Toxicity mitigation by N-acetylcysteine and synergistic toxic effect of nano and bulk ZnO to Panagrellus redivivus

Lola Virág Kiss 1 · Zoltán Sávoly 2 · András Ács 3 · Anikó Seres 1 · Péter István Nagy 1

Received: 19 October 2020 / Accepted: 22 January 2021 / Published online: 2 March 2021
© The Author(s) 2021

Abstract
To better understand the nanosize-relevant toxic effects and underlying mechanisms, N-acetylcysteine (NAC), as a mitigation agent, an ionic form of Zn (ZnCl₂), and the binary mixture of ZnO with different particle sizes (15 nm and 140 nm), was used in toxicity assays with the nematode Panagrellus redivivus. The ZnCl₂ concentrations were applied to show the amount of dissolved Zn ions present in the test system. Reactive oxygen species (ROS) measuring method was developed to fit the used test system. Our studies have shown that NAC can mitigate the toxic effects of both studied particle sizes. In the applied concentrations, ZnCl₂ was less toxic than both of the ZnO particles. This finding indicates that not only ions and ROS produced by the dissolution are behind the toxic effects of the ZnO NPs, but also other particle size-dependent toxic effects, like the spontaneous ROS generation, are also relevant. When the two materials were applied in binary mixtures, the toxic effects increased significantly, and the dissolved zinc content and the ROS generation also increased. It is assumed that the chemical and physical properties of the materials have been mutually reinforcing to form a more reactive mixture that is more toxic to the P. redivivus test organism. Our findings demonstrate the importance of using mitigation agent and mixtures to evaluate the size-dependent toxicity of the ZnO.

Keywords Zinc-oxide · N-acetylcysteine · Interaction · Nanomaterial · Nematode · Synergistic

Introduction
The prevalent use of nanotechnology in each part of human life results in an immense release of engineered nanomaterials to the environment. Some metal-based nanomaterials like zinc oxide (ZnO) reached significant importance for their extensive commercial applications. According to Coll et al. (2016) and Kiss et al. (2020), ZnO is the nanomaterial of the greatest concern because of its exhibited high toxicity and predicted exposure concentrations.

After being released to the soil environment, ZnO NPs (zinc-oxide nanoparticles) can be adsorbed onto soil particles, react with organic materials, and even be transported to groundwater. As a result of its increasing released amounts (Gottschalk et al. 2009, Sun et al. 2016) and its potential leaking into pore water, ZnO can pose a risk to soil microfauna, including nematodes. Soil living nematodes are highly exposed to ZnO NP pollution in the environment. There is vast evidence in the scientific literature showing that ZnO NPs are toxic in the applied concentrations (Ma et al. 2011, Wu et al. 2013, Khare et al. 2014, Sávoly et al. 2016, Kiss et al. 2018). Several studies observed significant mortality trends for various nematode species at concentrations as low as 0.32–0.65 mg/l (Khare et al. 2011, Kiss et al. 2018). Nanosize-relevant toxicity varied from laboratory to laboratory. Different test methods and the physical and chemical differences in the materials used may have been lied behind that. In most of the cases, however, ZnO NPs with smaller particle size were more toxic (Ma et al. 2011, Khare et al. 2011). Free-living soil nematodes are part of the food chain, are involved in degradation and remediation processes and have great importance in the global biogeochemical cycles of various
substances (Vinciguerra 1979, Sochová et al. 2006). Therefore, it is crucial to investigate the effects on soil nematodes exposed directly to ZnO NPs, preferably in the most environmentally relevant test medium.

Due to their varied applicability, the release of ZnO NPs is expected to increase, despite the vast amount of data available on potential hazards and risks, making reducing the toxic effects of ZnO NPs a paramount task. A suitable method is to mitigate or even eliminate the negative effects in a way that beneficial properties are maintained, with the help of various mitigating agents. The N-acetylcysteine (NAC) antioxidant can be suitable for this task because NAC is considered an important antioxidant and free radical scavenger by increasing intracellular glutathione levels (Sun 2010) and downregulating AP-1 luciferase activity (Shi et al. 2017). Moreover, thiol groups in NAC reduce free radicals and also provide the chelating site for metals (Flora and Pachauri 2010). Due to these abilities, NAC has been widely used in several research fields for investigating the toxic effects of ZnO NPs (Ma et al. 2014, Wang et al. 2014, El-Shorbagy et al. 2019). Toxicity mitigation effects were successfully observed in the relation of nematodes tested with TiO2 NPs (Wu et al. 2012, 2013), Al2O3 NPs (Li et al. 2012), and also ZnO NPs (Wu et al. 2013). Reports about the environmental effects of NAC are quite scarce, and its effect on the biota is relatively unknown. No safety concerns were described in human and animal studies collected in Bhatti et al.’s (2018) review about the topic. Moreover, they reported reduced apoptosis and oxidative stress. However, NAC can be overdosed and cause severe health problems such as hemolysis, thrombocytopenia, and death (Mahmoudi et al. 2015). NAC also have antibacterial properties; it is able to disperse biofilms of both Gram-negative and Gram-positive bacteria (Nowacka et al. 2018).

The ZnO NP toxicity can result from multiple properties, including photo-induced and regular dissolution of zinc ions, generation of reactive oxygen species (ROS), and other potential particle-specific effects like direct contact between the particles and the cells of an organism (Brunner et al. 2006, Khare et al. 2014, Starnes et al. 2019). Toxicity mitigation effects were successfully observed in the relation of nematodes tested with TiO2 NPs (Wu et al. 2012, 2013), Al2O3 NPs (Li et al. 2012), and also ZnO NPs (Wu et al. 2013). Reports about the environmental effects of NAC are quite scarce, and its effect on the biota is relatively unknown. No safety concerns were described in human and animal studies collected in Bhatti et al.’s (2018) review about the topic. Moreover, they reported reduced apoptosis and oxidative stress. However, NAC can be overdosed and cause severe health problems such as hemolysis, thrombocytopenia, and death (Mahmoudi et al. 2015). NAC also have antibacterial properties; it is able to disperse biofilms of both Gram-negative and Gram-positive bacteria (Nowacka et al. 2018).

The ZnO NP toxicity can result from multiple properties, including photo-induced and regular dissolution of zinc ions, generation of reactive oxygen species (ROS), and other potential particle-specific effects like direct contact between the particles and the cells of an organism (Brunner et al. 2006, Khare et al. 2014, Starnes et al. 2019). Moreover, size relevant effects of ZnO nanoparticles (NPs) and excess release of Zn2+ can also result in the generation of ROS. Even so, most studies point to ZnO NPs dissolution to ionic Zn playing the most significant role in eliciting toxicity (Ma et al. 2014, Wang et al. 2016, Sávoly et al. 2016, Lead et al. 2018).

