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Environmental Significance

CeO_2 nanoparticles (NPs) have shown great application potential in agriculture. However, there is no clear consensus on the biomagnification and transformation of CeO_2 NPs along terrestrial food chain. Also, the effect of trophic levels on the transfer of CeO_2 NPs is still unknown. Our results provide direct evidence that CeO_2 NPs could be transferred along a tri-trophic terrestrial food chain (lettuce-hornworm-chicken), whereas the biomagnification and biotransformation depended on the specific organisms within food chain rather than the number of trophic levels. These findings are useful for understanding the transfer of CeO_2 NPs along different terrestrial food chains and thus provide important information on their potential risk to food safety and human health.

Transfer and Transformation of CeO_2 NPs along a Terrestrial Trophic Food Chain

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Abstract

CeO_2 NPs could be inevitably exposed to terrestrial organisms and food chains with their agricultural applications. The transfer and transformation of CeO_2 NPs were therefore investigated within a tri-trophic terrestrial food chain, i.e., lettuce-hornworm-chicken. The results showed that CeO_2 NPs were accumulated by lettuce (*Lactuca sativa* L.) roots and translocated to leaves in a dose-dependent manner after exposure to CeO_2 NPs (2-1000 mg/L) for 10 days via roots. The obtained CeO_2 NP-contained leaves (14.37, 35.71 mg Ce/kg dry weight) were fed to hornworms (*Spodoptera litura* F.). The Ce contents in hornworms were 6.78 and 8.78 mg/kg dry weight, respectively, and the majority of Ce (13.51 and 26.03 mg/kg dry weight, respectively) was excreted to feces. In the case of chickens (*Gallus gallus domesticu*), Ce was mainly distributed in their intestines and stomachs from leaf and hornworm dietaries. Biomagnification factors of CeO_2 NPs were 0.179, 0.246 and 0.403 after the transfers through lettuce-chicken, lettuce-hornworm, and hornworm-chicken, respectively, suggesting that no biomagnification occurred during both bi- and tri-trophic food chain exposures. As detected by X-ray absorption near edge structure, Ce was primarily remained as CeO_2 in the lettuce roots (83.4%) and leaves (72.4%) after root exposure, Ce(III)-cysteine (52.1%) in the hornworms under trophic exposure, and CeO_2 in the intestines (85.1 and 87.1%, respectively) of chickens after leaf and hornworm dietaries. These results indicated that the transfer and transformation of CeO_2 NPs are independent of the number of trophic levels. This research provides further insight into the fate of NPs in terrestrial ecosystems and possible risk to food safety.

Keywords:

CeO_2 nanoparticles; terrestrial food chain; X-ray absorption near edge structure; biomagnification; translocation factor; biotransformation

1. Introduction

Nanotechnology, as an emerging technical revolution, has shown huge market potential and application foreground, with increasing productions of engineered nanoparticles (NPs) in recent years. One of the most widespread applications of NPs is in the agricultural industries as nanofertilizers and nanopesticides for the purpose of improving food quality and enhancing production.[1] CeO_2 NPs, the most consumed rare earth NPs, have great application potential in agriculture. CeO_2 NPs could scavenge reactive oxygen species (ROS) to protect plant from oxidative stress and increase plant growth such as cucumber and *Arabidopsis thaliana*.[2-4] In addition, Adisa et al. demonstrated that CeO_2 NPs (250 mg/L), applied by both root and foliar exposures, significantly suppressed the pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) in tomato (*Solanum lycopersicum*).[5] Along with these agricultural applications, it is inevitable that CeO_2 NPs could be transferred along terrestrial food chains. It should be noted that CeO_2 NPs are toxic to microbes, animals, and human cells.[6-8] Therefore, the transfer of CeO_2 NPs through terrestrial food chains and possible risks to food safety deserve careful investigations.

