Physiological and genotoxic responses of the earthworm *Aporrectodea caliginosa* exposed to sublethal concentrations of AgNPs

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**KEYWORDS**

AgNPs; *Aporrectodea caliginosa*; Cellulase; Cocoon; Coelomocytes

**Abstract** The purpose of the current study was to measure the ecotoxicity of silver nanoparticles (AgNPs) on *Aporrectodea caliginosa* earthworm. No adult earthworm mortality was observed at any treatment during the sub-chronic exposure period (28 d). Biomass and cellulase levels reduced in a concentration-dependent manner in the exposed earthworms compared to those of the control. The hatched cocoons from the contaminated substrates were significantly ($p < 0.05$) fewer than that of the control substrate. This finding provided further support for the conclusion that AgNPs may affect cocoon hatchability. Cocoon hatchability could therefore be a more reliable endpoint at a specific concentration than cocoon production. A significant increase in DNA damage was revealed in the earthworms treated with AgNPs compared to the untreated ones. The results denoted the effectiveness of measuring cellulase activity, biomass, reproduction and DNA damage and reinforced the application of the present methods in nanoparticles pollution biomonitoring studies.

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**Introduction**

Nanotechnology is rapidly becoming integrated into everyday products and uses. It swiftly enlarged by producing new nanoparticles. AgNPs, which are widely used in medicine, physics, chemistry and material sciences, are pivotal to develop nanotechnology (Sharma et al., 2014; Zhang and Zhang, 2014; Murphy et al., 2015). Until the arrival of antibiotics, silver nanoparticles cured wound infection (Edwards-Jones, 2009). AgNPs with novel properties of effective antibacterial activity have attracted the attention of many scientists and have enabled the technologists to develop nanosilver-based dental materials, catheters and burn wound. With greater AgNPs production and integration with consumer products, experts expect that AgNPs will provide an increasing plenty of anthropogenic Ag into the environment (Blaser et al., 2008). Despite the large number of studies concerning the effect of AgNPs in the aquatic environment, few have considered their impact on terrestrial ecosystems. Soil represents a major recipient of nanoparticles entering the environment. Engineered nanoparticles may enter soil via biosolids originating from wastewater treatment or the effluent from industrialization processes and can inhibit organisms in the terrestrial ecosystems (Schlich et al., 2012). Earthworms constitute...
Sensitive assays are becoming essential in the assessment of environmentally induced genotoxicity. Developing procedures and techniques allowed accurate surveys of living organisms in polluted environments. DNA is a supreme target of environmental stress in aquatic and terrestrial organisms (Frenzilli et al., 2001). Cells can take NPs up to bind with DNA when they come in contact by a variety of mechanisms (AshaRani et al., 2009). This can lead to the activation of cellular signaling processes producing reactive oxygen species (ROS), inflammation and eventually cell cycle arrest or cell death (Ahamed et al., 2010). Measuring DNA injury and assaying micronuclei (MN) frequency have markedly improved the potential for studying populations at risk. MN assay is a rapid and responsive test which detects genomic damage due to both clastogenic (chromosome damaging) effects and alteration of mitotic spindle (Gudi et al., 1992). In earthworms, coelomocytes are the circulating leukocytes present in the coelomic cavity. They play an important role in immune defense. Besides, they are used to study the effect of nanoparticle genotoxicants such as silver nanoparticles (AgNPs) and titanium dioxide nanoparticles (TiO2NPs) (Wang et al., 2009). Itziou et al. (2011) reported that hemocytes of the land snail Eobania vermiculata respond to environmental and experimental challenge by the activation of stress markers, such as DNA damage, detection of ROS production and lipid peroxidation.