The wide use of diverse engineered nanomaterials can also lead to the release of different mixtures of nanomaterials into the environment. However, little is known about the combined toxicity of various nanomaterials (Mott et al. 2007, Jafari et al. 2011, Li et al. 2011, Tong et al. 2014, 2015). No information was found on the combined toxicity of ZnO NPs and its bulk form, although exposure to such a complex is a realistic scenario as well. Furthermore, experiments dealing with a mixture of differently sized nanomaterials can also elucidate the size relevant effects of them.

In the present study, the main aim was to better understand the size relevant toxic effects of the ZnO on the nematode Panagrellus redivivus and mechanisms behind them. The usage of a mitigation agent, an ionic form of Zn, and a binary mixture of two ZnO agents with different sizes helped to explore size-dependent toxicity of the ZnO particles. In this research, we hypothesized that the nano-relevant toxic effects could be identified through mitigation assays with NAC, as it influences both the ionic and the ROS induced toxicity. It was assumed that ZnCl2 in the concentration series set by the dissolution rate of the ZnO compounds could elucidate the role of ionic toxicity in the toxicity mechanism of ZnO NPs. Furthermore, we hypothesized that the environmentally relevant combination of nano and bulk ZnO particles (15 and 140 nm) has different effects on used test species as compared with the same particles applied separately. For the aim of better clarifying the difference between compound effects, combined toxicity was also tested with the addition of NAC. In addition, we developed a technique to measure ROS production in a way that fits to the test system we used.

Materials and methods

Particle size characterization and sample preparation

Two ZnO particles of different nominal particle sizes were applied as (i) 10–30 nm (referred to as 15 nm average particle size) with purity 99+% and (ii) 80–200 nm (referred to as 140 average particle size) ZnO with purity 99.9%. Both materials were purchased from US Research Nanomaterials, Inc. The particle shape of the 15 nm ZnO was nearly spherical, and that of the 140 nm ZnO was irregular. ZnCl2 was used as a free ionic positive control. For the mitigation experiments, N-acetylcysteine (NAC) was the mitigation agent. Both ZnCl2 and NAC were purchased from Sigma-Aldrich.

The primary particle size and particle morphology were measured by scanning electron microscopy (SEM, FEI Quanta 3D, Eötvös Loránd University, Hungary). The materials were examined individually in previous experiments (Kiss et al. 2018). Both ZnO compounds contained nano and bulk particles; however, based on the definition of nanomaterials—the particle size of at least half of the particles in the number size distribution must measure 100 nm or below—the 15 nm was in nanosize range and the 140 nm was in bulk size range (Kiss et al. 2018). Images were recorded from the binary mixture of the two, and the average size and size distribution were ascertained by measuring approximately 100 particles from representative images by the ImageJ software.
software package. The ZnO particles were also checked with the addition of N-acetylcysteine after 24 h incubation by SEM.

New stock suspensions were made from the 15 and 140 nm and the binary mixture of them (referred to as Mix or mixture) (160.64 mg Zn/l) and ZnCl₂ (67.68 mg Zn/l) powders with Milli-Q water before each experiment. The mixture contained an equal amount (50–50%) of ZnO particles with an average size of 15 and 140 nm, respectively. The stock suspensions were dispersed by sonication for 20 min every time. Tested concentrations were chosen based on our previous experiments (Kiss et al. 2018). Environmentally relevant concentration of ZnO NPs was about 1.52–21 μg/l in 2016 (Sun et al. 2016); however, this value is increasing day by day due to the huge amount of released ZnO NPs to the environment. The concentration series were prepared from the stock immediately after sonication and added to the test media. They were set up based on pre-tests of our research group. No lethal effects were observed in the environmentally relevant concentration range, so higher values were used to get the desirable effects.

**Dissolution of ZnO nanoparticles**

The dissolution of materials in Milli-Q water (from 5 ml stock, 10.4 mg Zn/l) after 24 h of incubation time in a dark thermostat chamber was evaluated by centrifugation, followed by chemical analysis of complex supernatant zinc using inductively coupled plasma atomic emission spectrometry (ICP-AES, Horiba Jobin-Yvon Activa-M, SZIE, Hungary) (Ma et al. 2014). Dissolution was assessed for pure 15, 140 nm, mixture, and with the addition of NAC with all the mentioned compounds. Zn particles held in a complex by NAC were also included in the measurement.

**The mitigation effect of N-acetylcysteine and toxicity of ZnCl₂ compared with ZnO NPs on Panagrellus redivivus**

To facilitate the evaluation of results, an easily and quickly operated test system was chosen: a dose-response study with a bacterivore nematode, *Panagrellus redivivus*. *P. redivivus* can be found in a variety of nutrient-rich habitats such as soil, rotting fruits, insects, wheat paste, or beer yeast. It is an excellent species for studying the toxic effects of nanomaterials being easy to work with, having a high reproduction rate, and showing relatively minor differences from *Caenorhabditis* elegans, the most commonly used nematode species, in cell lineage (Sternberg and Horvitz 1982).

Toxicity assays were carried out based on modifications prompted by previously published methods (Ma et al. 2014, Hrčcs et al. 2018, Kiss et al. 2018). In a 96-well microplate (Bioster S.p.A., Italy), 5 adult nematode females were placed in each unit for testing acute mortality in Milli-Q water test media. The tests were performed with pure nano, bulk ZnO particles and ZnCl₂, and with added NAC (5 mg/l concentration). The used NAC concentration was based on previous research of Ma et al. (2014) and Wu et al. (2013). Applied concentrations for ZnO and ZnCl₂ compounds were 0.32, 0.63, 1.26, 2.51, 5.02, and 10.04 mg Zn/l and 0.13, 0.26, 0.53, 1.06, 2.12, and 4.23 mg Zn/l, respectively. The ZnCl₂ concentrations were set up based on the measured mean dissolution rate. Four replicates for each concentration and control were applied. Furthermore, a negative control (320 μl Milli-Q water) and a positive control containing NAC (160 μl Milli-Q water and 160 μl NAC suspension) were also set up.

A group of animals was randomly sampled from the stock culture into a counter filled with Milli-Q water for pure toxicity assay or filled with 10 mg/l NAC suspension for mitigation assay. From here, female specimens were selected with a pipette. Females are usually bigger and more frequent than males. The females’ vulva opening is located in the midline of the dorsal side, while the male orifice is located at the posterior end of the body, where the short, bent mating spike (spicule) can be easily recognized. Before the placement of the animals, 100 μl of Milli-Q water or NAC solution (10 mg/l concentration) was pipetted onto microplates to create a wet environment. Animals were then relocated with 2×30 μl Milli-Q water or NAC suspension into each well. Since the wells contained liquid by the time the solutions were added, the solutions with twofold concentrations were prepared before the experiments. After that, 160 μl of the test solution or Milli-Q water, in the case of the control group, was added to the test system to reach the final amount of 320 μl liquid. This way, the achieved final concentration of NAC was 5 mg/l. The microplates were incubated in a thermostat chamber under dark conditions at 20 ± 1 °C. Surviving specimens were counted after 24 h under a transmission stereomicroscope (Olympus SZH 10).

