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Environmental Pollution 261 (2020) 114117

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Foliar versus root exposure of AgNPs to lettuce: Phytotoxicity, antioxidant responses and internal translocation☆

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ARTICLE INFO

Article history:
Received 18 December 2019
Received in revised form
22 January 2020
Accepted 31 January 2020
Available online 1 February 2020

Keywords:
Nanosilver
Vascular plant
Uptake
Biodistribution
Oxidative stress

ABSTRACT

Whether toxicity of silver nanoparticles (AgNPs) to organisms originates from the nanoparticles themselves or from the dissolved Ag-ions is still debated, with the majority of studies claiming that extracellular release of Ag-ions is the main cause of toxicity. The objective of this study was to determine the contributions of both particles and dissolved ions to toxic responses, and to better understand the underlying mechanisms of toxicity. In addition, the pathways of AgNPs exposure to plants might play an important role and therefore are explicitly studied as well. We systematically assessed the phytotoxicity, internalization, biodistribution, and antioxidant responses in lettuce (*Lactuca sativa*) following root or foliar exposure to AgNPs and ionic Ag at various concentrations. For each endpoint the relative contribution of the particle-specific versus the ionic form was quantified. The results reveal particle-specific toxicity and uptake of AgNPs in lettuce as the relative contribution of particulate Ag accounted for more than 65% to the overall toxicity and the Ag accumulation in whole plant tissues. In addition, particle toxicity is shown to originate from the accumulation of Ag in plants by blocking nutrient transport, while ion toxicity is likely due to the induction of excess ROS production. Root exposure induced higher toxicity than foliar exposure at comparable exposure levels. Ag was found to be taken up and subsequently translocated from the exposed parts of plants to other portions regardless of the exposure pathway. These findings suggest particle related toxicity, and demonstrate that the accumulation and translocation of silver nanoparticles need to be considered in assessment of environmental risks and of food safety following consumption of plants exposed to AgNPs by humans.

1. Introduction

Due to their excellent catalytic and superconducting properties and their strong antibacterial activity, engineered silver nanoparticles (AgNPs) are present in a large variety of consumer, agricultural and medical products and are produced in large amounts (Starnes et al., 2015; Samarajeewa et al., 2017). However, with the accelerating production and application, there is the likelihood of release into the environment with emissions expected to increase (Quadros et al., 2013; Benn et al., 2010). The released AgNPs are expected to end up and accumulate in soil due to biosolids fertilization application, sewage disposal, irrigation, and waste landfills (Yu et al., 2013; Wang et al., 2016; Li et al., 2017a). Likewise, AgNPs also can be disproportionately emitted into the atmosphere and adsorbed onto fine atmospheric dust as a consequence of industrial activities, waste incineration, spray application by households (e.g. disinfection and anti-odor sprays) and the application of agricultural products (Yu et al., 2013; Park et al., 2009; Holder and Marr, 2013). Plants are in direct interaction with air, soil and water, and as primary producers are vital for the functioning of ecosystems, supplying food to different consumer levels. It is therefore needed to properly understand how enhanced exposure to synthetic AgNPs induces their uptake and subsequent translocation in plants as originating from the soil based uptake routes as well as from the air-borne route. This knowledge will allow to provide relevant

☆ This paper has been recommended for acceptance by Jörg Rinklebe.
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https://doi.org/10.1016/j.envpol.2020.114117

information for the evaluation of the potential risks of AgNPs to plants, being of great importance given their position within ecosystems as well as being a food source for humans.

After root exposure, the uptake and translocation from roots to leaves were reported, as well as adverse responses on plants. These responses include inhibition of seed germination and root elongation, reduction of biomass, and impacts on the photosynthetic system of plants (Yan and Chen, 2019). However, the current understanding of the impacts of foliar exposure on i) plant growth, and ii) AgNPs uptake and translocation from leaves to roots is rather limited. This information may carry important implications regarding the effect of atmospheric deposition on the concentrations of pollutants in above-ground plant portions as well as on the safety of AgNPs-added agricultural products applications(Kranjc et al., 2018). Based on the limitedly available studies, there have been contradictory reports where foliar exposure induced more metal accumulation but less toxicity (Li et al., 2017b), or more metal accumulation and higher toxicity (Salehi et al., 2018), or less metal accumulation and less toxicity in plants (Lian et al., 2020) as compared to root exposure. These apparent inconsistencies regarding the relationship between the toxicity and metal accumulation in plants highlight that the interactions of plants and nanoparticles involved in different exposure pathways should be investigated in greater detail.

In addition, it remains controversial whether the toxicity of a AgNPs suspension is specifically caused by nanoparticle itself or is due to the released ionic Ag. Although Ag-ions released from the AgNPs are often seen as the main cause of observed toxicity (Navarro et al., 2015)' (Navarro et al., 2008)' (Tripathi et al., 2017)' (Li et al., 2015)' (Zhang and Wang, 2019), the particle-specific toxicity has been reported and was in some cases shown to be important (Yin et al., 2011)' (Qian et al., 2013). Moreover, plants are known to take up particles/ions through cuticular pores and stomata in case of foliar exposure (Raliya et al., 2016; Wang et al., 2013a), and through the root epidermis in case of root exposure (Achari and Kowshik, 2018). These difference might change the ratio of ions versus particles that are taken up by plants. Whether this would lead to differences in Ag-ions/NPs biodistribution across the plant organs remains unclear. Furthermore, Ag-ions have a different mode of action and bioavailability compared to the particulate form (Poynton et al., 2011). However, differentiating the contribution of particulate Ag versus dissolved Ag-ions on the overall toxicity of AgNPs suspensions is challenging due to their common co-occurrence. This type of comparative toxicity assessments of AgNPs suspensions and Ag-ions are mostly performed with freshwater species in parallel experiments using identical concentrations of total Ag (Tripathi et al., 2017; Yin et al., 2011)' (Qian et al., 2013). However, it should be noted that the dissolution of Ag-ions from the particulate Ag in AgNPs suspension is a dynamic process, and the ratio of occurrence of particle forms versus ionic forms alters over time and is influenced by the concentration of AgNPs suspension as well as by water chemistry and/or soil properties (Xiao et al., 2018; Arenas-Lago et al., 2019). To address this issue, time weighted average concentrations and standardized aqueous test media instead of soil were used in this study.

In the present study, we exposed lettuce (*Lactuca sativa*) which is a widely cultivated vegetable having a large foliar surface, to different concentrations of AgNPs and/or Ag-ions following root or foliar exposure. The aims of the study were to: 1) investigate the relative contribution to toxicity and accumulation of dissolved Ag versus particulate Ag of AgNPs suspension, and 2) determine the difference in uptake, translation and phytotoxic responses of lettuce in both exposure pathways. Knowledge on uptake routes and toxic species provides building blocks to generate a mechanistic-based effect assessment for the plants, which is of great importance given their position within ecosystems as well as being a food source for humans.

2. Materials and methods

2.1. Characterization of AgNPs suspensions and quantification of dissolved Ag-ions

Suspensions of spherical AgNPs (RAS AG, Regensburg, Germany) with a nominal size of 20 nm were obtained at a concentration of 100 g/L Ag in water under nitrogen gas. $AgNO_3$ was purchased from Sigma−Aldrich (Zwijndrecht, Netherlands). Stock suspensions were freshly prepared in 1/4 Hoagland solution (pH 6.0 ± 0.1; without EDTA or chloride to avoid Ag chelation or precipitation, Hoagland solution compositions are described in Table S1, Supplementary material) after 5 min sonication at 60 Hz (USC200T, VWR, Amsterdam, The Netherlands). The size distribution and zeta potential of the nanoparticle suspensions at the exposure concentrations were analyzed at 1, 24, 48 and 72 h after incubation in 1/4 Hoagland solution using a zetasizer Nano-ZS instrument (Malvern, Instruments Ltd., Royston, UK).

The dissolution kinetics of AgNPs suspension at 0.1, 0.5 and 1 mg/L in 1/4 Hoagland solution over 72 h were investigated to obtain the actual exposure concentrations of soluble Ag. After being exposed to 1/4 Hoagland solution for 0, 6, 16, 24, 48 and 72 h, the suspensions (defined as $AgNPs_{(total)}$) were taken from the tubes (top 10 cm) and centrifuged at 30,392 g for 30 min at 4 °C (Sorvall RC5Bplus centrifuge, Bleiswijk, Netherlands) to remove the particulate Ag remaining in suspension. The supernatants obtained in this step were used as the corresponding dissolved Ag suspension (defined as $AgNPs_{(ion)}$). Next, the concentrations of $AgNPs_{(total)}$ and $AgNPs_{(ion)}$ were measured by Atomic Absorption Spectroscopy (AAS, PerkinElmer 1100 B,Waltham, MA, USA) after adding a drop of 65% HNO_3 into the solution. Accordingly, the concentration of $AgNPs_{(particle)}$ is the difference between the Ag measured as $AgNPs_{(total)}$ and $AgNPs_{(ion)}$(Zhai et al., 2016). All experiments were run in triplicate.