As with pollution of surface waters, contamination of land has increased with anthropogenic activities. Past industrial activities, waste disposal and agricultural practices are sources of soil pollution in the long term. As a result, toxicity toward terrestrial species cannot be extrapolated from aquatic species. Specific approaches and models are needed to assess the impact of soil pollutants on terrestrial biota. Earthworms among other soil invertebrates have received more attention because of their ecological importance. Earthworms represent a significant, if not a dominant part of the soil biomass, where they may represent 60–80% of the total soil biomass (Rida, 1994) and are soil engineers regulating important soil processes, notably fertilization. They appear to be suitable as biomonitoring organisms, particularly for their strong interaction and permanent direct contact with soil. Moreover, earthworms are common in a wide range of soil and are readily available, sensitive, and easy to handle and historical data are available from their use in toxicity tests. Therefore, earthworms are effectively used in the research of nanomaterial interaction with living organisms and in assessing environmental nanosafety. Spurgeon and Hopkin (1996) reported that earthworms are great bioindicators in the monitoring of soil polluted by metals. Limited information is available concerning the reproduction of toxicity of nanoparticles (NPs). In the last decade, there was an increasing concern for the potential effects of the use of NPs on reproductive health. Nevertheless, only few studies were performed on the invertebrates. Schlich et al. (2012) used the earthworm Eisenia fetida, as described in the reproduction test guideline 222 (OECD, 2004) by spiking the soil, to gain more information about the effect of AgNPs in the terrestrial ecosystem. Gomes and Soares (2013) studied the mechanisms of response to AgNPs on Enchytraeus albidus (Oligochaeta) in terms of survival, reproduction and gene expression profile.

Aporrectodea caliginosa is found in a large number in null soil having moderate moisture and forms a large proportion of the earthworms in the farmlands of Egypt. In spite of their widespread occurrence and distribution in Egyptian soil, little is known about their ecological, physiological and ecotoxicological characters. The main objectives of the present study was to better understand the effects of AgNPs on A. caliginosa and the underlying mechanisms in order to provide more information on their toxicological effects in soil ecosystem.

Materials and methods

Chemicals

AgNPs (80 nm) were purchased from Sigma–Aldrich, Cairo, Egypt with a purity of 99.9%. Nominal ranges of particle diameters as provided by the manufacturer were 30 ± 5. Fig. 1 shows transmission electron micrograph (TEM) of AgNPs, which were dispersed in deionized water. The solution was sonicated in a low power ultrasonication bath for 30 s and 1 drop (20 μL) was deposited on a carbon coated Cu TEM grid. Samples were dried at room temperature for several hours before examination in the TEM. On the TEM images a small (approx. 20–25) number of particles were measured from about 4 or 5 TEM micrographs to get a rough particle size distribution for the primary particles. Each particle was measured individually from the TEM micrographs using

![Image](https://example.com/figure1.png)

**Figure 1** Characterization of AgNPs (a) TEM image and (b) the size-distribution histogram generated by using TEM images.
Digital Micrograph program, which is a standard TEM instrument control and analysis program. The TEM images show that the AgNPs were mainly equiaxial and rounded. All other chemicals were of highest purity and were purchased from Al Gomhoria Pharm. Ind., Cairo, Egypt.

Test soil

The test soil through the application was natural top soil (5–20 cm), loamy, medium acidic and lightly humic sand (pH 5.57, Organic matters 1.12%, sand 68%, clay 11%, silt 21%). This soil matches the properties as described in the OECD terrestrial ecotoxicological guideline (OECD, 2004). Appropriate amount of natural soil was sampled one to two weeks before the test. If the soil was too wet for sieving, it was dried at room temperature to 20 and 30% of the maximum water-holding capacity (WHC<sub>max</sub>) with periodic turning to avoid surface drying.

Earthworm collection and culture

Healthy earthworms of the species <i>A. caliginosa</i> were collected during summer 2014 from rice fields located near Belbeis city (30° 25' N, 31° 34' E), Sharkia Governarate, Egypt. Seven days prior to the experiments, the earthworms were maintained in their soil of origin in large plastic containers (40 × 60 × 10 cm) covered with coarse canvas woven from jute (burlap). The earthworms were bred under defined conditions 23 ± 2°C with 12 h of light per day to mimic a realistic diurnal cycle, water hold capacity (WHC, 60%) and free access to food (2 mm sieved air dried cow-dung).

