential toxicity of released nanoparticles (Handy et al., 2008).
- 4) Revising personal protection equipment and work routines for handling nano-waste to ensure safety (Bystrzejewska-Piotrowska et al., 2009).

Conclusion:

In conclusion, the widespread use of silver nanoparticles (AgNPs) in various products, coupled with the inadequate management of nanowaste, poses a significant threat to the environment, particularly in terms of its cytotoxic effects on plant life. The research reviewed herein underscores the intricate relationship between AgNPs and plant cell cycle dynamics, growth, and development. The evidence presented reveals that AgNPs, especially in smaller sizes and higher concentrations with prolonged exposure times, induce substantial cytotoxicity, leading to a range of chromosomal aberrations, decreased mitotic indices, and adverse effects on seed germination.

Moreover, the findings emphasize the need for a specialized nano-waste management system to address the unique challenges posed by nanoparticles in terms of their disposal and potential environmental impact. The current methods of wastewater treatment and traditional waste disposal may prove ineffective in comprehensively removing and neutralizing AgNPs, necessitating innovative approaches for sustainable waste management.

As we move forward, a life cycle approach encompassing the production, utilization, and disposal of nanomaterials becomes imperative. Precautionary measures, including labeling of products containing nanoparticles, understanding water-mediated leaching, comprehensive toxicity assessments, and revising safety protocols for nano-waste handling, are crucial steps toward minimizing the environmental risks associated with AgNPs.

The complex interplay between nanotechnology and the environment demands interdisciplinary research, collaboration between industries and regulatory bodies, and proactive measures to safeguard our ecosystems. Implementing these strategies is vital not only for curtailing the potential threats posed by silver nanoparticles but also for ensuring the responsible and sustainable integration of nanomaterials into our daily lives.

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HOW TO CITE

Alokemoy Basu (2023). Cytotoxic Effects of Silver Nanoparticles on Plants: A Potential Threat to the Environment and Its Management. © International Academic Publishing House (IAPH), Shubhadeep Roychoudhury, Tanmay Sanyal, Koushik Sen & Sudipa Mukherjee Sanyal (eds.), *A Basic Overview of Environment and Sustainable Development [Volume: 2]*, pp. 231-243. ISBN: 978-81-962683-8-1. DOI: https://doi.org/10.52756/boesd.2023.e02.015