2.2. Plant growth and experimental design

Lactuca sativa seeds were sterilized for 15 min with NaClO (0.5% w/v), rinsed three times with tap water, and then immersed in deionized water for 24 h. The seeds were germinated in a rolled paper towel suspended in deionized water. After 3 d, the seedlings were placed in Petri dishes (10 seedlings/dish) with 50 mL of 1/8 Hoagland solution for one week and then the young plants were transferred to 22 mL tubes (one seedling per tube) containing 1/4 Hoagland solution for a further week of growth. The seeds germination and growth were kept in a climate room at a 20/16 °C day/night temperature and 60% relative humidity set to a 16 h photoperiod.

The plants were exposed to $AgNPs_{(total)}$ and $AgNPs_{(ion)}$ for 15 days via the root or leaves (see below for details). The exposure procedure was modified from a previous study (Li et al., 2017b). In all cases, the tubes that contained exposure medium and the control treatments with 1/4 Hoagland solution had lids with a small hole and were covered with aluminum foil to minimize the impact of light-induced transformations of AgNPs and to avoid evaporation of water. Plants were placed with their roots within the tubes, and the upperparts such as leaves were placed above the foil. All exposure tests were performed under the same conditions as described above for seeds growth.

Root exposure. Uniform pre-grown lettuce plants were selected and were exposed through the roots to either $AgNPs_{(total)}$ suspensions at nominal concentrations of 0.1, 0.5 and 1 mg/L, or the corresponding dissolved concentration of Ag ($AgNPs_{(ion)}$) released from the above concentrations of $AgNPs_{(total)}$ using $AgNO_3$ (12 replicates per treatment). The AgNPs suspensions were prepared by mixing different volumes of the AgNPs stock suspension into 1/4 Hoagland solution and sonicating for 10 min at 60 Hz to facilitate dispersion prior to application. The $AgNPs_{(total)}$ were chosen based on our preliminary tests which showed that the highest concentration (1 mg/L) reduced the fresh biomass of lettuce by *ca.* 40% after one week, and $AgNPs_{(ion)}$ concentrations were selected according to the dissolution kinetics of AgNPs suspensions. The exposure media were renewed every 3 d.

Foliar exposure. No significant effects on biomass production were found during preliminary tests in which lettuce leaves were exposed to AgNPs suspensions at the same concentrations as used for root exposure, and roots were exposed to AgNPs continually. Thus, uniformly grown lettuce plants were divided into two groups for foliar exposure. In one group (defined as *foliar exposure*), which is mainly used to study the effects of foliar application of AgNPs, the freshly prepared $AgNPs_{(total)}$ suspensions with nominal concentrations of 1, 10, and 50 mg/L (fresh biomass decreased by around 40% after one week preliminary exposure under the highest concentration) were carefully dropped onto lettuce leaves. A volume of 0.5 mL of the AgNPs suspensions was applied to each plant seven times per day (every 2 h during daytime). The small volume and high application frequency ensured effective exposure of the leaves to AgNPs suspensions and minimal Ag loss due to dripping off the leaves. To avoid Ag contamination of the hydroponic medium, dry cellulose tissues were added to the small hole in the lids. The Ag content in the 1/4 Hoagland solution was below the detection limit, indicating that the foliar applied Ag was the only source for the plants.

In the other group (defined as *single-leaf immersed exposure*), which is only used for comparison with the uptake and accumulation of Ag via root exposure, one of the lettuce leaves was immersed in AgNPs suspensions at nominal concentrations of 0.1, 0.5 and 1 mg/L (same as root exposure).

2.3. Biomass and Ag accumulation measurement

All treated plants were harvested after 15 d of exposure and subsequently thoroughly washed with flowing deionized water and rinsed with ultrapure water three times. Next, the plants were separated into the root and shoot. For the leaf immersed exposure treatments, plants were separated into three parts: root, unexposed leaves (shoot) and exposed leaf. After measuring the fresh biomass, half of samples were flash-frozen in liquid nitrogen and stored at −80 °C for further biochemical analysis.

To determine the total Ag content in plant tissues, the attached AgNPs/Ag-ions were removed by immersing the whole plant for 20 min in 10 mM HNO_3, followed by immersion for 20 min in 10 mM EDTA, and finally thoroughly rinsing with Milli-Q water (Li et al., 2017b; Jiang et al., 2017). Samples were oven-dried for 72 h at 70 °C and weighed to determine dry weight. The weighed root and shoot samples were digested by adding 3 mL of HNO_3 (65%) at 120 °C for 40 min on a hotplate and then 1.5 mL of H_2O_2 (30%) was added and heated at 120 °C for another 20 min (Ma et al., 2016). Following digestion, the samples were diluted with deionized water to 3 mL and analyzed on their metal content by using AAS (PerkinElmer 1100 B, Waltham, MA, USA). Ten blanks were used to calculate the detection limit of Ag for AAS. Standard Ag solutions of 0.5 mg/L and 1 μg/L were measures every 20 samples to monitor the stability of AAS. Recoveries were found to be in between 95% and 110% for AAS. Blanks and Ag standard solutions were included in the digestion procedure for quality control purposes.

2.4. Biochemical analysis of plant tissue

The variations in chlorophyll pigment could affect plant growth as chlorophyll has an important role in photosynthesis. In addition, NPs toxicity to plants has been related to oxidative stress as a result of increasing reactive oxygen species (ROS) productions and disturbance in defense mechanisms (Yan and Chen, 2019). Therefore, chlorophyll pigment, ROS production and the related antioxidants were measured as following.

Photosynthetic Pigment Measurement. Fresh leaves (0.1−0.2 g) were homogenized in liquid nitrogen and extracted with 80% acetone for 24 h at 4 °C in the dark followed by centrifuging for 10 min at 4500 g at 4 °C. Chlorophyll *a* and *b*, and carotenoids were determined by using a UV−vis spectrophotometer at 663, 646 and 470 nm respectively (Lichtenthaler, 1987).

ROS production analysis. The superoxide anion (O_2^-) assay in root and shoot tissues of different treatments was performed according to the method of Wang and Lou (Wang and Luo, 1990) with a modification by oxidizing hydroxylamine hydrochloride. This procedure yields nitrite which can react with sulphanilamide and a-naphthylamine to form a red azo dye with a maximum absorbance at 530 nm. Hydrogen peroxide (H_2O_2) was quantified according to Mosa et al. (2018) by incubating the plant extracts with potassium iodide and reading the absorbance at 390 nm. The content of H_2O_2 was obtained based on a H_2O_2 standard curve (R=0.99) (Samarajeewa et al., 2017). Malondialdehyde (MDA) was measured to analyze lipid peroxidation following the method of Mosa et al. (2018) using a UV−vis spectrophotometer.

Enzymatic antioxidants. Fresh roots or leaves tissues (0.1−0.2 g) were separately homogenized in ice cold extraction buffer. After centrifugation at 10,000 g for 20 min at 4 °C, the supernatants were used for superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) activities analysis following the protocols as described by Ma et al. (2016).

Non-enzymatic antioxidants. The ascorbate (ASA) content in plant tissues was estimated spectrophotometrically at 525 nm according to the method of Kampfenkel et al. (1995) by quantifying on the basis of a standard curve of L-ascorbic acid (Sigma-Aldrich, Zwijndrecht, Netherlands). The extracts were obtained by grinding 0.1 g leaf tissues in 0.8 mL 6% (v/v) trichloroacetic (ice cold) and centrifuging at 15,600 g for 10 min at 4 °C. The reduced glutathione (GSH) level was assayed by the method modified from Xia et al. (2018) based on the fact that the sulfhydryl groups present in the tissue homogenates react with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) to form a yellow dye with maximum absorbance and read at 412 nm.

More detailed information about the biochemical parameters methodology and quantifications can be found in the supplementary material.