Acute toxicity

Exposure concentrations were based on previous literature reports (Hu et al., 2010; Schlich et al., 2012; Gomes and Soares, 2013). Enough dry powder AgNPs were thoroughly mixed into the test soil to achieve the desired final concentrations of 0, 10, 100, 1000 mg kg<sup>-1</sup> dry soil. For every test concentration, the mixed soil was adjusted to 60% WHC using distilled water (Schlich et al., 2012). Clitellated <i>A. caliginosa</i> earthworms (690–730 mg fresh weight) were added to 500 g of the contaminated soil of different concentrations along with a control group (distilled water) contained in 1 L plastic boxes. During the 28-day sublethal exposure to AgNPs, the earthworms were fed on 30 g of uncontaminated cow-dung (2 mm sieved and moistened before application) spread over the surface of each pot each week. The water content was checked each week, and the evaporated water was replaced. The pots were incubated at room temperature 23 ± 2°C and a light:dark cycle of 12:12 h. The contents were then placed in a tray, and the surviving adults were removed, washed, blotted with filter papers and weighed. The contents of all pots were wet sieved through a 1-mm mesh recovering cocoons. The number of cocoons produced was determined each seven days. The cocoons of each pot were then placed on moist filter paper in petri dishes and incubated at room temperature until viable cocoons had hatched. Adverse effects were expressed as percentage mortality. The number of defunct <i>A. caliginosa</i> in each box was recorded after 28 days. Earthworms that did not respond to a gentle prodding with forceps were considered to be dead.

Gut cells and coelomocytes collection

The gut regions from two randomly chosen live earthworms were excised with sharp scissors and removed under dissecting microscope. Guts were minced into small pieces and transferred to a flask containing 10 mL dissociating solution (3:1 ethanol acetic acid solution). The mixture was then gently stirred for 1 h and filtrated through 250–60 mm diameter nylon filters. Earthworms were subjected to 4.5 V electric current for 30 s to expel coelomic fluid with coelomocytes through the dorsal pores according to the procedures modified by Roch (1979). Briefly, after weighing, washing and dry-blotting, the earthworms were placed individually in petri dishes containing 1–4 mL (depending on the earthworm body weight) of extrusion fluid. This fluid is composed of phosphate buffered saline (PBS) supplemented with 2.5 g L<sup>-1</sup> EDTA to prevent cell aggregation (Kurek and Plytycz, 2003). Gut cells and coelomocytes were monitored by light microscope and counted with the chamber method in Neubauer hemocytometer. Cell concentrations were adjusted to 10<sup>3</sup> cells mL<sup>-1</sup>.

Cellulase assay

Cellulase activity was assayed in earthworm gut using the methods described by Zhang et al. (1993). The enzyme was measured at 37 °C for 24 h in 10 mM sodium phosphate buffer (pH 7.5) with carboxymethyl cellulose (CMC) as substrate. The release of glucose was monitored continuously at 490 nm. Specific cellulase enzymatic activities were expressed as U mg<sup>-1</sup> protein.

Nuclear abnormalities assay

The micronucleus test was performed with earthworm coelomocytes according to the methodology of Hooftman and de Raat (1982) and the analysis of hemocyte nuclear abnormalities according to Carrasco et al. (1990). Immediately after sampling, hemolymph was smeared on clean glass slides, dried
overnight, fixed with methanol for 10 min and stained with Giemsa (5%). A total 3000 hemocytes per snail were examined under an optical microscope (1000 x magnification). The mean frequencies of micronucleus (MN) and binucleated cells (BN) found in each experimental group were calculated and expressed per 1000 cells (%).

Protein content determination

The protein content was determined by coomassie brilliant blue method developed by Bradford (1976) using bovine serum albumin as standard.

Micronuclei assay

Aliquots of 20 µL of coelomic and gut cell suspensions were smeared on clean microscopic slides using one slide for each replicate (a total of three slides per concentration). After the slides were air-dried, the cells were fixed in absolute ethanol for 15 min, stained with 5% Giemsa solution for 8 min and washed under the tap water (Rita et al., 2007). The mitotic metaphase stage was examined with a binuclear microscope. The frequencies of MN and BN cells were evaluated as the number of abnormalities per 1000 cells (%) scored (Barsiene et al., 2010).

Statistical analyses

The results were expressed as mean ± SD. The p-values less than 0.05 were considered statistically significant. The Shapiro-Wilk’s test and Levene’s test were used to ensure the normality assumption and the homogeneity of variances, respectively. Significant differences of a treatment on mortality, biomass, cellulase, reproduction and nuclear abnormalities between treatments and controls were tested using a one-way analysis of variance (ANOVA), and followed Bonferroni’s multiple comparison test (p < 0.05). *Indicates significance difference (p < 0.05) between the exposed groups and the control.