Actually, there are only three reports on the trophic transfer of CeO_2 NPs along terrestrial food chains.[9-11] Ma et al. found that CeO_2 NPs were not magnified along the soil-cultured lettuce and snail, and the trophic transfer of CeO_2 NPs from lettuce to snail was much more efficient than that under direct exposure.[9] Hawthorne et al. investigated the transfer of CeO_2 NPs through zucchini-crickets-spiders, and observed that CeO_2 NPs were not magnified along with this food chain, with the Ce contents decrease by more than an order of magnitude at each level.[10] On the contrary, Majumdar et al. demonstrated that CeO_2 NPs magnified along kidney bean plants (*Phaseolus vulgaris* var. red hawk, KBPs), Mexican bean beetles (*Epilachna varivestis*, MBB) adults and spined soldier bugs (*Podisus maculiventris*, SSBs), but did not magnify from KBPs to MBB larvae due to the different feeding habits and metabolic rates between larvae and adults.[11] There is no definitive mechanism that could explain the above inconsistent results. The transfer of CeO_2 NPs may be dependent on the

number of trophic levels within agricultural food chains, which deserves further investigation.

It should be noted that CeO_2 NPs could undergo transformation during the internalization by different organisms including plants and animals. CeO_2 NPs were reported to be transformed to Ce(III) during interaction with plants, and the Ce(III) species in plants are mainly $CePO_4$, Ce(III)-hydroxide and Ce(III)-acetate. [9, 12, 13] However, the transformation of CeO_2 NPs in animals may be in a large degree different from that in plants. To the best of our knowledge, there are currently three reports on the transformation of CeO_2 NPs in animals. For instance, the main Ce species were reported as Ce(III)-cysteine in the digestive gland of snail (*Planorbarius corneus*),[14] while Ce(III)-sulfate in *Caenorhabditis elegans*.[15] There is only one report on the transformation of CeO_2 NPs along a terrestrial food chain, in which more than 85% of Ce(IV) was reduced to Ce(III) in the digestive glands of snails (*Achatina fulica*) through the lettuce (*Lactuca sativa*)-snail food chain as detected by X-ray absorption near edge structure (XANES).[9] Whether CeO_2 NPs (and/or the produced Ce(III) species) could be further transformed by other animals or the predators (e.g., chicken) at a higher trophic level is unknown. As far as we know, there is currently no report on the transformation of CeO_2 NPs along a tri-trophic food chain. Through the investigation on a tri-trophic food chain, the transformation of CeO_2 NPs along different animals (primary and secondary consumers) will be demonstrated.

Overall, a tri-trophic terrestrial food chain along lettuce-hornworm-chicken was selected to investigate the transfer and transformation of CeO_2 NPs. The objectives of our work were: (1) to investigate the transfer and biomagnification of CeO_2 NPs within two terrestrial food chains with different trophic levels: lettuce-hornworm, lettuce-chicken and lettuce-hornworm-chicken; and (2) to determine the transformation of CeO_2 NPs at each trophic level of the tri-trophic food chain. This study will provide insights into the transfer and transformation of NPs in the agricultural food chains.

2. Materials and methods

2.1 Characterization of CeO_2 NPs

CeO_2 NPs were purchased from Sigma-Aldrich (USA). The morphology and individual size of the CeO_2 NPs were examined using transmission electron microscopy (TEM, JEM-2100, Japan) operated at 200 kV. The hydrodynamic diameters and zeta potentials of CeO_2 NPs in the plant nutrient solution (pH 6.8) as a function of exposure concentrations and times were determined by Zetasizer (Nano-ZS90, Malvern Instruments, Ltd., UK). Briefly, CeO_2 NP suspensions at the nominal concentrations of 2, 10, 100, 500 and 1000 mg/L were prepared by suspending the NPs in plant nutrient solution with sonication (100 W, 40 kHz, 30 min). These CeO_2 NP suspensions (50 mL) were treated under aeration, and 1.5-mL suspension was sampled after 2, 4, 6, 8, and 10 days for hydrodynamic diameter or zeta potential measurement. In addition, the suspended CeO_2 NPs in the suspensions (nominal concentrations: 2, 10, 100, 500 and 1000 mg/L) were digested with a microwave digestion unit (Mars, CEM, USA), and determined by inductively coupled plasma-mass spectrometry (ICP-MS, iCAPTM Q, Thermo Scientific, Germany). To detect the dissolution, the CeO_2 NP suspensions (500 and 1000 mg/L) in the plant nutrient solution were prepared by sonication in a water bath at 25 °C for 30 min, and kept in dark for 10 days. Then, the suspensions were centrifuged (10,000 rpm, 30 min) and filtered by a 0.22-μm glass filter. Ce concentration in the supernatant was then determined by ICP-MS. The plant nutrient solution (pH 6.8) is composed of (mmol/L): K_2SO_4, 0.75; $Ca(NO_3)_2$, 2.0; KCl, 0.1; KH_2PO_4, 0.25; $MgSO_4 \cdot 7H_2O$, 0.65; H_3BO_3, 0.01; $MnSO_4 \cdot H_2O$, 1.0×10^{-3}; $ZnSO_4 \cdot 7H_2O$, 1.0×10^{-3}; $CuSO_4 \cdot 5H_2O$, 1×10^{-4}; $(NH_4)_6Mo_7O_{24}$, 5×10^{-6}; Fe-EDTA, 0.1.