2.5. Data analysis

The behavior of AgNPs during the exposure period involves dynamic processes, especially in the root exposure. Time weighted average concentrations (C_{TWA}) were therefore used to assess the actual exposure concentration of $AgNPs_{(total)}$, $AgNPs_{(particle)}$ and $AgNPs_{(ion)}$ over each 3 d refreshment period. The TWA concentration was calculated based on the following equation (Zhai et al., 2016):

$$C_{TWA} = \frac{\sum_{n=0}^{N}\left(\Delta t_n \frac{C_{n-1}+C_n}{2}\right)}{\sum_{n=1}^{N}\Delta t_n} \quad (1)$$

where Δt is the time interval, n is the time interval number, N is the total number of intervals (N = 5), C is the concentration at the end of the time interval.

To calculate the relative contribution of $AgNPs_{(partices)}$ and $AgNPs_{(ion)}$ to the effects induced by the suspensions of AgNPs, the decrease of biomass as compared to the control was chosen as the endpoint of assessment. Based on previous literature (Liu et al., 2016), it is widely believed that the modes of actions of nanoparticle$_{(particle)}$ and nanoparticle$_{(ion)}$ are likely to be independent, which is in line with the assumption of the response addition model:

$$E_{(total)} = 1 - \left(\left(1 - E_{(particle)}\right)\left(1 - E_{(ion)}\right)\right) \tag{2}$$

where $E_{(total)}$ and $E_{(ion)}$ represent the effects caused by the nanoparticle suspensions and their corresponding released ions, which were quantified experimentally. This makes $E_{(particle)}$ as the only unknown, allowing for direct calculation of the effects caused by the $AgNPs_{(particle)}$.

The Ag enrichment factor (EF), defined to evaluate the ability of plants to accumulate Ag, was calculated using the following equation:

$$EF = \frac{M_{plant}}{M_{medium}} \tag{3}$$

The Ag content in plants (M_{plant}) was calculated as follows:

$$M_{Plant} = C_{leaves} \times DW_{leaves} + C_{roots} \times DW_{root} \tag{4}$$

where C_{leaves} and C_{roots} represent the Ag concentration in leaves and roots, in units of milligrams per kilogram.

The Ag content in the medium (M_{medium}) was calculated as follows:

Root exposure:

$$M_{medium} = C_{TWA} \times V_{exposure} \tag{5}$$

Foliar *exposure*:

$$M_{medium} = \frac{\sum_{n=0}^{N}\left(\Delta t_n \times \left(C_{exposure \times V_{exposure}}\right)\right)}{\sum_{n=1}^{N}\Delta t_n} \tag{6}$$

Where Δt is the time interval between each drop, n is the time interval number, N is the total number of intervals (N = 104), C is the exposure concentration of AgNPs suspensions (mg/L), V is the exposure volume dropped onto the leaves each time (L).

The Ag translocation factor (TF), defined to evaluate the capacity of plants to transfer Ag from exposed parts to the remainder of the plant, was calculated as follows:

$$TF = \frac{C_{shoots}}{C_{roots}} \text{ for root exposure; } TF = \frac{C_{roots}}{C_{shoots}} \text{ for foliar exposure} \tag{7}$$

Statistically significant differences among different concentrations in the same group were analyzed by one-way ANOVA followed by Turkey's honestly significant difference tests at $\alpha < 0.05$ using IBM SPSS Statistics 25 (Data were tested for normal distribution and homogeneity of variance with Shapiro-Wilk test and Bartlett test prior to running the ANOVA, with no deviations from both found). The T-test was performed to analyze the significance between $AgNPs_{(ion)}$ and $AgNPs_{(total)}$ ($p < 0.05$). Results are expressed as mean ± standard error of 12 replicates for biomass and 4 replicates for biochemical parameters and Ag bioaccumulation. All test statistics (p-values) are presented in Table S2, supplementary material.

3. Results

3.1. AgNPs suspension characterization

The DLS results showed that the AgNPs aggregated rapidly in the 1/4 Hoagland solution as the hydrodynamic diameter increased over time (Table S2). The Zeta-potential of the AgNPs suspensions of all concentrations ranged between −9.5 and −15.4 mV and their changes were slight over the test period (Table S3). The ionic Ag concentration increased gradually over time while the concentrations of total and particulate Ag decreased over time (Fig. 1). The extent of ionic Ag released was found to be related to the concentrations of the AgNPs suspensions as the percentage of $AgNPs_{(ion)}$ increased by 38%, 29% and 24% after 72 h of incubation in the exposure medium at nominal concentrations of 0.1, 0.5 and 1 mg/L, respectively. Based on the dynamic dissolution behaviors of $AgNPs_{(total)}$, TWA concentrations of $AgNPs_{(ion)}$ were chosen as the exposure concentration of ionic Ag (corresponding dissolved Ag released from AgNPs) to plants, that is: 6.3, 36.6 and 85.0 μg/L are the average Ag-ions concentrations present in AgNPs suspensions of nominal concentrations of 0.1, 0.5 and 1 mg/L, respectively (Table S4).

3.2. Impacts on growth of lettuce

Shoot and root biomass of the lettuce plants were significantly reduced for the $AgNPs_{(total)}$ and $Ag_{(ion)}$ treatments with a dose-dependent effect regardless of exposure pathway (Fig. 2; Table S2). Following root exposure to 0.1, 0.5 and 1 mg/L of $AgNPs_{(total)}$, the biomass of lettuce significantly decreased by 24, 48 and 78% for the roots and 27, 52 and 70% for the shoots relative to the controls, respectively. For the corresponding concentrations of dissolved $AgNPs_{(ion)}$, only the highest exposure concentration caused significant effects on root/shoot biomass with a reduction of 26/20% compared to the control, respectively. The results indicated a particle-specific toxicity to plants, in addition to the particles being a potential source of Ag-ions. Following foliar exposure, a significant decrease on root/shoot biomass (42/28%) was observed at the highest exposure concentration of $AgNPs_{(total)}$, while a significant increase was observed only in root biomass (34%) at 1 mg/L. On the other hand, the highest actual amount of $AgNPs_{(total)}$ based on the TWA method in case of foliar exposure was 1.12 mg, which was 10 times higher than the highest amount (0.048 mg) in case of root exposure. However, the corresponding effects on biomass reduction were much lower in case of foliar exposure than in case of root exposure. This indicated higher $AgNPs_{(total)}$ toxicity following root exposure when considering exposure on the basis of a similar dose expression.

The chlorophyll content in leaves was measured as an indicator of the photosynthetic performance of the plants. AgNPs had no significant impacts on total chlorophyll content of lettuce (Table S2), regardless of Ag forms or exposure pathways, although a trend toward a decreasing chlorophyll content with increasing dose was noted (Fig. S1 Supplementary).

3.3. Analysis of oxidative stress

Exposure to increasing concentrations of $AgNPs_{(ion)}$ under root exposure significantly increased the accumulation of O_2^{-}, H_2O_2 and MDA in lettuce roots and shoots (Fig. 3; Table S2). For root exposure to $AgNPs_{(total)}$, the content of O_2^{-} and MDA in shoots, and the content of H_2O_2 in roots were significantly increased upon increasing exposure concentrations. Even though not significant, a