Cellulase activity and biomass

The activity of cellulase in the gut of A. caliginosa earthworms decreased as the concentrations of the AgNPs increased (Fig. 2). Although activity of cellulase appeared to drop in the worms exposed to the lowest concentration of AgNPs (10 mg kg⁻¹), there was no significant difference observed compared to the control (p < 0.05). In contrast, cellulase showed a significant decrease (p < 0.05) compared to the control when the earthworms were exposed to high concentrations. However, there were no significant differences (p < 0.05) in cellulase inhibition values among all tested concentrations. There were concomitant significant decreases in the biomass of the earthworms exposed to AgNPs compared to the control. Biomasses of the earthworms exposed to 50, 100 and 200 mg kg⁻¹ for 28 days were 89.19, 81.08 and 72.97 percent of the control, respectively (Table 1).

Reproduction test

Reproduction test results are summarized in Table 2. The mean number of cocoons produced in the control group after

![Cellulase activity in gut of A. caliginosa earthworms after exposure to different concentrations of AgNPs for 28 days. Each value represents the mean ± SD. of three replicates. Different letters indicate significant differences among treatments (one-way ANOVA, followed by Bonferroni multiple comparison test p < 0.05). *Indicates significance difference (p < 0.05) between the exposed groups and the control.](image.png)

### Table 1 Mean biomass ± SD of A. caliginosa after exposure to different concentrations of AgNPs (mg kg⁻¹) for 28 days. n = 36 earthworms per treatment.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mean biomass (g)</th>
<th>Percent of the control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.74 ± 0.02</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>0.72 ± 0.04</td>
<td>97.29</td>
</tr>
<tr>
<td>50</td>
<td>0.66 ± 0.03*</td>
<td>89.19</td>
</tr>
<tr>
<td>100</td>
<td>0.60 ± 0.07*</td>
<td>81.08</td>
</tr>
<tr>
<td>200</td>
<td>0.54 ± 0.06*</td>
<td>72.97</td>
</tr>
</tbody>
</table>

Mean biomass significantly different than control at *p < 0.05.
28 days was 151 ± 9. No significant ( \( p < 0.05 \) ) difference in cocoon production was observed between earthworms of the control group and those exposed to 10 and 50 mg kg\(^{-1}\) AgNPs. During experiment, earthworms of the control group produced one cocoon per earthworm each week while at exposure to 200 mg kg\(^{-1}\) AgNPs, the production dropped to be one cocoon per earthworm each 2 weeks. Cocoon production reduced to be 87.72 and 83.18% of the control value at 50 and 200 mg kg\(^{-1}\) AgNPs, respectively. There was no significant ( \( p < 0.05 \) ) difference between the number of hatched cocoons produced by 10 mg kg\(^{-1}\) treated earthworms and that of the control. The percent of hatched cocoons was 35.11 ± 4.1, 33.60 ± 2.7 and 30.22 ± 3.6 in the earthworms exposed to 50, 100 and 200 mg kg\(^{-1}\), respectively.

Table 2 Reproduction test in \( A. \) caliginosa earthworm exposed to different concentrations (mg kg\(^{-1}\) ). \( n = 36 \) earthworms per treatment.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Total no. of cocoons</th>
<th>No. of cocoons/worm</th>
<th>Cocoon inhibition %(^1)</th>
<th>Hatchability %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>151 ± 9</td>
<td>4.40 ± 0.7</td>
<td>–</td>
<td>55.12 ± 6.2</td>
</tr>
<tr>
<td>10</td>
<td>139 ± 10</td>
<td>3.86 ± 1.06</td>
<td>87.72</td>
<td>53.68 ± 4.4</td>
</tr>
<tr>
<td>50</td>
<td>132 ± 12</td>
<td>3.66 ± 0.9</td>
<td>83.18</td>
<td>35.11 ± 4.1(^1)</td>
</tr>
<tr>
<td>100</td>
<td>109 ± 11(^1)</td>
<td>3.02 ± 0.5(^*)</td>
<td>68.63</td>
<td>33.60 ± 2.7(^1)</td>
</tr>
<tr>
<td>200</td>
<td>97 ± 15(^1)</td>
<td>2.69 ± 0.5(^*)</td>
<td>61.13</td>
<td>30.22 ± 3.6(^1)</td>
</tr>
</tbody>
</table>

\(^*\) Statistically significant decrease than the control ( \( p < 0.05 \) ).