**Interaction between ZnO nanoparticles**

The *P. redivivus* acute mortality test was used in the experiments investigating the interaction between nano and bulk ZnO particles. The concentration series were made from the 50–50% mixture of ZnO particles with an average size of 15 nm and 140 nm. Experiments were carried out the same way as described in “The mitigation effect of N-acetylcysteine and toxicity of ZnCl₂ compared with ZnO NPs on Panagrellus redivivus.” In preliminary experiments, only the three highest concentrations (2.51; 5.02 and 10.04 mg/l Zn) were tested on their own and also with the addition of NAC to see if a valid result can indeed be obtained. Since there was a great difference between the toxicity of the mixture and that of the original substances, mainly with the addition of NAC, the entire experiment was repeated using the whole concentration series (0.32, 0.63; 1.26; 2.51; 5.02 and 10.04 mg/l Zn). After
24 h of incubation, the microplate was examined under a stereomicroscope. Milli-Q water was used as a negative control.

**Measuring the intracellular reactive oxygen species generation**

Intracellular ROS production was measured using aminophenyl fluorescein (APF; Thermo Fisher) assay. APF is a relatively new and more specific indicator for ROS measurement than the hitherto used dyes (such as 2′,7′-dichlorodihydrofluorescein diacetate). It is more tolerant of light-induced oxidation and becomes fluorescent in the presence of hydroxyl radical (OH·), peroxynitrite anion (ONOO−), and hypochlorite anion (OCl−) (Nagano 2009). APF reacts three times more strongly to hydroxyl radicals than to other ROS radicals, e.g., superoxide (·O2−), hydrogen peroxide (H2O2), or singlet oxygen (¹O2) (Setsukinai et al. 2003). Several studies have suggested that the photocatalytic and antibacterial properties of nanoparticle oxides are mainly due to free and surface-bound hydroxyl radicals, although superoxide and hydrogen peroxide play a vital role in the processes (Ma et al. 2013). Thus, we can assume that OH· production is a representative of total ROS formation by ZnO NPs.

No standard guideline is available for measuring intracellular ROS with APF. Therefore, a modified method was used based on other ROS measuring methods (Wang et al. 2018, Sarasija and Norman 2018, Yoon et al. 2018). In those studies, *Caenorhabditis elegans* was the tested nematode species. The two different nematode species length is almost the same (approx. 1 mm). The diameter for *P. redivivus* on average is 50 μm (Sautter et al. 2007), while for *C. elegans*, it can vary between 45 and 80 μm (Maguire et al. 2011, Pallikaras and Tavermanakis 2013, Desta et al. 2017). Therefore, *P. redivivus* was an adequate choice to replace *C. elegans* in this experiment. The measurement was set up similarly to the toxicity assay except the exposure time being only 3 h, and for all six types of compounds, three concentrations were tested: 2.51, 5.02, and 10.04 mg Zn/l (in Milli-Q water). Instead of the 96-well microplate, the test compounds were placed inside well microplate, the test compounds were placed inside 5.02, and 10.04 mg Zn/l (in Milli-Q water). Instead of the 96-well microplate, the test compounds were placed inside well microplate, the test compounds were placed inside

**Data analysis**

Median lethal and effective concentrations (LC50, EC50) and associated confidence intervals (95% CI) were calculated by Probit analysis using the ToxRat program (Light Version 2.08) (TOXRATLIGHT2.08 n.d.). This was repeated with the Microsoft Excel Solver plug-in to verify the results (Microsoft Corporation 2010). For the statistical analysis, R Statistics 3.5.2. program was used (RCoreTeam 2013). In those experiments where assumptions were met (balanced standard deviation, normal distribution), the p values were calculated by one-way and two-way ANOVA (F) with Tukey’s honestly significant difference (t) post hoc test. If the conditions were not met, linear model (LM) was used when examining the relationship of concentrations to control. For comparing different curves, generalized least squares (GLS) technique and, in some cases, an interactional linear model (ILM) were used. The GLS model can also be used well for unequal variances. Normality was checked in all cases with the Shapiro-Wilk test.

**Results**

**Particle characterization by SEM**

The average size for the mixture, based on measuring the diameter of 100 particles, was 130 ± 118 nm (n = 100). As revealed by looking at the mixture size distribution (Fig. 1e), the most common particle size by mode is 30 nm, but also larger aggregates (877 nm) may be present. In addition, as the picture clearly shows, both forms (spherical, irregular) are present in samples (Fig. 1c, d). The binary mixture of 15 nm and 140 nm ZnO individually and all three materials (15 nm, 140 nm, binary mixture) were investigated with N-acetylcysteine (Fig. 1). The addition of NAC did not
significantly change the size or morphology of the materials (Fig. 1a, b, c, d). Coating around the materials by the NAC was not visible by SEM.

**Zinc ion dissolution**

There was no significant difference in the complex Zn$^{2+}$ dissolution associated with mitigation studies between the two pure ZnO particles of different sizes (Table 1). On the other hand, the dissolution of the mixture (pure and with the addition of NAC) (mg/l) is shown in Table 1.

**Table 1** The dissolution of 15 nm ZnO, 140 nm ZnO, and the mixture (pure and with the addition of NAC) (mg/l)

|            | 15 nm ZnO | 140 nm ZnO | Mixture  |
|------------|-----------|------------|---------|
| Pure       | 4.13±0.01 | 4.31±0.09  | 5.78±0.07 |
| Addition of NAC | 5.87±0.05 | 5.30±0.05  | 6.01±0.04 |
hand, significantly more zinc ions (~30–40%) were dissolved from the binary mixture of the two substances (GLSDF:2.6, 15 nm, \( t = 40.42, p < 0.001 \); 140 nm, \( t = 22.33, p < 0.001 \)). The measured data did not follow normal distribution (W = 0.80; \( p < 0.05 \)). An increase in zinc dissolution was also observed with the addition of NAC to all test materials (15 and 140 nm ZnO, 20–30%; mixture, 4%). There was a significant difference for 15 nm (GLSDF:2.6, \( t = -58.10, p < 0.001 \)) and 140 nm ZnO (GLSDF:2.6, \( t = -16.65, p < 0.001 \)) and a less prominent but also significant difference for the mixture (GLSDF:2.6, \( t = -4.04, p < 0.05 \)) compared with the dissolution values of untreated pairs of materials. Furthermore, in all cases, significant difference was found between the NAC-treated compounds (GLSDF:2.6, 15 nm vs. 140 nm, \( t = 13.96, p < 0.001 \); 15 vs. Mix, \( t = 2.77, p < 0.05 \); 140 nm vs. Mix, \( t = 15.28, p < 0.001 \)). For the 15 nm and the mixture, this difference was relatively slight (~3%).