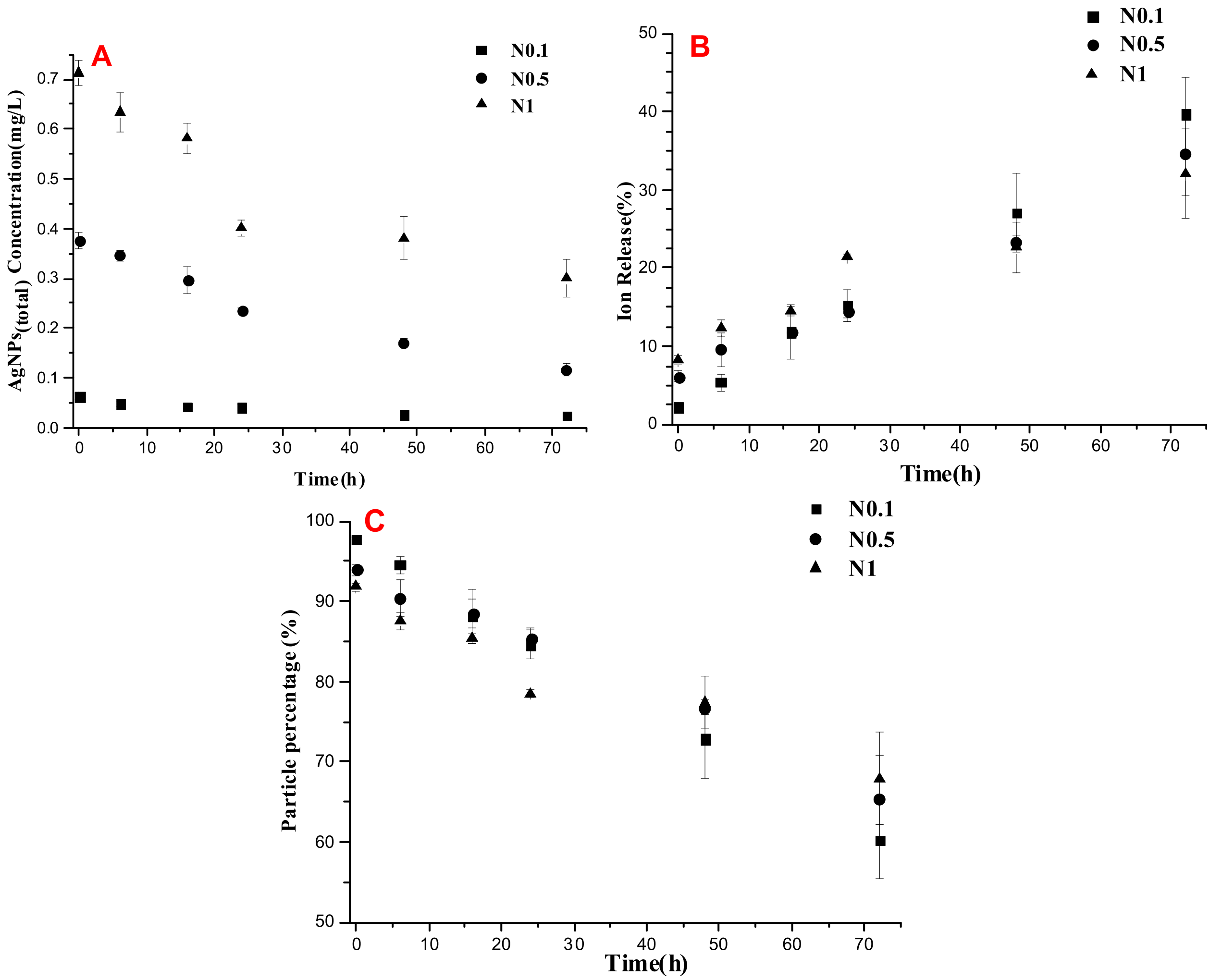

Fig. 1. Ion release profiles of AgNPs suspensions at the concentrations of 0.1 mg/L, 0.5 mg/L and 1 mg/L(N0.1, N0.5, N1) in the exposure medium over time. (A) Total Ag concentrations in the AgNPs suspension. (B) Percentages of dissolved Ag released in the AgNPs suspension. (C) Percentages of particulate Ag present in the AgNPs suspensions. Data are the mean ± SE (n = 3).

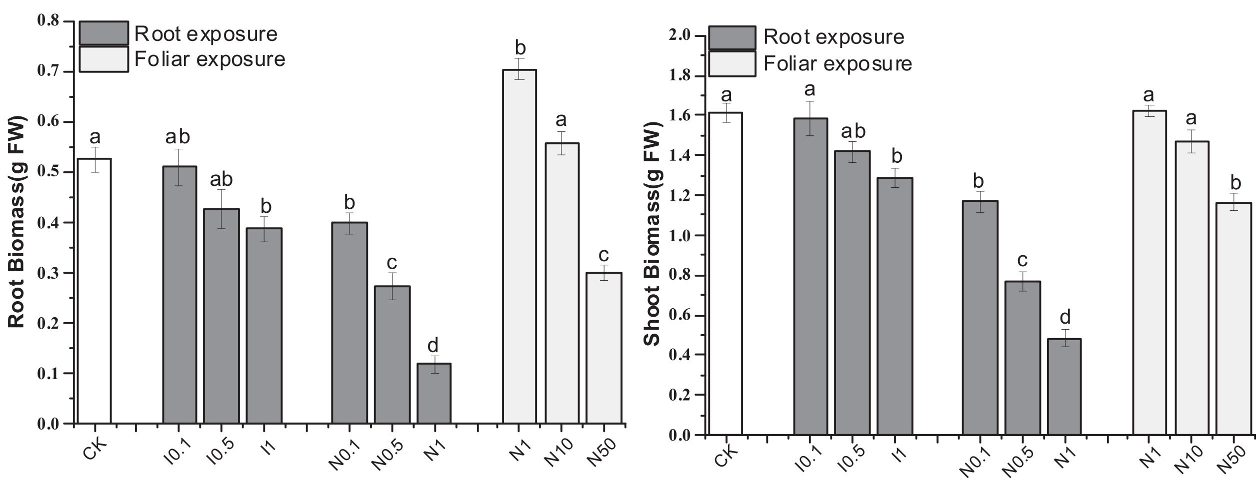

Fig. 2. Root and shoot fresh biomass of lettuce (*Lactuca sativa*) exposed to different concentrations of $AgNPs_{(total)}$ and $AgNPs_{(ion)}$ after 15 days of exposure. Data are the mean ± SE (n = 12). Different letters in the same group indicate statistically significant differences between treatments at $p < 0.05$. I0.1, 0.5 and 1 represent *Lactuca sativa* exposed to the $AgNPs_{(ion)}$ concentrations as released from AgNPs suspensions with nominal concentrations of 0.1,0.5 and 1 mg/L; N0.1, 0.5, 1, 10 and 50 represent *Lactuca sativa* exposed to nominal $AgNPs_{(total)}$ concentrations of 0.1, 0.5, 1, 10 and 50 mg/L.