\(^1\) Percent of cocoons compared to control.

DNA damage

The baseline of MN frequencies in the coelomocytes was significantly higher (4.67 ± 0.57‰) than that of the gut cells (2.33 ± 0.57‰) (Fig. 3a). In gut cells of earthworms exposed to AgNPs, Bonferroni test, \( p < 0.05 \) showed that the frequency of MN did not depend on the concentration of exposure, whereas the values obtained for all treated groups were significantly higher as compared to values of the control. The frequency of MN in coelomocytes of the exposed groups significantly increased in a dose-dependent manner after treatment with AgNPs compared to the control group ( \( p < 0.05 \) ). Exposure of earthworms to the AgNPs showed an increase in BN in the gut cells and coelomocytes compared to their relative unexposed groups, with slight differentiation among the experimental groups ( \( p < 0.05 \) ) (Fig. 3b). At all tested concentrations, BN frequency was significantly ( \( p < 0.05 \) ) higher in coelomocytes than that in gut cells. The morphological features of the studied nuclear abnormalities are shown in Fig. 4a and b.

Discussion

The purpose of this study was to evaluate the toxicity of AgNPs in soil exposures containing \( A. \) caliginosa earthworm. This earthworm is common and abundant in agriculture soils and therefore, a relevant test organism when considering the side effects of AgNPs on soil organisms. The study was designed to examine mortality, potential physiological and molecular responses, including reproduction, biomass and genotoxicity. Natural soil was used in the present study so that the results were relevant to environmental conditions. It seems that earthworms are generally tolerant of AgNPs. Total 28-d earthworm mortality in the control replicates was nil. According to the validity criteria in the test protocols of OECD (1984) for earthworms, it should be less than 10%, and therefore, this study fully complies with OECD guidelines. The results indicated that AgNPs did not induce significant mortality in \( A. \) caliginosa population at a concentration up to 200 mg kg\(^{-1}\) soil. Similar results were observed in \( E. \) fetida (Shoults-Wilson et al., 2011a) and \( E. \) andrei (Schlich et al., 2012). Schlich et al. (2012) interpreted their data as the earthworms attempted to avoid the contaminated soil during the first day of the experiment and then preferred to remain in the thin food layer spread over the top of test soil. Shoults-Wilson et al. (2011b) noticed that \( E. \) fetida avoid soil contaminated with AgNPs at