The mitigation effect of N-acetylcysteine and toxicity of ZnCl₂ compared with ZnO NPs on Panagrellus redivivus

The addition of NAC significantly reduced the toxic effects of 15 nm ZnO (GLSDF:4.60, \( t = -4.44, p < 0.001 \)) (Fig. 2a). In the case of 140 nm ZnO, a slight mitigating effect was observed (GLSDF:4.56, \( t = -3.34, p < 0.05 \)) (Fig. 2b). The mitigating effect showed a decreasing tendency above 2.51 mg/l and 1.26 mg/l for 15 nm and 140 nm ZnO, respectively. In contrast, when using ZnCl₂, mitigation was only observed if the highest concentration (4.23 mg/l Zn) was excluded (LM DF:3.44, \( t = 2.07, p < 0.05 \)) (Fig. 2c). The two different particle sizes were also affected differently by mitigation treatment. Notwithstanding the two highest concentrations, where none of the substances had a mitigating effect, a greater decrease in toxicity was observed in the presence of 15 nm ZnO than at 140 nm ZnO (GLSDF:4.44, \( t = 2.09, p < 0.05 \)). These results are also apparent from the LC₅₀ values (Table 2). Our data did not follow normal distribution (W = 0.85; \( p < 0.05 \)).

When testing the pure materials, no particle size-dependent toxicity was observed. ZnCl₂ was significantly less toxic than the two ZnO particles in the concentration series based on the dissolved zinc content (GLSDF:4.56, 15 nm, \( t = -3.16, p < 0.05 \); 140 nm, \( t = -3.16, p < 0.01 \)).

Interaction between ZnO nanoparticles

A synergistic increase in toxicity was observed when using the binary mixture of the two particle-sized ZnO (Fig. 3a). Compared with the 15 nm ZnO particle, the mixture proved to be significantly more toxic, even when used without the addition of NAC (GLSDF:4.56, 15 nm, \( t = -2.05, p < 0.05 \)). Moreover, this difference in toxicity was further increased by the addition of NAC. Thus, in that case, the toxic effect of the mixture was stronger than both of the used ZnO size type alone (GLSDF:4.56, 15 nm, \( t = -3.33, p < 0.01 \); 140 nm, \( t = -2.50, p < 0.01 \)) (Fig. 3b). There was no statistically demonstrable mitigating effect of the antioxidant on the mixture when studying all of the concentrations, although in up to 1.26 mg/l Zn, a slight decrease in toxicity was observed as compared with the mixture without NAC (GLSDF:4.28, \( t = -2.33, p < 0.05 \)) (Fig. 2d). This can also be supported by LOAEC values (pure, 0.31 mg/l; NAC, 1.26 mg/l). The

![Fig. 2](https://example.com/fig2.png)

**Fig. 2** Mitigating effect of N-acetylcysteine on P. redivivus in the presence of 15 nm ZnO (a), 140 nm (b), ZnCl₂ (c) and mixture of 15 nm + 140 nm ZnO (d) after 24 h exposure. Four replicates were used per concentration. Significance levels: *\( p < 0.05 \); **\( p < 0.001 \). Linear model shows the difference between the control (0 mg/l) and the individual concentrations.
mitigating effect was less evident for the mixture than for the two ZnO particles alone, as can be seen from the LC50 values (Table 2).

**Evaluation of reactive oxygen species generation method**

The newly developed method was repeated six times to make sure it was functional. In 5 out of 6 experiments, the ROS content was between 0.374 and 0.425 μM/mg in the Milli-Q water control groups. A peak value of 0.716 μM/mg was experienced only in one case. Control values per treatment are shown in Table 3. When comparing the Milli-Q water control values, SD was 0.018 and CV 4.6%, excluding the peak value with outlier analysis by graphic representation (0.716 μM/mg) (see Appendix A for Q-Q plot).

**Measuring the intracellular reactive oxygen species generation**

Among the three measured pure materials, the highest ROS production was observed for the mixture and the lowest for the

| Pure | 15 nm | 140 nm | ZnCl2 | Mixture |
|------|-------|--------|-------|---------|
|      | 1.85  | 2.66   | 4.96  | 0.65    |
| (CI 95%: 1.1–3.13) | (CI 95%: 1.6–4.53) | (CI 95% n.d.) | (CI 95% n.d.) |
| Addition of NAC | 14.091 | 10.725 | 5.80  | 1.62    |
| (CI 95% n.d.) | (CI 95% n.d.) | (CI 95% n.d.) | (CI 95% n.d.) |
Therefore, 140 nm ZnO was significantly different from the mixture (LMDF: 3.8, t = 8.930, p < 0.001) and 15 nm ZnO (LMDF: 3.8, t = 5.322, p < 0.01) (Fig. 4a). A concentration-dependent ROS increase and a significant difference were observed in the case of 15 nm ZnO and the binary mixture (LMDF: 3.8, t = 4.440, p < 0.01). In the presence of NAC, a completely different tendency was observed from when the materials were applied alone (Table 5), as there was no significant difference between the materials (Fig. 4b). Both for 140 nm ZnO and the mixture, a sharp increase in ROS production was observed at 2.51 mg/l Zn concentration, followed by a substantial decrease. For the data obtained during the reactive oxygen species measurement, a normal distribution was observed (W = 0.94; p > 0.05).

**Discussion**

**The mitigation effect of N-acetylcysteine on Panagrellus redivivus**

So far, the mitigating effect of N-acetylcysteine on nano-metal oxides has been demonstrated mainly in a human cell test system (Wang et al. 2014, Liu et al. 2017, El-Shorbagy et al. 2019). Wu et al. (2013) have shown the mitigation effect of NAC (5 mM) on ZnO NPs (50 μg/l) in another nematode test species. Their results were comparable with our findings. We also experienced 20% mitigation in 0.63 mg/l concentration in the case of 15 nm ZnO on the nematode P. redivivus, similarly as stated in Wu et al. (2013). In higher concentrations (1.26–10.04 mg/l Zn), NAC successfully reduced the toxic effects of both 15 nm and 140 nm ZnO particles on average by 50% and 30%, respectively.

Particle size-dependent mitigation is less known since bulk controls were lacking in most studies (Wang et al. 2014, Yang and Ma 2014, El-Shorbagy et al. 2019). Liu et al. (2017) compared the effects of two nano-sized ZnO particles (18.5 ± 1.2 nm and 47.1 ± 5.1 nm ZnO) on the human neuroblastoma SHSY5Y cell line. When tested alone, they found stronger toxic effects from the smaller particle size ZnO, and the addition of NAC reduced the toxicity of both particle sizes. Mitigation was the strongest at concentrations below 40 mg/l.

| 15 nm | 140 nm | Mixture |
|-------|-------|--------|
| Pure  | 0.698 | 0.380  | 0.399  |
|       | 0.734 | 0.374  | 0.420  |
| Addition of NAC | 0.399 | 0.425  | 0.425  |
|       | 0.420 | 0.415  | 0.415  |

The peak values were indicated in italics.

140 nm ZnO (Table 4). Therefore, 140 nm ZnO was significantly different from the mixture (LMDF: 3.8, t = 8.930, p < 0.001) and 15 nm ZnO (LMDF: 3.8, t = 5.322, p < 0.01) (Fig. 4a). A concentration-dependent ROS increase and a significant difference were observed in the case of 15 nm ZnO and the binary mixture (LMDF: 3.8, t = 4.440, p < 0.01). In the presence of NAC, a completely different tendency was observed from when the materials were applied alone (Table 5), as there was no significant difference between the materials (Fig. 4b). Both for 140 nm ZnO and the mixture, a sharp increase in ROS production was observed at 2.51 mg/l Zn concentration, followed by a substantial decrease. For the data obtained during the reactive oxygen species measurement, a normal distribution was observed (W = 0.94; p > 0.05).