2.2 Lettuce growth under CeO_2 NP exposure

The seedlings (3 cm) of lettuce (*Lactuca sativa* L.) were obtained from Qingdao Seed Administration Station (China). The selected lettuce seedlings were grown hydroponically in plastic containers amended with plant nutrient solution. Lettuce seedlings were planted under 12-h light (16500 lx) at 20-25/15-20 °C (day/night) with relative humidity of 60%-70%. When the fourth leaf of each plant had emerged, the plants were exposed to the plant nutrient solution (un-exposed

control), or CeO_2 NP suspensions (at nominal concentrations of 2, 10, 100, 500, and 1000 mg/L) prepared in the plant nutrient solution via roots (root exposure). Each treatment had five replicates. After 10-day exposure, the plants from each group were collected, and washed 5 minutes with tap water and three times with deionized water. The shoot and root lengths were measured by a ruler. The leaves and roots of lettuce after root exposure were oven-dried at 60 °C to a constant weight. Dried tissues were digested with pure HNO_3 by a microwave digestion instrument. The total Ce contents in plant samples were then measured by ICP-MS. The remaining fresh leaves were obtained to feed hornworms in the following food chain experiments.

2.3 Hornworm under trophic and direct exposures

Hornworm (*Spodoptera litura* F.) larvae (second instar, about 1 cm) were purchased from Jiyuan Baiyun Industry Co., Ltd. (Henan, China), and cultured in plastic cases (12 × 10 × 3 cm). When grown to the third instar, the hornworms were randomly divided into three groups: control (fed with un-exposed lettuce leaves), trophic exposure (fed with CeO_2 NPs-contained leaves obtained from root exposure, Ce contents: 14.4 and 35.7 mg/kg dry leaves, respectively), and direct exposure (fed with CeO_2 NPs-leaf mixture, Ce content: 166.2 mg/kg dry leaves). For direct exposure, 10 μL of CeO_2 NP suspension (1000 mg/L) were dropped on each piece of leaf (with the diameter at 1.5 cm by punching) for further feeding. The high NP concentrations were selected in order to better quantify the magnitude of CeO_2 along the trophic transfer. Each group had five separate replicates and each replicate contained 20 hornworms. All three groups of hornworms were fed for 7 days, and each hornworm was fed with four pieces of leaves (diameter: 1.5 cm). During exposures, the feces of hornworms were collected for Ce content determination. Hornworm weights were recorded at 1, 3, 5, 6, and 7 days. Hornworms were sacrificed for further analysis at the 7th day by liquid nitrogen, and the Ce contents in the hornworms and feces were measured by ICP-MS after HNO_3 digestion. The remaining hornworms were stored in a freezer (-80 °C) for further chicken feeding.

2.4 Chickens under hornworm and leaf dietaries

Chickens (*Gallus gallus domesticus)* (≈120 g, 14 days of age) were divided into three groups: un-exposed control, hornworm dietary and leaf dietary. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Ocean University of China and approved by the Animal Ethics Committee of Ocean University of China. Chickens fed with feedstuff (corn 62%, corn bran 10%, soya-bean cake 17%, fish meal 9%, and bone meal 2%) only for 10 days served as the un-exposed control. For the hornworm dietary, chickens were fed with feedstuff and hornworms (8.78 mg Ce/kg dry hornworms) that were pre-fed with lettuce leaves (35.7 mg Ce/kg dry leaves). For the leaf dietary, chickens were fed with feedstuff and leaves of lettuce (35.7 mg Ce/kg dry leaves). After 10-day trophic transfer, the chickens were immediately euthanatized and dissected. The heart, liver, spleen, kidney, brain, intestine, stomach, muscle, and blood of the chickens were collected and then stored under liquid nitrogen. The feces of chickens in each group were also collected. The whole body, feces and organs/tissues were weighed, freeze-dried and ground to powder for further Ce content determination and XANES analysis.