Figure 3 Frequencies of micronuclei (a) and bi-nucleated cells (b) of \( A. \) caliginosa earthworms after exposure to different concentrations of AgNPs for 28 days. Different letters indicate significant differences among treatments (one-way ANOVA, followed by Bonferroni multiple comparison test \( p < 0.05 \)).
concentration 6.92 mg kg$^{-1}$. The low toxicity of AgNPs could be attributed to the release of Ag$^+$ ions from the particles or by a slower uptake of AgNPs (Gomes and Soares, 2013). Yang et al. (2012) observed that AgNPs are more toxic than the equivalent mass of dissolved Ag or the generation of reactive oxygen species in studies with a single-cell system (e.g. nitifying bacteria, human cells). Several studies have been carried out on the digestive enzymes of some earthworms (Mishra and Dash, 1980; Lattaud et al., 1998; Trigo et al., 1999). Garvin et al. (2000) and Zhang et al. (1993) reported that cellulase cannot be synthesized in the gut of *Hormogaster elisae* and *Pontoscolex corethrurus* to digest the cellulose. In contrast, Nozaki et al. (2009) identified a novel cellulose-encoding gene (phhEg) in the gut of *Phresetim hilgendorfi* and concluded that the earthworms themselves have the ability to produce the endogenous and functional cellulase. Occurrence of cellulase in the earthworms’ gut denotes its role in the decomposition of plant litter and other cellulosic materials (Dash, 1987). Zhang et al. (2000) found that cellulase activity was 7-fold greater in *E. fetida* than that in guts of *Metaphire guilelmi*. They attributed the difference in cellulase activities of the two various earthworms to their ecological categories and feeding habitats. Urbasik (1990) found that epigeic species had a higher gut cellulase activity than endogeic earthworms that feed on soil less enriched in organic matter. Results of the present study on *A. caliginosa*, the endogeic species, accorded with the results of Hu et al. (2010) in which nanoparticles depressed the cellulase activity resulting in growth inhibition in *E. fetida* earthworms. Biomass alterations could be a good signal of chemical stress, which may link chemical effects to energy reserves and eventually inhibit growth of treated organisms. In the present study, there was a significant decrease in the biomass of the earthworms exposed to AgNPs compared to the control. Seemingly opposing findings are reported in the literature, e.g. Shoults-Wilson et al. (2011c) found no concentration-dependent changes in growth in *E. fetida* caused by AgNPs exposure up to 791.7 mg kg$^{-1}$. Interestingly, Schlich et al. (2012) observed a statistically significant increase in the biomass of the adult *E. andrei* earthworms exposed to AgNPs. They attributed the increase in biomass to the tendency of the earthworms to favor the food layer, which leads to the ingestion of more food and consequently, increases in biomass. Since a decrease in the reproductive potential would affect population density, the sublethal effects of the AgNPs are of prime importance for purposes of ecotoxicological evaluation. The present study showed that in comparison to the control, the inhibition in reproduction ranged from 7.94% at the lowest concentration to 35.76% at the highest concentration. This result is in agreement with that of Schlich et al. (2012) who found a concentration-effect relationship and observed a strong significant inhibition of earthworm reproduction in their test with AgNPs. Quantity and quality of available food could be expected to affect cocoon production. AgNPs could, of course, have an influence over the feeding behavior and thus affect biomass and reproduction indirectly. In this study, the significantly decreased hatchability following exposure to AgNPs occurred in a concentration-dependent manner compared with the control group (Table 2). The facts that cocoons hatched from the contaminated substrates were fewer than those hatched from the control substrates, provided further support for the conclusion that AgNPs may affect cocoon hatchability detrimentally. Cocoon hatchability could therefore be a more reliable endpoint for measuring sublethal effects of certain nanoparticles at a specific concentration than cocoon production. Genotoxicity studies providing the estimation of different DNA damage after exposure to xenobiotics are important for most chemical toxicity testing and risk assessment. Among nuclear contents, DNA is an important target of environmental stress in aquatic and terrestrial organisms (Frenzilli et al., 2001). Previous studies demonstrating changes in DNA caused by pollutants have referred to DNA damage as a biomarker of contamination stress (Reinecke and Reinecke, 2004; Hu et al., 2010; Dobryńska et al., 2014). There are not many genotoxicity papers with nanoparticles, especially regarding *in vivo* effects. Nanoparticles which are smaller than hundred nanometers are able to penetrate cells and to bind macromolecules including protein and DNA (Chen and Mikez, 2005; AshaRani et al., 2009). According to the nuclear abnormalities test results, there was a significant increase in MN and BN in coelomocytes of *A. caliginosa* earthworms exposed to various concentrations of AgNPs. The slight reduction in MN frequency of the earthworm exposed to 50 mg kg$^{-1}$ may be due to the process of continuous renewal of coelomocytes including those which contain MN. The BN of coelomocytes were significantly enhanced at 50, 100, and 200 mg kg$^{-1}$ ($p < 0.05$) than those observed in the gut cells exposed to the corresponding concentrations, suggesting that DNA of coelomocytes had suffered more damage even at the lowest concentration (50 mg kg$^{-1}$). In this study, it appeared that the coelomocytes were more sensitive and appropriate biomarker than gut cells of *A. caliginosa* to AgNPs genotoxicity.

**Conclusion**

The present study shows that AgNPs can have adverse impact on earthworms, but they are not lethal to earthworms with exposure to 50–1000 mg kg$^{-1}$ and abnormal physiological changes occur at various concentrations during the experimental period (28 days). Cocoon production, cocoon hatchability, biomass and cellulase activity in the exposed earthworms significantly decreased compared to the untreated earthworms. In addition, AgNPs can cause genotoxic effects, leading to the elevation of MN and BN frequencies in coelomocytes and gut cells of *A. caliginosa* earthworms.
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References


Impact of AgNPs on \textit{A. caliginosa}