**Table 3** Measured ROS (μM/mg) control values

|          | 15 nm | 140 nm | Mixture |
|----------|-------|--------|---------|
| Pure     | 0.698 | 0.380  | 0.399   |
|          | 0.734 | 0.374  | 0.420   |
| Addition of NAC | 0.399 | 0.425  | 0.425  |
|          | 0.420 | 0.415  | 0.415  |

The peak values were indicated in italics.
l, above which a reduced effect was observed, as well as a slight difference in toxicity between the two particle sizes. In the case of the larger particle size, the mitigation was smaller, similarly to present studies, where a milder effect was observed in the case of 140 nm ZnO. These results are supported by our earlier findings where we found 140 nm ZnO to be more toxic in the presence of soil solution (to the nematode *P. redivivus* and artificial soil (to the springtail *Folsomia candida*) (Kiss et al. 2018). From the two substances applied in our study, the larger particle size toxicity was more difficult to mitigate by the NAC. This is presumably because the two materials had different surface charge densities, distributions, and electrical potentials due to their different size (Abbas et al. 2008, Holmberg et al. 2013) as well as different morphology (Andelman 1995).

Liu et al. (2017) also compared the effects of nano and bulk ZnO with ZnCl₂ similarly to our experiments. Although mitigation was observed in this case at two lower concentrations (122.9 μM and 245.7 μM ZnCl₂), above this, cell survival rate decreased below 10% even with the addition of NAC. Therefore, NAC had a much weaker effect on ZnCl₂ than on the nanoforms, similarly to our results. According to the present experiments, NAC has a lower effect on ionic toxicity.

No specific reference has been found regarding the effect of N-acetylcysteine on the dissolution of Zn, but the chelating properties of the material have been described in several studies (Rossignol 2005, Flora and Pachauri 2010, Giampreti et al. 2016). Solubility values in the present study were significantly higher when NAC was added, as here the total Zn content was measured. While from the two tested ZnO particles alone, approximately the same amount of Zn dissolved, after the addition of NAC, significantly lower values were obtained from ZnO with the larger particle size. Our experiments have shown that N-acetylcysteine addition reduces the toxicity of 15 and 140 nm ZnO particles on *P. redivivus*.

Based on our test results, NAC can be favorable as a mitigation agent; however, before the environmental applications, the effects of NAC require more research with relevant test systems.

### Elucidate the role of ionic toxicity in the toxicity mechanism of ZnO NPs

The concentrations of ZnCl₂ used in these experiments represented the amount of dissolved Zn ions present in the test system. It can be seen that behind the toxic effects of ZnO particles, there could be additional properties apart from the toxicity of dissolved ions, like spontaneous and size relevant ROS generation and interaction between the cell and the particle. These results are in agreement with the findings of Song et al. (2010) who showed that the amount of Zn²⁺ dissolved and the amount of ROS generated could not induce the degree of cytotoxicity that was observed. Thus, it is assumed
that additional toxicity factors must be present to produce the observed effects.

**Synergistic toxicity increases due to mixing the ZnO particles**

Studies have also shown an increase in synergistic toxicity when mixing two different nanomaterials, e.g., ZnO NP + AG NP (Jafari et al. 2011), Au NP + Pt NP (Mott et al. 2007), and Ag NP + TiO₂ NP (Li et al. 2011). However, a reduction in toxicity was detected when nanoparticulate ZnO and TiO₂ were mixed, and this effect was explained by the Zn²⁺ adsorption on the TiO₂ surface and thus became less available for test animals (Tong et al. 2014). In the present study, the binary mixture of two different particle sizes ZnO showed a substantial increase in toxicity, in the Zn dissolution rate and the ROS production. The increase in toxicity was also observed with the addition of N-acetylcysteine, as the lowest mitigating effect was detected in the case of the mixture. There is no literature available on testing mixture toxicity with the addition of NAC. According to Liu et al. (2017) and the present experiments with NAC, the antioxidant has less influence on the toxic effects caused by dissolved ions. The measured amount of dissolved ions was 1.5 times higher in the mixture than in the two substances individually. This could be one reason behind the milder mitigation effect on the mixture.

SEM images of the mixture compared with the pure compounds images (Kiss et al. 2018) confirm that both materials are present in the new mixture and a new ZnO with an average particle size between the two other materials (130 ± 118 nm),
with both spherical and irregular particles, has been generated. Particle distributions also show that approximately 30% of the particles found in the 140 nm ZnO are in the nano-size range (37–97 nm, most often 37 nm) (Kiss et al. 2018), so the ratio of nanoparticles has increased above 50% due to the mixing of the two materials. Moreover, larger particles are believed to have a dispersing effect on the test system. In the mixture, particles aggregated more with each other—due to the different charge distribution—than with particles of their size group. As a result, small particles aggregated on the surface of 140 nm ZnO particles fixed the large surface, increasing dissolution, reactivity, and thus toxicity. This is also probably because of the different surface properties (Abbas et al. 2008, Holmberg et al. 2013) and morphology (Andelman 1995). Therefore, by mixing the particles, the different adverse effects of the two materials (15 nm—particle size, 140 nm—irregular morphology) reinforce each other, which may be the reason for the stronger toxic effects.

**Evaluation of reactive oxygen species generation method**

The method suggested by literature proved to be unsuitable for measuring the produced ROS in our test system. Sarasija and Norman (2018) recommend removing supernatant from above the animal with 100 μl of liquid remaining in the tubes. This is not feasible since the amount of liquid remaining in the Eppendorf tube cannot be accurately determined. Even though animals settle to the bottom of the tube, for such a small amount, it is inevitable to enter the pipette. Yoon et al. (2018), on the other hand, suggests a more feasible method by pipetting the animals onto a glass slide. However, the recommended volume of 10 μl was very low for the 40 animals used in our experiments. As based on our own experience, the animals can be moved with 80 μl of liquid. In both protocols and Wang et al. (2018) suggest adding an indicator to the sample after lysis, but in our tests, it was found that too much time elapses between incubation and measurement. Consequently, animals still alive can degrade the produced reactive oxygen species. The APF was added immediately after the test end and lysed with, thus preventing the breakdown of ROS. After these changes, the method became usable, and protein and fluorescence measurements of the samples were successful.

During the method development, we managed to modify the ROS measurements described by Sarasija and Norman (2018), Wang et al. (2018), and Yoon et al. (2018), so they would be usable and reproducible with the test species and indicator material used in the present study. Without improvements, we failed to use the method. Subsequently, we applied the refined method successfully several times. In the future, it can be used to measure the induced intracellular reactive oxygen species with APF indicator in the P. redivivus and probably in comparable test species.