2.5 Lettuce nutritional quality and hornworm malondialdehyde (MDA) content determination

After exposure to CeO_2 NPs at nominal concentrations of 0, 2, 10, 100, 500, and 1000 mg/L for 10 days via roots, the protein, vitamin C and Fe contents of lettuce leaves were measured. For protein, the fresh leaves were collected from the lettuce plants, frozen under liquid nitrogen, and finally homogenized with water using a homogenizer. The homogenate was centrifuged at 3,000 r/min for 10 min. The soluble protein content of the supernatant was measured by the Coomassie brilliant blue method.[16] For vitamin C, the fresh leaves were collected, cooled, and homogenized with water and 6% $HClO_4$. The homogenate was centrifuged at 3,000 r/min for 10 min. The supernatant was used to quantify the vitamin C content following the method of Yabuta et al.[17] For Fe Content, the lettuce leaves were digested with HNO_3 in a microwave digestion system. The total Fe concentrations in the leaves were measured by ICP-MS.

The MDA contents of hornworms after trophic exposure (pre-fed with leaves that contained

35.7 mg Ce/kg dry weight) were also determined. The obtained guts of hornworms after exposure were divided into foregut, midgut and hindgut. The MDA contents of the foregut, midgut and hindgut were measured by MDA assay kits (A003-1, Nanjing jiancheng bioengineering institute, China) using a Microplate Reader (Thermo, USA) at 532 nm.[10]

2.6 Identification of Ce species based on XANES analysis

Specific Ce species of CeO_2 NPs during trophic transfer were analyzed by XANES. The samples of lettuce roots and leaves after CeO_2 NP exposure (at nominal concentration of 1000 mg/L) via roots, hornworms after fed with fresh lettuce leaves (35.7 mg Ce/kg dry leaves), and intestines of chickens after fed with hornworms (8.78 mg Ce/kg dry hornworms) or leaves (35.7 mg Ce/kg dry leaves) were freeze-dried (FD5-series, SIM, USA) and ground into powders. The analysis of Ce species by XANES was performed on the beamline BL14W1 at Shanghai Synchrotron Radiation Facility (SSRF Shanghai, China). CeO_2, $CePO_4$, Ce(III)-acetate, and Ce(III)-cysteine were used as reference compounds representing Ce(IV) and Ce(III) in their solid powder forms. Ce(III)-cysteine was synthesized in the laboratory (equimolar solution of $Ce(SO_4)_2$ and L-cysteine at pH 1.0),[14] while other reference compounds were purchased from Sigma. Cerium L_{III} (5723 eV) edge absorption spectra of all the reference compounds were determined by the transmission mode. The spectra of the samples were taken in fluorescence mode with a 19-elements solid-state detector due to the low concentrations of Ce in the samples. The Ce XANES data were analyzed by linear combination fitting (LCF) using the Athena software. LCF result with the small R-factor indicated the data were valid.

2.7 Statistical Analysis

Biomagnification factor (BMF) shows the degree of biological amplification along the food chain and was estimated using eq (1). The translocation factor (TF), defined as the ratio of the Ce content (dry weight) in receiving level to that in source level, was calculated using eq (2).

$$\mathrm{BMF} = \frac{Ce\ concentration\ in\ trophic\ level\ n+1\ (mg/\mathrm{kg})}{Ce\ concentration\ in\ trophic\ level\ n\ (mg/\mathrm{kg})} \qquad \text{eq (1)}$$

$$TF = \frac{Ce\ concentration\ in\ receiving\ level\ (mg/\mathrm{kg})}{Ce\ concentration\ in\ source\ level\ (mg/\mathrm{kg})} \qquad \text{eq (2)}$$

All treatments had a minimum of three replicates and presented as the mean ± SD (the standard deviation). Statistical analysis was performed using one-way variance (ANOVA) with the Fisher LSD post hoc test by SPSS 19.0 software at $p < 0.05$.