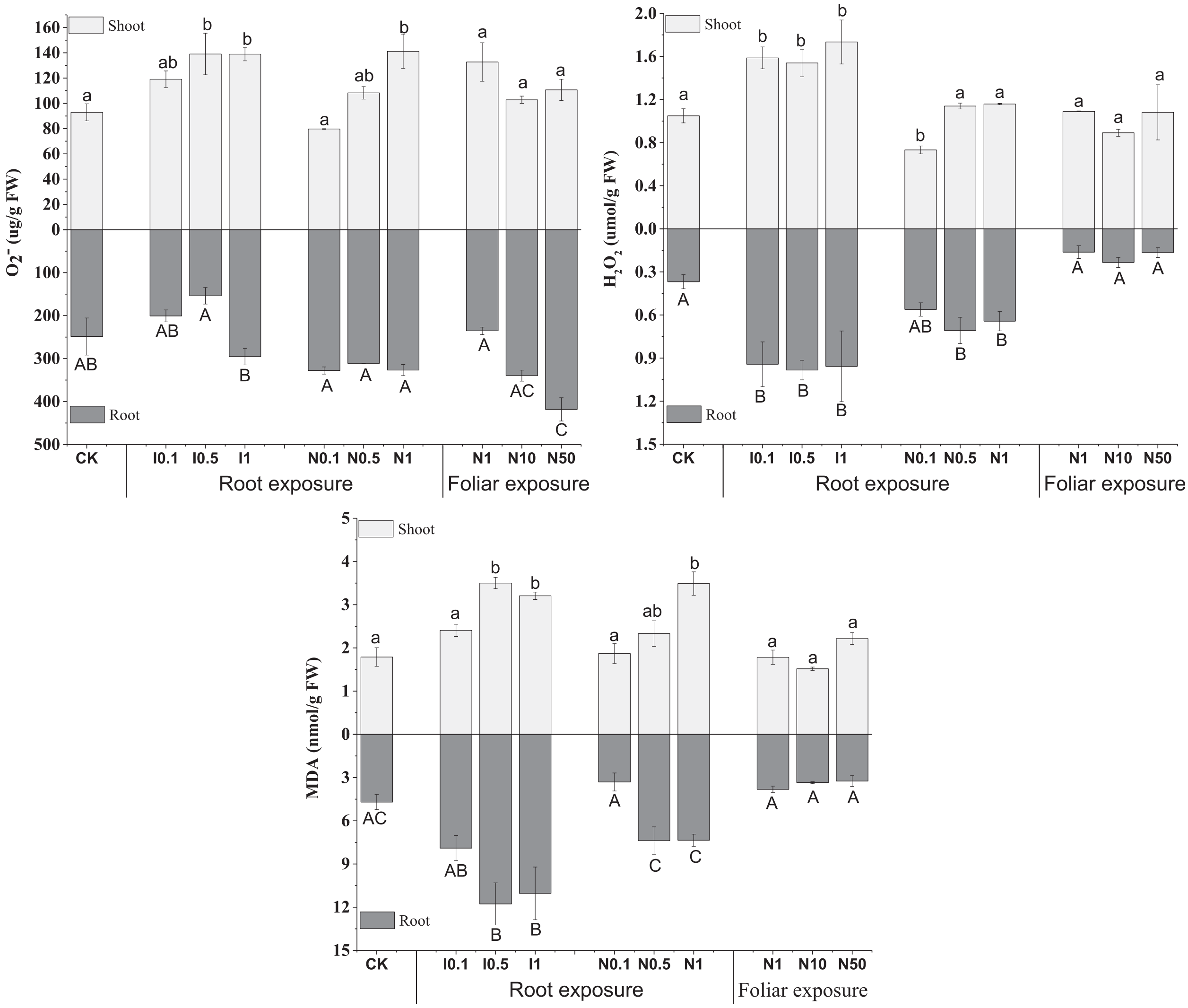

Fig. 3. O_2^{-}, H_2O_2 and MDA production in *Lactuca sativa* exposed to different concentrations of $AgNPs_{(total)}$ and $AgNPs_{(ion)}$ after 15 days of exposure. Data are mean ± SE (n = 4). Within the same plant tissue, the different letters in the same group indicate statistically significant differences between treatments at p<0.05. I0.1, 0.5 and 1 represent *Lactuca sativa* exposed to the $AgNPs_{(ion)}$ concentrations as released from AgNPs suspensions with nominal concentrations of 0.1,0.5 and 1 mg/L; N0.1, 0.5, 1, 10 and 50 represent *Lactuca sativa* exposed to nominal $AgNPs_{(total)}$ concentrations of 0.1, 0.5, 1, 10 and 50 mg/L.

slight increase of O_2^{-} and MDA in roots and of the H_2O_2 contents in shoots in comparison with control also should be noted following root exposure to $AgNPs_{(total)}$ (Fig. 3 and Table S5).

For foliar exposure, no significant differences (Fig. 3; Table S2) in the contents of O_2^{-}, H_2O_2 and MDA were found in roots and shoots of lettuce exposed to $AgNPs_{(total)}$, the exception being the O_2^{-} contents in the group of root tissues (ANOVA, P = 0.01; Fig. 3), as the O_2^{-} content was significantly increased by 68% at the highest exposure concentration compared to the control (Table S5).

In general, the accumulation of ROS in roots/shoots following root exposure to $AgNPs_{(ion)}$ was higher or equal to the ROS production in case of exposure to the corresponding concentration of $AgNPs_{(total)}$ (Fig. 3). This finding suggests that $AgNPs_{(particle)}$ contributed only to a limited extent to the induction of oxidative stress and/or its effects are being efficiently counteracted by the antioxidants system. There was an exposure pathway dependent pattern for the alterations of the ROS production in plants, with an increasing tendency for root exposure to $AgNPs_{(total)}$, whereas a slight decrease of H_2O_2 and MDA contents in roots via leaf exposure to $AgNPs_{(total)}$ was observed (Fig. 3 and Table S5).

3.4. Antioxidants responses

A clear dose-dependent effect on the activity of enzymatic antioxidants activities was observed following root exposure. Compared to the control, the changes of the enzymatic antioxidants activity were significantly increased upon increasing exposure concentrations (Table S2) in plant roots and shoots regardless of the form of Ag, except for the APX activity in plant roots (Table 1, P > 0.1). In addition, the alterations of SOD, CAT and POD activities in plants exposed to $AgNPs_{(total)}$ were comparable to, or slightly higher than, the changes in case of exposure to the corresponding concentration of $AgNPs_{(ion)}$, with the exception of APX activity (Table 1). This suggests that the alterations of the enzymatic antioxidants activity triggered by the $AgNPs_{(ion)}$ was stronger than in case of corresponding $AgNPs_{(particle)}$.

For foliar exposure, significant differences were found for APX and CAT activity (Table 1; Table S2). Interestingly, there was no consistent concentration dependent pattern with regard to enzyme type and plants organ. For instance, the APX and CAT activities decreased in shoots and increased in roots as the exposure concentration increased. The SOD and POD activity decreased in roots

Table 1
Variations enzymatic antioxidants (SOD, CAT, APX and POD) and non-enzymatic antioxidants activity (ASA, GSH and Carotenoid) in *Lactuca sativa* exposed to different concentrations of AgNPs(total) and AgNPs(ion) after 15 days of exposure. Data are mean ± SE (n = 4). Within the same plant tissue, the different letters in the same group indicate significant differences between treatments at p < 0.05.

	Treatments		Enzymatic antioxidants				Non-enzymatic antioxidants[a]		
			SOD	APX	CAT	POD	ASA	GSH	Carotenoid
Root tissues		CK	107.1 ± 16.8a	99.8 ± 27.1a	128.8 ± 12.1a	460.9 ± 41.8a			
	Root exposure	I0.1	144.8 ± 22.5a	200.8 ± 33.2a	136.3 ± 8.4a	183.3 ± 19.2 b			
		I0.5	237.8 ± 28.6 ab	120.7 ± 7.5a	137.1 ± 12.9a	198.1 ± 12.3 b			
		I1	341.9 ± 46.8 b	129.8 ± 12.7a	287.5 ± 16.4 b	313.9 ± 39.2 b			
		N0.1	285.0 ± 9.4 ab	137.3 ± 16.0a	140.9 ± 8.0a	268.1 ± 19.7 b			
		N0.5	330.7 ± 84.4bc	142.4 ± 13.6a	189.2 ± 18.6a	232.9 ± 26.9 b			
		N1	494.6 ± 35.6c	78.1 ± 10.0a	304.1 ± 18.3 b	307.5 ± 6.15 b			
	Foliar exposure	N1	93.0 ± 9.7a	72.2 ± 2.3a	229.0 ± 17.9 ab	438.6 ± 55.7a			
		N10	74.2 ± 13.4a	85.8 ± 2.4a	245.3 ± 18.5 b	375.5 ± 37.8a			
		N50	52.9 ± 11.2a	181.8 ± 1.2 b	395.3 ± 49.9c	377.0 ± 33.5a			
Shoot tissues		CK	82.8 ± 18.8a	112.0 ± 20.6a	18.7 ± 7.9a	123.3 ± 21.1a	331.9 ± 25.6 ab	0.70 ± 0.03a	0.19 ± 0.02a
	Root exposure	I0.1	138.8 ± 11.7a	118.5 ± 12.4a	51.1 ± 5.1 ab	139.3 ± 27.9a	370 ± 18.1 b	0.63 ± 0.03a	0.18 ± 0.01a
		I0.5	169.1 ± 9.5a	165.2 ± 34.3 ab	97.1 ± 18.0bc	204.4 ± 15.5 ab	270.3 ± 23.3a	0.74 ± 0.07a	0.16 ± 0.01a
		I1	312.5 ± 23.2 b	257.1 ± 19.7 b	134.7 ± 21.6c	284.7 ± 28.0 b	228.6 ± 37.7a	0.75 ± 0.04a	0.15 ± 0.01a
		N0.1	294.5 ± 25.6 b	52.9 ± 5.7a	24.4 ± 4.0a	124.1 ± 8.2a	260.6 ± 18.3a	0.66 ± 0.01a	0.16 ± 0.01 ab
		N0.5	375.6 ± 13.6 b	49.2 ± 2.6a	74.4 ± 11.9a	201.8 ± 18.4 ab	256.0 ± 45.5a	0.70 ± 0.01a	0.13 ± 0.01 b
		N1	371.7 ± 27.8 b	191.7 ± 31.0 b	140.3 ± 18.8 b	257.01 ± 27.7 b	280.0 ± 54.2a	0.74 ± 0.03a	0.13 ± 0.01 b
	Foliar exposure	N1	99.0 ± 25.3a	154.7 ± 6.1a	41.9 ± 4.1a	135.0 ± 12.9a	296.9 ± 31.5a	0.70 ± 0.08a	0.18 ± 0.01a
		N10	25.7 ± 4.5a	23.9 ± 6.9 b	23.8 ± 4.8a	117.8 ± 6.1a	359.5 ± 18.5a	0.81 ± 0.02a	0.17 ± 0.02a
		N50	56.6 ± 12.2a	20.7 ± 5.8 b	23.1 ± 3.7a	141.7 ± 9.6a	347.5 ± 31.8a	0.83 ± 0.07a	0.14 ± 0.01a

[a] Non-enzymatic antioxidants only analyzed for shoot tissue.

with increasing exposure concentrations, but their changes are irregular in shoots.