**Concentration reactive oxygen species production**

Concentration-dependent production of reactive oxygen species is generally detected in ZnO NPs assays (Xia et al. 2008, Song et al. 2010, Liu et al. 2017, Huang et al. 2019). Compared with other metal oxides, H2O2 production is medium dependent, but superoxide production was always the highest in the case of ZnO NPs (Xia et al. 2008). In most instances, comparisons of nano and bulk ZnO were found to have a visible particle size-dependent effect; ZnO NPs induced higher ROS generation (Song et al. 2010, Liu et al. 2017) than their bulk counterparts. In the present experiments, the highest amount produced was found after 3 h of exposure time in the mixture, and the lowest appeared in the 140 nm ZnO. When NAC was added, ROS were produced to a much greater extent in the case of 140 nm and the mixture than independently. In literature, ROS production is generally reduced by NAC (Wang et al. 2014). It is hypothesized that NAC-induced GSH production has not yet begun during the 3-h ROS exposure (Farbiszewski et al. 2000), so the decrease was not studied. Further experiments should be performed with longer exposure times (6 and 12 h).

**Summary of discussion**

*P. redivivus* was sensitive to both 15 and 140 nm ZnO treatment. Our studies have shown that N-acetylcysteine can mitigate the toxic effects of both studied particle sizes. From the applied two particle sizes, 140 nm ZnO toxicity was found to be harder to mitigate by NAC. This can be explained by morphological differences (Iswarya et al. 2015, Tong et al. 2015), the difference in charge distribution (Andelman 1995), and the fact that N-acetylcysteine was less able to make complexes with this material than with smaller particle-sized one, likely also due to morphological differences. The NAC had less mitigation on the toxic effect of zinc ions (Liu et al. 2017). This was possibly one of the reasons why lower mitigating effects were found in the case of the mixture of the two substances, where the solubility was significantly higher. The ZnCl2 concentrations were used to represent the amount of dissolved Zn ions present in the test system, which proved that other particle size-dependent toxic effects are also important in ZnO NP toxicity besides dissolution. Previous findings (Song et al. 2010) are supported by our results. When the two materials were applied in binary mixtures, the toxic effects increased significantly. Besides, the dissolved zinc content and the ROS generation also increased. It is assumed that the chemical and physical properties of the materials (several smaller particles—higher bioavailability, increased toxicity from a fixed, large surface area, morphological aspects) have
been mutually reinforcing each other to form a much more reactive mixture that is more toxic to *P. redivivus* test organism. Studies have shown that 15 nm ZnO alone can generate higher amounts of ROS and dissolved ions than 140 nm ZnO.

On the other hand, such different trends in toxicity can be changed by influencing some parameters of the test system in order to neutralize the toxic effects of 15 nm ZnO. This happened in present study when adding NAC as a mitigation agent or in Kiss et al. (2018) when soil solution as an alternative test media was applied. In both cases, 140 nm ZnO will immediately become more toxic than its counterpart with a smaller size. This is presumably due to the irregular particle morphology. ROS production was induced by all used materials measured by the modified method. When testing the substances by themselves for ROS production, it was the highest in the mixture and the lowest in the 140 nm ZnO. With the addition of NAC, due to the low toxicity of the substance itself, the control ROS values were somewhat higher than with the individually tested compounds. Higher exposure times are required for the assay for the substance to exert its effect (Farbiszewski et al. 2000).

**Conclusion**

Our findings testify the need to investigate the mechanism behind ZnO nanoparticles toxicity. Toxicity mitigation by special antioxidants is a new way to decrease the environmental risk of nanoparticles. As proved by our study, N-acetylcysteine can mitigate the effect of ZnO NPs on nematodes. It is also important to investigate the same compound in different test systems, as only one way of testing can lead to false assumptions. In the present study, we only experienced size related toxicity difference with the addition of NAC. Moreover, our findings highlight the role of dissolution unrelated ROS production in toxicity. Our study suggests taking into consideration the interaction between compounds as a hazard and risk assessment for nanomaterials. Future studies need to focus more on morphology and charge density distribution of the researched nanomaterials.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-12674-7.

**Acknowledgements** We would like to express our thanks to Eötvös Loránd University, Central Research and Instrument Center for their help with scanning electron microscopy and to the Department of Chemistry at Szent István University for their help with inductively coupled plasma atomic emission spectroscopy. Special thanks are due to Professors Zoltán Hórvölgyi, Miklós Mézes, and Gábor Bakonyi for their advice.

**Author contribution** All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Kiss, L.V. Besides Kiss, L.V., Sávoly, Z., and Ács, A. took part in the method development, and Ács, A. also took part in the measuring process of the produced reactive oxygen species. Seres, A. and Nagy, P. I. coordinated and supervised the whole research process. The first draft of the manuscript was written by Kiss, L.V., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** Open access funding provided by Szent István University. This study was supported by the New National Excellence Program under Grant (ÚNKP-18-3-III-SZIE-7) and by 2017-1.3.1-VKE-2017-00001 grant.

**Data availability** The data that support the findings of this study are available online (https://doi.org/10.6084/m9.figshare.12933284.v1) and from the corresponding author (lolavirag.kiss@gmail.com).

**Declarations**

**Ethics approval and consent to participate** Research involving invertebrates, like nematodes, does not require ethical approval from the ethics committee. Consent to participate is not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare that they have no competing interests.

**References**

Abbas Z, Labbez C, Nordholm S, Ahlberg E (2008) Size-dependent surface charging of nanoparticles. J Phys Chem 112:5715–5723. https://doi.org/10.1021/jp709667u

Andelman D (1995) Electrostatic properties of membranes: the Poisson-Boltzmann theory. In R Lipowsky, E Sackmann (eds) Handbook of biological physics. Elsevier, pp 603–642

Bhatti J, Nascimento B, Akhtar U, Rhind SG, Tien H, Nathens A, and da Luz LT (2018) Systematic review of human and animal studies examining the efficacy and safety of N-acetylcysteine (NAC) and N-acetylcysteine amide (NACA) in traumatic brain injury: impact on neurofunctional outcome and biomarkers of oxidative stress and inflammation. Front Neurol 8: https://doi.org/10.3389/fneur.2017.00744

Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, Brunink A, Stark WJ (2006) In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol 40:4374–4381. https://doi.org/10.1021/es052069i

Coll C, Notter D, Gottschalk F, Sun T, Somc C, Nowack B (2016) Probabilistic environmental risk assessment of five nanomaterials (nano-TiO2, nano-Ag, nano-ZnO, CNT, and fullerenes). Nanotoxicology 10:436–444. https://doi.org/10.3109/17435390.2015.1073812

Desta IT, Al-Sharif A, AlKhariheb N, Mustafa N, Orozaliev A, Giakoumidis N, Gunsalus KC, Song YA (2017) Detecting and trapping of a single C. elegans worm in a microfluidic chip for automated microplate dispensing. SLAS Technol 22:431–436. https://doi.org/10.1177/2211068216669088