3. Results and Discussion

3.1 CeO_2 NP characterization and the interaction of CeO_2 NPs with lettuce plants

The shape of CeO_2 NPs was irregular with the sizes varying from 5 to 50 nm as shown from the TEM image in Figure S1A. Zeta potential results showed that CeO_2 NPs (2-1000 mg/L) were negatively charged in the nutrient solutions over 10 days (Figure S1B). The hydrodynamic diameters of CeO_2 NPs were much larger than their individual sizes, indicating stronger aggregation of CeO_2 NPs in nutrient solutions (Figure S1C). The released Ce ions from CeO_2 NPs were not detected by ICP-MS after 10 days, indicating negligible dissolution of CeO_2 NPs in the nutrient solutions. After 10-day incubation, the measured concentrations of CeO_2 NPs (at nominal concentrations of 2, 10, 100, 500 and 1000 mg/L) in the plant nutrient solutions were 0.19±0.03, 0.30±0.04, 2.50±0.57, 77.75 ±5.90, and 150.77±25.79 mg/L, respectively.

As shown in Figure 1A, lettuce roots could adsorb and accumulate CeO_2 NPs at all the nominal exposure concentrations (2-1000 mg/L). The internalized CeO_2 NPs in roots could be further transported to leaves of lettuce, especially for CeO_2 NPs at higher nominal concentrations (e.g., 500, 1000 mg/L) (Figure 1B). Ce contents in leaves were 14.37 and 35.71 mg Ce/kg dry weight of CeO_2 NP treatments at nominal concentrations of 500 and 1000 mg/L, respectively. In addition, the growth of lettuce plants was also examined (Figure S2). CeO_2 NPs at low nominal concentrations (2-100 mg/L) did not affect the lengths of shoots and roots, while those at high nominal concentrations (500 and 1000 mg/L) exhibited significantly growth inhibition.

Lettuce is rich in vitamins and minerals, which are essential to organisms at higher trophic levels. The changes of protein, vitamin C and Fe contents in lettuce plants upon CeO_2 NP exposure

were therefore investigated (Figure 1C-E). Protein contents in lettuce leaves significantly increased at low nominal CeO_2 NP concentrations (2-100 mg/L), while decreasing at high nominal concentrations (500 and 1000 mg/L). The change in protein contents is in good agreement with the growth of lettuce, which should be a response to NP stress.[18] Similar changes also occurred in vitamin C (Figure 1D), in which vitamin C contents were significantly higher at low nominal concentrations of CeO_2 NPs (2 and 10 mg/L), but were significantly decreased at 500 and 1000 mg/L. Vitamin C (Ascorbic Acid) with an excellent ability to scavenge reactive oxygen species (ROS), plays important roles in the plant antioxidant defense system against external stresses.[19-21] The increased vitamin C may be a response to oxidative stress induced by NPs. In the case of Fe content, it was significantly increased by 113.67%, 90.47%, and 101.44% after exposure to CeO_2 NPs at nominal concentrations of 2, 10, and 100 mg/L, respectively. Fe acts as the cofactor of many antioxidant enzymes such as catalase and superoxide which could scavenge the ROS in plants. Therefore, the enhanced Fe content may also play a role in protecting plant against oxidative stress. All these results suggested that CeO_2 NPs changed nutrient compositions in lettuce plants, and the nutritional quality was improved at low CeO_2 NP concentrations while reduced at high concentrations.

3.2 Transfer of CeO_2 NPs from lettuce to hornworms under trophic and direct exposures

Two different exposure pathways for the transfer of CeO_2 NPs from lettuce leaves to hornworms were conducted: (1) Trophic exposure, hornworms were fed with CeO_2 NP-contained leaves of lettuce under root exposure (14.37 and 35.71 mg Ce/kg dry leaves); (2) Direct exposure, hornworms were fed with CeO_2 NP-mixed leaves of lettuce upon foliar exposure (166.2 mg Ce/kg dry leaves). After exposure for 7 days, Ce contents in hornworms and feces were determined and shown in Figure S3. Clearly, CeO_2 NPs were significantly accumulated in hornworms for both exposure pathways. Ce contents in hornworms under trophic exposure were lower than that after direct exposure due to much lower Ce content in the leaves. In addition, Ce contents in feces were

much higher than that in hornworms for both trophic and direct exposures, suggesting that most of the CeO_2 NPs (or their transformed products) were excreted through their feces. These results strongly suggest that CeO_2 NPs could be transferred along terrestrial food chains and excreted into the environment after digestion by the consumers.