The contents of the non-enzymatic antioxidants ascorbic acid (ASA), reduced glutathione (GSH) and the carotenoid did not change significantly following any of the exposure modalities (Table 1; Table S2).

3.5. Accumulation and translocation of silver in lettuce tissue

Significant differences (Fig. 4; Table S2) were found in Ag accumulation in both roots and shoots of lettuce after 15 d of exposure for all exposure scenarios with a general concentration-dependent increase. An exposure pathway- and a particle-specific effect on the accumulation were observed as well. For instance, the Ag accumulation in whole plants (root + shoot) for $AgNPs_{(total)}$ was much higher than the accumulation for the corresponding $AgNPs_{(ion)}$ concentrations and differed by a factor of 2.7–17.4 times for root exposure and 2.9–4.1 times for single leaf exposure. In addition, at equivalent exposure concentrations, more Ag accumulated in lettuce plants following root exposure than following foliar exposure (Fig. 4 A and B), with a significant difference observed in N0.5 and N1 treatments (*t*-test, $p < 0.05$).

Regarding Ag enrichment factors (EFs), significant differences (Table 2; Table S2) among different exposure concentrations were observed for all groups with the exception of the group of root exposure to $AgNPs_{(total)}$ (ANOVA, $p = 0.285$). The EFs of $AgNPs_{(total)}$ were higher than the EFs for the corresponding $AgNPs_{(ion)}$ for root exposure (*t*-test, $p < 0.05$; Table 2) with the treatment at the lowest concentration of 0.1 mg/L as the exception, while the corresponding concentration of $AgNPs_{(ion)}$ was higher than the concentration of $AgNPs_{(total)}$ for single leaf exposure (*t*-test, $p < 0.05$; Table 2). This suggests that $AgNPs_{(particle)}$ are more inclined to be taken up by root exposure whereas $AgNPs_{(ion)}$ was more inclined to be taken up via leaf exposure. This indicates a Ag form-dependent uptake for different exposure ways. The EFs of $AgNPs_{(total)}$ in lettuce via different exposure routes follow the order: root exposure > foliar exposure > exposure via single leaf immersion. This suggests an exposure pathway-specific impact on Ag accumulation in lettuce plants.

Likewise, significant differences (Table 2; Table S2) among the translocation factors (TFs) for different exposure concentrations were only observed in the $AgNPs_{(total)}$ exposure groups via root exposure and foliar exposure, with a decreasing tendency upon increasing exposure concentration. In addition, the TFs of the $AgNPs_{(ion)}$ were higher than the corresponding $AgNPs_{(total)}$ for root exposure at all concentrations (*t*-test, $p < 0.05$; Table 2) while no significant differences were observed between $AgNPs_{(total)}$ and $AgNPs_{(ion)}$ for single leaf exposure (*t*-test, $p > 0.05$; Table 2). For $AgNPs_{(total)}$ exposure, the TFs decreased in the following order for different exposure pathways: foliar exposure > single leaf immersion exposure > root exposure. This order indicates that Ag is more inclined to be transmitted from the shoots to the roots instead of being translocated from the roots to the shoots.

3.6. Relative contribution of $AgNPs_{(particle)}$ and $AgNPs_{(ion)}$ to toxicity and accumulation

As can be seen from Table 3, in case of root exposure, the $AgNPs_{(particle)}$ contributed more to toxicity than $AgNPs_{(ion)}$ regardless of the plant tissue (root, shoot, or the whole plant). The $AgNPs_{(particle)}$ accounted for more than 65% to the overall toxicity. The contributions of the $AgNPs_{(particle)}$ to the overall toxicity show a decreasing tendency upon increasing exposure concentration. Similarly, the ratio of particles versus ions in the AgNPs suspensions decreased from 5.0 to 4.1 when the exposure concentrations increased from 0.1 to 1 mg/L. Additionally, the relative contribution of the particulate Ag form to the overall Ag accumulation in plants was much higher than the contribution of the corresponding $AgNPs_{(ion)}$ as well, accounting for about 67–95% for root exposure and 78 - 63% for leaf exposure in whole plant at all exposure concentrations. In summary, exposed plants to $AgNPs_{(total)}$ following different exposure pathways caused differences in the

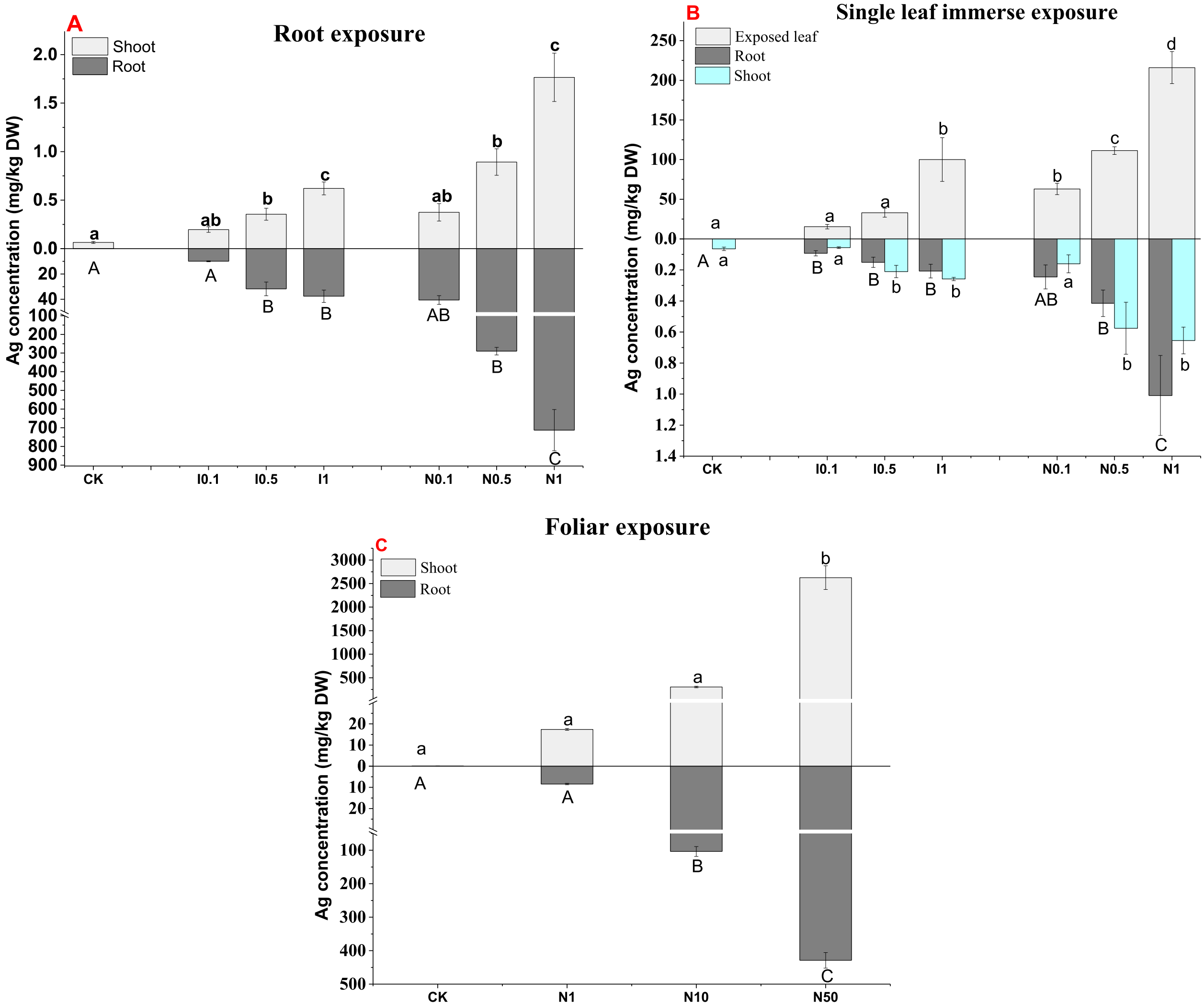

Fig. 4. Ag concentration in lettuces after exposure to different concentrations of AgNPs and corresponding dissolved Ag^{+} for 15 days. Data are mean ± SE (n = 4). Within the same plant tissue, the different letters in the same group represent statistically significant differences between the treatments at p< 0.05. I0.1, 0.5 and 1 represent *Lactuca sativa* exposed to the $AgNPs_{(ion)}$ concentrations as released from AgNPs suspensions with nominal concentrations of 0.1,0.5 and 1 mg/L; N0.1, 0.5, 1, 10 and 50 represent *Lactuca sativa* exposed to nominal $AgNPs_{(total)}$ concentrations of 0.1, 0.5, 1, 10 and 50 mg/L.