El-Shorbagy HM, Eissa SM, Sabet S, El-Ghor AA (2019) Apoptosis and oxidative stress as relevant mechanisms of antitumor activity and genotoxicity of zno-NPs alone and in combination with N-acetyl cysteine in tumor-bearing mice. Int J Nanomedicine 14:3911–3928. https://doi.org/10.2147/IJN.S204757
Farbiszewski R, Wittek A, Skrzylewska E (2000) N-acetylcysteine or trolox derivative mitigate the toxic effects of methanol on the antioxidant system of rat brain. Toxicology 156:47–55. https://doi.org/10.1016/S0300-483X(00)00333-4

Flora SJS, Pachauri V (2010) Chelation in metal intoxication. Int J Environ Res Public Health 7:2745–2788. https://doi.org/10.3390/ijerph7072745

Giampreti A, Lonati D, Ragghianti B, Ronchi A, Petrolini VM, Vecchio S, Locatelli CA (2016) N-acetyl-cysteine as effective and safe chelating agent in metal-on-metal hip-implanted patients: two cases. Case Rep Orthop 2016:1–7

Gottschalk F, Sonderer T, Scholz RW, Nowack B (2009) Modeled environmental concentrations of engineered nanomaterials (TiO2, ZnO, Ag, CNT, fullerene) for different regions. Environ Sci Technol 43:9216–9222. https://doi.org/10.1021/es9015553

Holmberg JP, Ahlberg E, Bengten M, Hasselöv M, Abbas Z (2013) Surface charge and interfacial potential of titanium dioxide nanoparticles: experimental and theoretical investigations. J Colloid Interface Sci 407:168–176. https://doi.org/10.1016/j.jcis.2013.06.015

Hrác K, Sávoly Z, Seres A, Kiss LV, Papp IZ, Kuvoczev Á, Záráy G, Nagy P (2018) Toxicity and uptake of nanoparticulate and bulk ZnO in nematodes with different life strategies. Ecotoxicology 27:1058–1068. https://doi.org/10.1007/s10646-018-0195-8

Huang C-WW, Li S-WW, Liao VH-CC (2019) Long-term sediment exposure to ZnO nanoparticles induces oxidative stress in Caenorhabditis elegans. Environ Sci Nano 6:2602–2614. https://doi.org/10.1039/c9en00039a

Iswarya V, Bhuvaneshwari M, Ann S, Iyer S, Chaudhuri G, Chandrasekaran PT, Bhalerao GM, Chakravarty S, Raichur AM, Chandrasekaran N et al (2015) Combined toxicity of two crystalline phases (anatase and rutile) of Titania nanoparticles towards freshwater microalgae: Chlorella sp. Aquat Toxicol 161:154–169. https://doi.org/10.1016/j.aquatox.2015.02.006

Jafari A, Ghane M, Arastoo S (2011) Synergetic antibacterial effects of nano zinc oxide combined with silver nanocrystals. Afr J Microbiol Res 5:5465–5473. https://doi.org/10.5897/AJMR11.392

Khare P, Sonane M, Pandey R, Ali S, Gupta KC, Satish A (2011) Adverse effects of TiO2 and ZnO nanoparticles in soil nematode, Caenorhabditis elegans. J Biomed Nanotechnol 7:116–117. https://doi.org/10.1016/j.bjnn.2011.12.029

Khare P, Sonane M, Nagar Y, Moin N, Ali S, Gupta KC (2014) Size dependent toxicity of zinc oxide nanoparticle in soil nematode Caenorhabditis elegans. Nanotoxicology 5:5390:1–10. https://doi.org/10.1080/17435390.2014.940403

Kiss LV, Hrác K, Nagy PJ, Seres A (2018) Effects of zinc oxide nanoparticles on Panagrellus redivivus (Nematode) and Folsomia candida (Collembola) in various test media. Int J Environ Res 12:233–243. https://doi.org/10.3390/s1472-018-0086-y

Kiss LV, Boros G, Seres A, Nagy PJ (2020) Nano-fémoxidok kulcsfontosságú talajállat csoportokra gyakorolt hatásainak áttekintése [Toxic effects of nanosized metal oxides on soil-living organisms with particular ecological importance - a review]. Állattani Közlemények 105:29–57. https://doi.org/10.20331/allkoz.2020.105.1-2.29

Lead JT, Batley GE, Alvarez PJJ, Croteau MN, Handy RD, McLaughlin MJ, Judy JD, Schirmer K (2018) Nanomaterials in the environment: behavior, fate, bioavailability, and effects—an updated review. Environ Toxicol Chem 37:2029–2063. https://doi.org/10.1002/etc.4147

Li M, Noriega-Trevino ME, Nino-Martinez N, Mambiamo-Jones C, Wang J, Damoiseaux R, Ruiz F, Hoek EMV (2011) Synergistic bacterial activity of Ag-TiO2 nanoparticles in both light and dark conditions. Environ Sci Technol 45:8989–8995

Li Y, Yu S, Wu Q, Tang M, Pu Y, Wang D (2012) Chronic Al2O3-nanoparticle exposure causes neurotoxic effects on locomotion behaviors by inducing severe ROS production and disruption of ROS defense mechanisms in nematode Caenorhabditis elegans. J Hazard Mater 219–220:221–230. https://doi.org/10.1016/j.jhazmat.2012.03.083

Liu J, Kang Y, Yin S, Song B, Wei L, Chen L, Shao L (2017) Zinc oxide nanoparticles induce toxic responses in human neuroblastoma SH-SY5Y cells in a size-dependent manner. Int J Nanomedicine 12:8085–8099. https://doi.org/10.2147/IJN.S49070

Ma H, Kabengi NJ, Bertsch PM, Urine JM, Glenn TC, Williams PL (2011) Comparative phototoxicity of nanoparticulate and bulk ZnO to a free-living nematode Caenorhabditis elegans: the importance of illumination mode and primary particle size. Environ Pollut 159:1473–1480. https://doi.org/10.1016/j.envpol.2011.03.013

Ma H, Williams PL, Diamond SA (2013) Ecotoxicity of manufactured ZnO nanoparticles. A review. Environ Pollut 172:76–85. https://doi.org/10.1016/j.envpol.2012.08.011

Ma H, Wallis LK, Diamond S, Li S, Canas-Carrell J, Parra A (2014) Impact of solar UV radiation on toxicity of ZnO nanoparticles through photocatalytic reactive oxygen species (ROS) generation and photo-induced dissolution. Environ Pollut 193:165–172. https://doi.org/10.1016/j.envpol.2014.06.027

Maguire SM, Clark CM, Nunnari J, Pirri JK, Alkema MJ (2011) The C. elegans touch response facilitates escape from predacious fungi. Curr Biol 21:1326–1330. https://doi.org/10.1016/j.cub.2011.06.063

Mahmoudi GA, Astaraki P, Mohtashami AZ, Ahadi M (2015) N-acetylcysteine overdose after acetaminophen poisoning. Int Med Case Rep J 8:65–69. https://doi.org/10.2147/IMCRJ.S74563