BMFs between lettuce-hornworm and TFs between hornworm-feces were further calculated (Figure S4). The BMFs related to trophic and direct exposures were 0.246 and 0.369, respectively. The calculated BMFs were also the bioaccumulation factor (BAF) because CeO_2 NP-containing lettuce was the only Ce source for hornworm intake. Considering that Ce content in the leaves (166.2 mg Ce/kg) under direct exposure was even much higher than that (35.7 mg Ce/kg) under trophic exposure, direct exposure may be a more effective pathway for accumulation of CeO_2 NPs in hornworms. This finding is different from that reported by Ma et al., in which BMF of CeO_2 NPs from lettuce to snail under trophic exposure was higher than that under direct exposure.[9] The snails tended to consume more leaves under trophic exposure than those under direct exposure,[9] while the hornworms in the present study ate up all the leaves and did not show any selection tendency on lettuce leaves through trophic and direct exposures, which may explain the above inconsistent results. Thus, it can be concluded that biomagnification did not occur through this bi-trophic food chain in the present work. Majumdar et al. reported that CeO_2 NPs magnified from KBPs to MBB adults rather than MBB larvae.[11] The hornworm larvae used in this study may be a reason for the low BMF values. In addition, the TFs from hornworms to feces under trophic and direct exposures are 2.96 and 1.74, respectively. The low TFs from hornworms to feces indicated systemic saturation or a low metabolic rate of Ce in the hornworms during direct exposure.

The mortality of hornworms upon trophic and direct exposures over 7 days is shown in Figure 2A-B. At the first five days, hornworm mortalities were slowly increased for all the exposures, while significantly increased at Day 6 and Day 7 for both trophic and direct exposures. On Day 7, the mortalities reached 20% and 46.1% after trophic exposure (35.7 mg Ce/kg) and direct exposure

(166.2 mg Ce/kg), respectively. Ecdysis occurred between Day 4 and Day 5. The hornworms may be more sensitive to the accumulated CeO_2 NPs after ecdysis, which could be a reason for the increased mortality. The body weights of hornworms during trophic exposures constantly increased over 7 days (Figure S5). However, at Day 7, the weight of the hornworms under CeO_2 NP trophic exposure (35.7 mg Ce/kg) significantly decreased by 13.0% in comparison with the un-exposed control hornworms ($p < 0.05$), suggesting that CeO_2 NPs were toxic to hornworms during food chain transfer.

The digestion system acts as the main exposure and retention organs of NPs. Therefore, the MDA contents of the main digestion system (foregut, midgut and hindgut) of hornworms after trophic exposure (35.7 mg Ce/kg) were measured to assess the lipid peroxidation and toxicity of the accumulated CeO_2 NPs (Figure 2C). The MDA contents in all parts of the hornworm guts were significantly higher than those of the un-exposed control, suggesting the CeO_2 NP-treated hornworms suffered higher oxidative stress and lipid peroxidation. Marie et al. showed that CeO_2 NPs resulted in the oxidative stress and lipid peroxidation of snails after 3-week exposure.[14] This oxidative damage could be a reason for the high mortality of hornworms after trophic transfer of CeO_2 NPs. It is noted that MDA content in the midgut was much higher than that in the foregut and hindgut, suggesting that CeO_2 NPs resulted in the greatest membrane damage to the midgut. This is because that midgut is the main digestion and absorption site, playing a vital role in metal regulation and mineral accumulation.[22] It is well established that the midgut accumulates more metals (e.g., Pb) than foregut, hindgut, and other organs,[22] which could be a main target organ for the damage of CeO_2 NPs in hornworms.

3.3 CeO_2 NPs transfer in lettuce-hornworm-chicken food chain

The transfer of CeO_2 NPs in the lettuce-hornworm-chicken tri-trophic food chain was further investigated along with a bi-trophic food chain (lettuce-chicken) (Figure 3). For both food chains, Ce was detected in chickens, suggesting that CeO_2 NPs did transfer along terrestrial food chains. In the tri-trophic food chain, the Ce content in whole chickens was 3.54 mg/kg (dry weight), which was

much lower than that (6.4 mg/kg) after bi-trophic food chain transfer. However, the BMFs from both lettuce to hornworm (0.246) and hornworm to chicken (0.403) in the tri-trophic food chain were higher than that from leaf to chicken (0.179) in the bi-trophic food chain. Given that the BMFs in these two food chains were well below 1.0, the biomagnification of CeO_2 NPs did not occur. CeO_2 NPs were not biomagnified from the KBPs-MBB larvae (BMF = 0.42 ± 0.12),[11] which was in agreement with our finding. On the contrary, biomagnification of CeO_2 NPs was found from KBPs to MBB adults (BMF = 5.32 ± 0.29), indicating that the growth stage of the consumer may play an important role in the biomagnification in the food chain. The used consumers (hornworms and chickens) in the present study were at their early growth stages, which may be a reason that no biomagnification of Ce could be found.[23]