Table 2
Enrichment (EF) and transfer (TF) factors of Ag for lettuces exposed to the indicated concentrations of $AgNPs_{(total)}$ or corresponding dissolved $AgNPs_{(ion)}$. The data represent the mean ± SE (n = 4). The different letters in the same group indicate statistically significant differences between treatments at p < 0.05. * represent statistically significant differences for EFs or TFs between $AgNPs_{(total)}$ and $AgNPs_{(ion)}$ in same row(*t*-test, p < 0.05).

	Nominal exposure concentrations of AgNPs suspension	EFs		TFs	
		$AgNPs_{(ion)}$	$AgNPs_{(total)}$	$AgNPs_{(ion)}$	$AgNPs_{(total)}$
Root exposure	0.1 mg/L	0.915 ± 0.093a	0.554 ± 0.036a*	0.072 ± 0.008a	0.037 ± 0.006a*
	0.5 mg/L	0.403 ± 0.032 b	0.639 ± 0.035a*	0.043 ± 0.007a	0.014 ± 0.002 b*
	1 mg/L	0.253 ± 0.019 b	0.614 ± 0.025a*	0.042 ± 0.005a	0.009 ± 0.002 b*
Single leaf immerse exposure	0.1 mg/L	0.130 ± 0.049a	0.084 ± nd a	0.078 ± 0.018a	0.045 ± 0.012a
	0.5 mg/L	0.051 ± 0.006 b	0.027 ± 0.003 b*	0.047 ± 0.006a	0.043 ± 0.007a
	1 mg/L	0.055 ± 0.008 b	0.027 ± 0.001 b*	0.029 ± 0.007a	0.047 ± 0.013a
Foliar exposure	1 mg/L		0.188 ± 0.005a		0.174 ± 0.017a
	10 mg/L		0.193 ± 0.020 ab		0.092 ± 0.006 b
	50 mg/L		0.271 ± 0.024a		0.036 ± 0.006c

phytotoxicity and total Ag accumulation in plants, but the dominant role of $AgNPs_{(particle)}$ in the contribution of Ag accumulation was similar for the two exposure pathway. In addition, when the exposure concentrations of $AgNPs_{(total)}$ increased, the relative contribution of $AgNPs_{(ion)}$ to the overall Ag accumulation decreased for root exposure whereas the $AgNPs_{(ion)}$ contributions increased in case of foliar exposure (Table 3).

4. Discussion

In this study, the uptake, translocation and various response endpoints in lettuce after 15 days of exposure to AgNPs suspensions

Table 3
Relative contribution (%) of $AgNPs_{(particle)}$ and $AgNPs_{(ion)}$ to toxicity and accumulation at different concentrations of AgNPs suspensions.

AgNPs suspension		Root exposure				Single leaf immerse exposure	
		Relative contribution to biomass decrease		Relative contribution to accumulation		Relative contribution to accumulation	
		$AgNPs_{(particle)}$	$AgNPs_{(ion)}$	$AgNPs_{(particle)}$	$AgNPs_{(ion)}$	$AgNPs_{(particle)}$	$AgNPs_{(ion)}$
Root	0.1 mg/L	88.5	11.5	75.5	24.5	62.2	37.8
	0.5 mg/L	65.5	34.5	89.0	11.0	63.7	36.3
	1 mg/L	72.5	27.5	94.7	5.3	79.5	20.5
Shoot	0.1 mg/L	94.8	5.2	47.6	52.4	65.0	35.0
	0.5 mg/L	79.2	20.8	60.3	39.7	63.5	36.5
	1 mg/L	75.8	24.2	64.8	35.2	60.5	39.5
Whole plant	0.1 mg/L	93.4	6.6	67.3	32.7	77.6	22.4
	0.5 mg/L	76.0	24.0	87.4	12.6	68.9	31.1
	1 mg/L	74.8	25.2	94.5	5.5	63.2	36.8

and dissolved Ag-ions following foliar versus root pathway were compared. Explicitly the effects induced by ionic Ag released from AgNPs versus the particle-related effects of $AgNPs_{(particle)}$ on phytotoxicity and ROS in lettuce were differentiated. This is one of the first studies focusing on higher plants in which the exposure pathways of foliar or root exposure are considered to calculate the relateve contribution of dissolved Ag and particulate Ag to the overall Ag accumulation in plants. AgNPs are one of the most commercialized nanomaterials available (Nanotechnology - Project on Emerging Nanotechnologies, 2019; Vance et al., 2015) and (unwanted) impacts on primary producers have been studied intensively, but the focus has been mostly on aquatic primary producers, such as algae and duckweed (Tripathi et al., 2017; Yin et al., 2011), (Qian et al., 2013), (Song et al., 2015).

The results of this study demonstrate that both the released ions and particulate Ag cause adverse impacts on the growth of lettuce in a dose-dependent manner when using biomass as the endpoint of effect assessment (Fig. 2). Importantly, the results of assessment of the relative contribution to biomass reduction revealed that particulate Ag was found to dominate the toxicity of AgNPs suspensions, although the contribution of particulate Ag to the overall toxicity decreased slightly with increasing exposure concentrations (Table 3). Similarly, previous studies also reported that particulate Ag outperforms the corresponding dissolved ions with regard to the overall toxicity to other vascular plants species, including *Arabidopsis thaliana* (Qian et al., 2013) and *Lolium multiflorum* (Yin et al., 2011).

Internalization of AgNPs was reported, with their bioavailability comparable to (Stegemeier et al., 2015), lower than (Piccapietra et al., 2012), or even higher (Geisler-Lee et al., 2013) than that of Ag-ions depending on experimental conditions and plant species. In present study, the relative contributions of $AgNPs_{(particle)}$ to the overall Ag accumulation were higher than that of the corresponding $AgNPs_{(ions)}$ regardless of exposure concentrations and pathways. Moreover, the EFs of $AgNPs_{(total)}$ were slightly higher than in case of the corresponding $AgNPs_{(ions)}$ via root exposure. Taken together, these observations confirmed that $AgNPs_{(particle)}$ play a dominant role in the accumulation of Ag in lettuce exposed to $AgNPs_{(total)}$. The results obtained in this study are not in line with understanding of other researchers of uptake, as Ag-ions are thought to be more readily internalized than particulate Ag in plant tissues (Stegemeier et al., 2017) because the cell wall and the cell membrane constitute a barrier for particle internalization (Piccapietra et al., 2012). The findings of present study could be in part caused by the large proportion of the $AgNPs_{(particle)}$ in AgNPs suspensions, as exposure concentrations of $AgNPs_{(particle)}$ were approximately 5 times higher than the exposure concentrations of $AgNPs_{(ions)}$ in the AgNPs suspensions. This is in line with others studies, where the accumulation of Ag in plants was found to be positively correlated with the amount of AgNPs in the medium (Jiang et al., 2017; Li et al., 2017b; Li et al., 2015). Similarly, previous studies have also discovered that the accumulation of Ag in the AgNPs treatments was much higher than in case of Ag-ions treatments (Zhang and Wang, 2019), even at the same exposure level (Yang et al., 2019). Yang et al. confirmed the direct uptake of Ag particles; and nanoparticulate Ag was the main Ag species accumulated in plants (Yang et al., 2019). The reason they suggested for this finding is that Ag-ions bind easily to hard and soft ligand residues on the cell wall (e.g., hydroxyl, carboxyl, amino, and thiol groups), which could immobilize Ag-ions on the root surface and limit their internalization (Yang et al., 2019).