Microsoft Corporation (2010) Microsoft Excel (No. 2010)

Mott D, Luo J, Njoki PN, Lin Y, Wang L, Zhong C (2007) Synergistic activity of gold-platinum alloy nanoparticle catalysts. Catal Today 122:378–385. https://doi.org/10.1016/j.cattod.2007.01.007

Nagano T (2009) Bioimaging probes for reactive oxygen species and reactive nitrogen species. J Clin Biochem Nutr 45:111–124

Nowacka M, Rygala A, Kregiel D, Kowalewska A (2018) Poly(siloxanesiloxane) and poly(siloxanes) grafted with N-acetylcysteine for eradicating mature bacterial biofilms in water environment. Colloids Surf B: Biointerfaces 172:627–634. https://doi.org/10.1016/j.colsurb.2018.09.017

Palkar J and Tavernarakis N (2013) Caenorhabditis elegans (Nematode). In: Brenner’s encyclopedia of genetics: second edition (vol. 1). Elsevier Inc. https://doi.org/10.1016/B978-0-12-374984-0.00186-8

RCoreTeam (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0

Rossignol DA (2005) The use of N-acetylcysteine as a chelator for metal toxicity. In: RE Frye & M Berk (eds) The therapeutic use of N-acetylcysteine in medicine. 1st edn. ADS, pp 169–179

https://doi.org/10.1016/S0097-9810-10-5311-5

Sarasija S, Norman KR (2018) Measurement of ROS in Caenorhabditis elegans using a reduced form of fluorescein. Bio Protoc 8:11. https://doi.org/10.21769/BioProtoc.2800. Measurement

Sautter J, Kaiser H, Focken U, Becker K (2007) Panagrellus redivivus (Nematoda) and toxicity of nano-ZnO in the plant-feeding nematode, Xiphinema spp. Environ Sci Pollut Res 14:1068. https://doi.org/10.1016/j.envpol.2011.03.013

Sékine K, Urano Y, Majima HJ, Chem JB, Kakimura K, Majima HJ, Nagano T (2003) Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. J Biol Chem 278:3170–3175. https://doi.org/10.1074/jbc.M209264200
Shi H, Gu Y, Xie Z, Zhou Q, Mao G, Lin X, Liu K, Liu Y, Zou B, Zhao J (2017) Mechanism of N-acetyl-cysteine inhibition on the cytotoxicity induced by titanium dioxide nanoparticles in JB6 cells transfected with activator protein-1. Exp Ther Med 13:3549–3554. https://doi.org/10.3892/etm.2017.4415

Sochová I, Hofman J, Holoubek I (2006) Using nematodes in soil ecotoxicology. Environ Int 32:374–383. https://doi.org/10.1016/j.envint.2005.08.031

Song W, Zhang J, Guo J, Zhang F, Meng F, Li L, Sun Z (2010) Role of the dissolved zinc ion and reactive oxygen species in cytotoxicity of ZnO nanoparticles. Toxicol Lett 199:389–397. https://doi.org/10.1016/j.toxlet.2010.10.003

Starnes D, Unrine J, Chen C, Lichtenberg S, Starnes C, Svendsen C, Kille P, Morgan J, Baddar ZE, Spear A et al (2019) Toxicogenomic responses of Caenorhabditis elegans to pristine and transformed zinc oxide nanoparticles. Environ Pollut 247:917–926. https://doi.org/10.1016/j.envpol.2019.01.077

Sun SY (2010) N-acetylcysteine, reactive oxygen species and beyond. Cancer Biol Ther 9:109–110. https://doi.org/10.4161/cbt.9.2.10583

Sun TY, Bornhöft NA, Hungerbühler K, Nowack B (2016) Dynamic probabilistic modeling of environmental emissions of engineered nanomaterials. Environ Sci Technol 50:4701–4711. https://doi.org/10.1021/acs.est.5b05828

Tong T, Fang K, Thomas SA, Kelly JJ, Gray KA, Gaillard JF (2014) Chemical interactions between nano-ZnO and nano-TiO2 in a natural aqueous medium. Environ Sci Technol 48:7924–7932. https://doi.org/10.1021/es501168p

Tong T, Wilke CM, Wu J, Thanh CT, Kelly JJ, Gaillard J, Gray KA (2015) Combined toxicity of nano-ZnO and nano-TiO2: from single- to multi-nanomaterial systems. Environ Sci Technol 49:8113–8123. https://doi.org/10.1021/acs.est.5b02148

TOXRATLIGHT2.08 (n.d.) Software for statistical evaluation of biotests in ecotoxicology. ToxRat Solutions GmbH, Germany, Alsdorf (2.08)

Vinciguerra MT (1979) Role of nematodes in the biological processes of the soil. Bolletino Di Zool 46:363–374. https://doi.org/10.1080/1125007909440312

Wang J, Deng X, Zhang F, Chen D, Ding W (2014) ZnO nanoparticle-induced oxidative stress triggers apoptosis by activating JNK signaling pathway in cultured primary astrocytes. Nanoscale Res Lett 9:1–12. https://doi.org/10.1186/1556-276X-9-117

Wang D, Lin Z, Wang T, Yao Z, Qin M, Zheng S, Lu W (2016) Where does the toxicity of metal oxide nanoparticles come from: the nanoparticles, the ions, or a combination of both? J Hazard Mater 308:328–334. https://doi.org/10.1016/j.jhazmat.2016.01.066

Wang K, Chen S, Zhang C, Huang J, Wu J, Zhou H, Jin L (2018) Enhanced ROS production leads to excessive fat accumulation through DAF-16 in Caenorhabditis elegans. Exp Gerontol 112:20–29. https://doi.org/10.1016/j.exger.2018.07.017

Wu Q, Wang W, Li Y, Li Y, Ye B, Tang M, Wang D (2012) Small sizes of TiO2-NPs exhibit adverse effects at predicted environmental relevant concentrations on nematodes in a modified chronic toxicity assay system. J Hazard Mater 243:161–168. https://doi.org/10.1016/j.jhazmat.2012.10.013

Wu Q, Nouara A, Li Y, Zhang M, Wang W, Tang M, Ye B, Ding J, Wang D (2013) Comparison of toxicities from three metal oxide nanoparticles at environmental relevant concentrations in nematode Caenorhabditis elegans. Chemosphere 90:1123–1131. https://doi.org/10.1016/j.chemosphere.2012.09.019

Xia T, Kovochich M, Lioung M, Ma L, Gilbert B, Shi KH, Yeh JI, Zink JI, Nel AE (2008) Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2:2121–2134

Yang Q, Ma Y (2014) Irradiation-enhanced cytotoxicity of zinc oxide nanoparticles. Int J Toxicol 33:187–203. https://doi.org/10.1177/1091581814529168

Yoon DS, Lee M, Cha DS (2018) Measurement of intracellular ROS in Caenorhabditis elegans using 2',7'-dichlorodihydrofluorescein diacetate. Bio Protoc 8:1–9. https://doi.org/10.21769/BioProtoc.2774

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.