The uptake and accumulation of Ag in organisms have been reported to be responsible for the toxicity of AgNPs in many cases. Our results also agree with this general finding as upon increased Ag accumulation in plants, increased reduction in biomass was found. The pattern of $AgNPs_{(particle)}$ contribution to the overall Ag accumulation is consistent with the contribution of $AgNPs_{(particle)}$ to the overall toxicity. This suggests that the toxicity induced by the uptake and accumulation of Ag was mainly due to the intracellular uptake and accumulation of particulate Ag. After uptake and accumulation of $AgNPs_{(total)}$, particles can deposit and/or aggregate in plasmodesmata and in the cell wall (Geisler-Lee et al., 2013), which might cause mechanical damage (Peng et al., 2011) and/or the blockage of intercellular communication. This could affect nutrient uptake and translocation, and the regulation of plasma membrane receptors, as well as plasma membrane recycling and signaling (Geisler-Lee et al., 2014) in plants. Additionally, once AgNPs accumulate in plants, small amounts of Ag-ions could be released *in vivo* from the particles (Li et al., 2017b; Wang et al., 2015; Wang et al., 2017). The released Ag-ions would in-place biological transform to secondary particles (e.g. AgNPs, Ag_2S, AgCl-NPs and others Ag-species) (Li et al., 2017b; Yang et al., 2019; Wang et al., 2015). It was reported that in general the newly formed particles were about 2–3 times larger than the originally dosed AgNPs (Li et al., 2017b). Both the dissolution from the accumulated AgNPs and progress of forming secondary particles *in vivo* could also partially inhibit the plant growth (Wang et al., 2015; Wang et al., 2017).

Based on previous literature assessing the overall toxicity of nanoparticle suspensions, the main mechanism driving the phytotoxicity of nanomaterials is the production of excess reactive oxygen species and/or the cellular uptake of metallic Ag (Wang et al., 2017; Ivask et al., 2014). It was reported that induced oxidative stress levels in plants can lead to lipid peroxidation and damaged cell membrane permeability, eventually resulting in growth inhibition in plants (Silva et al., 2019). This study confirmed that oxidative stress expressed as $O_2^{\cdot-}$, H_2O_2 and MDA contents was enhanced in roots and/or shoots at higher exposure concentrations of $AgNPs_{(total)}$ relative to the control in case of root exposure. Interestingly, the ROS production in ionic treatments was higher or not significantly different from the ROS production in the corresponding $AgNPs_{(total)}$ treatments. This can be explained by the activation of the antioxidant system to counteract the elevated ROS production and maintain the redox status. For instance, following root exposure, the SOD activity in plant roots and shoots of $AgNPs_{(total)}$ treatments were higher than for the corresponding $AgNPs_{(ion)}$ treatments, suggesting that more $O_2^{\cdot-}$ in $AgNPs_{(total)}$ treatments can be catalyzed to less toxic species by SOD (Ma et al., 2016). As a result, the $O_2^{\cdot-}$ contents in plant roots and shoots of $AgNPs_{(total)}$ treatments were similar to/lower than the corresponding $AgNPs_{(ion)}$ treatments. A concentration-dependent influence on the enzymatic antioxidants can be noted because higher $AgNPs_{(total)}$ and $AgNPs_{(ion)}$ exposure concentrations induced higher enzymes activity when compared to control plants. However, following root exposure APX activity in plant roots decreased with increasing exposure concentration and POD activity was lower than in the control. This implied that when the stresses exceed the tolerance threshold of plants, the antioxidant enzyme activity is depleted. Similar results were reported by Zhang et al. (2018), who found that exposure to copper nanoparticles and ionic copper significantly decreased the antioxidant enzyme activities in wheat (*Triticum aestivum* L.) as compared to the control. Considering the results of the $AgNPs_{(total)}$ and $AgNPs_{(ion)}$ treated plants, the SOD, CAT and POD activities in $AgNPs_{(total)}$ treated plant roots and shoots were just slightly higher than or similar to the corresponding $AgNPs_{(ion)}$ treatments, and hence an ionic-specific influence on enzymatic antioxidant activities became obvious. This means that the toxicity of $AgNPs_{(total)}$ caused by oxidative damage was predominantly from the Ag-ions.

The impact of exposure pathways on toxicity and uptake of NPs in plants is still an open question. The observations from this study clearly demonstrated that root exposure to AgNPs had a stronger negative effect on plants than foliar exposure when biomass was selected as the endpoint of assessment, even though the exposure amount of total Ag (0.048 mg) was 10 times lower than the amount (1.12 mg) in case of foliar exposure. Although an irregular trend was observed for antioxidants for foliar exposure, the MDA in different treatments also indicated that root exposure induced more toxicity than foliar exposure to some extent as MDA is an indicative of the extent of lipid peroxidation content. The accumulation and translocation of AgNPs depending on the exposure pathways were also observed in present study. The plants accumulated more Ag following root exposure, but the translocation of Ag inside the plants from the exposed part to the unexposed part is more efficient in case of foliar exposure (Table 2). Not only leaf-root translocation but also leaf-leaf translocation of Ag was observed for foliar exposure. This difference is likely due to the different pathways/mechanisms involved in Ag uptake and translocation between root exposure and foliar exposure. The entrance of AgNPs into plants by foliar exposure is most likely through stomatal openings, and across the cuticles via hydrophilic pores and/or via cuticle diffusion and direct disruption (Avellan et al., 2019). After foliar uptake, ions or particles are transported to other parts of plant (unexposed leaves, roots) through the phloem system (Raliya et al., 2016; Wang et al., 2013a; Wang et al., 2012). It is reported that the pressure gradient or the mass flow of photosynthate in leaves drive the flow stream of nanoparticles and assist them to move in the phloem through phloem loading mechanisms (Raliya et al., 2016; Shahid et al., 2016; Lv et al., 2019). For root uptake of NPs, the most accepted mode is that NPs are adsorbed onto the root surface firstly and then penetrated the cell walls and the plasma membranes of the epidermal layers in the roots. The ions and particles inside plants are transported from the root to the aerial part via xylem loading by either the apoplasmic pathway or the symplastic pathway, which in turn are driven by the transpiration stream (Shahid et al., 2016). As reported (Wang et al., 2013b; Musante and White, 2012), root exposure to AgNPs suspensions can significant reduce the water transpiration, thus the upwards movement of Ag could be inhibited. This pathway likely occurred as particles trafficked through the plant organs and induced biomass reduction were reported.

The results obtained from present study have implications for food safety as the fate of AgNPs in plants was affected by the exposure concentrations and the mode of application. Moreover, since NPs are not fully removed by washing with water, AgNPs in and on crops may potentially be transferred to humans. Strategies to limit human consumption of metallic NPs originating from soil fertilizer, atmospheric deposits and agricultural foliar sprays should therefore raise more attention. In addition, the results of this study provide information on the effects of environmental transfer of nanoformulated agricultural products that are applied intentionally to roots or leaves. Furthermore, the understanding of the mechanisms of AgNPs entrance and translocation to all the plant parts via foliar or root pathway are not well-developed. Studies at subcellular levels are thus required to explore this issue in detail. Finally, literature suggests that different NPs will present different solubility and plant homeostasis and regulation. Thus, more studies involving a wider range of NPs, exposure conditions, plant species and plant growth stages should be conducted to investigate the toxicity and internalization of NPs in the future.

5. Conclusions

This research has revealed the response chains within the plants for different forms of Ag in AgNPs suspensions following different exposure routes. The action chain of toxicity of particulate Ag was induced by the penetration of AgNPs into cells, followed by the translocation to various organs and by suggested blocking of internal trafficking, thus resulting in biomass reduction. The toxicity caused by the ions in AgNPs suspension was mainly due to the generation of oxidative stress, whether induced by extracellularly adherence of ions to the plants or by the accumulation of Ag in the plants. In addition, the relative contributions of $AgNPs_{(particle)}$ to the overall toxicity and the Ag accumulation in plants of AgNPs suspensions were 75–93% and 63%–95%, respectively, regardless of exposure pathway, indicating that the $AgNPs_{(particle)}$ dominated the toxicity of AgNPs suspensions to plants rather than $AgNPs_{(ion)}$. The exposure pathway significantly affects AgNPs uptake and phytotoxicity in lettuce, with the biomass decreasing and Ag accumulation via root exposure being much higher. Although particulate Ag contributed more to the accumulation of Ag in plants, the ionic Ag was more inclined to be transported to other parts of the plant as the TFs of $AgNPs_{(ion)}$ were higher than the TFs of $AgNPs_{(total)}$. Overall, our observations, together with mechanistic explanations, will improve the understanding of the interaction of AgNPs and terrestrial plants, as well as the hazard evaluation of AgNPs exposures either being intentionally added applications in agriculture as well as unintentionally exposures from air-born emissions and soil emissions.

Acknowledgements

The authors would like to thank Rudo Verweij (Vrije Universiteit Amsterdam) and Qi Yu (Institute of Environmental Sciences (CML), Leiden University) for the technical help with the AAS measurements. The China Scholarship Council (CSC) is gratefully acknowledged for its financial support to Juan Wu (201706750014).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114117.

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