TF of CeO_2 NPs from chickens to feces was 3.74 under hornworm dietary (Figure 3). This high TF value indicates that the feces was an important route for CeO_2 NP elimination. A lot of hornworm residues were observed in the chicken feces, which may be responsible for the high concentration of Ce detected in feces in comparison with the un-exposed control. However, TF value of chickens along the lettuce-chicken bi-trophic food chain was much lower (0.567). There are two possible reasons for the low TF in lettuce-chicken food chain: (1) CeO_2 NPs tended to be accumulated in the chicken body along the short bi-trophic food chain, rather than tri-trophic food chain; (2) the bioavailability of CeO_2 NPs (and/or their transformed products) was higher in lettuce-chicken than lettuce-hornworm and/or hornworm-chicken. The bioavailability of NPs could be changed by organic compositions (e.g., enzyme, protein, and sugar) during both ingestion and digestion processes.[24]

3.4 Distribution of CeO_2 NPs in chickens after hornworm and leaf dietaries

Ce contents in different organs/tissues of chickens after food chain transfers were further investigated (Table S1). The Ce contents were 5.28 ± 1.11 and 18.2 ± 3.0 mg/kg (dry weight) in the intestines, and 3.70 ± 1.21 and 6.96 ± 0.50 mg/kg (dry weight) in the stomachs of chickens upon

dietaries via hornworm and leaf, respectively, significantly higher than those in the un-exposed chickens. The contributions of different tissues/organs in total Ce distribution under hornworm and leaf dietaries over 10 days are shown in Figure 4. Under hornworm dietary, the contributions of different tissues/organs in total Ce distribution followed: residues > intestine > stomach > brain > muscle > kidney > spleen > heart > liver. Clearly, intestine and stomach showed higher retention capacity for CeO_2 NPs, and were the main accumulative organs/tissues for CeO_2 NPs in comparison with other organs/tissues. The gastrointestinal tract is the main place for nutrient digestion and absorption. Therefore, the retention of CeO_2 NPs was high in intestine and stomach. After absorption, CeO_2 NPs were transferred to the remaining organs such as brain, muscle, kidney, spleen, heart and liver through the blood stream. Similarly, under leaf dietary, intestine and stomach were also the main Ce accumulation organs/tissues (together accounted for 87% of the total Ce).

Additionally, after hornworm and leaf dietaries, TFs from hornworm/leaf to different organs/tissues of chickens, and from different organs/tissues of chickens to feces were calculated (Table S2). Intestine and stomach showed the highest TFs under both hornworm and leaf dietaries. Notably, in the case of blood, no Ce was detected which may be due to the detoxification function of the kidney.[25] As we can see from Table S2, TFs from kidney to hornworm/leaf were higher than those from the liver, spleen and heart. A previous study showed that the kidney accumulates Cd for the purpose of eliminating toxins.[25] Additionally, Dan et al. found small size (5-10 nm) CeO_2 NPs could be eliminated from the blood by the kidney.[26]

3.5 Speciation of Ce within a tri-trophic food chain

The species of Ce in lettuce, hornworms, and chickens during food chain transfer were analyzed using XANES. Normalized XANES spectra of the reference compounds and samples are presented in Figure 5A. The spectra of Ce in all of the five samples exhibited a mixture of Ce(III) and Ce(IV), indicating the biotransformation of CeO_2 NPs during trophic transfer. To obtain the quantitative data on Ce speciation, LCF was performed on the normalized XANES spectra. As shown in Figure 5B,

83.4% Ce(IV) and 16.6% Ce(III) were identified in the lettuce roots, and $CePO_4$ was the main Ce(III) species. It is confirmed that CeO_2 NPs could be adsorbed on the root surfaces of hydroponic cucumber plants, and then partially dissolved to Ce(III) ions or transformed to Ce(III) on the particulate surface via root exudates, and finally, precipitated as $CePO_4$ in the presence of phosphate, [27] which can clearly explain our finding on Ce bio-reduction. After transferring to the leaves, the proportion of Ce(III) significantly increased from 16.6% (in roots) to 27.6%. The dissolved Ce(III) ions may be transferred into vascular tissue, where they combined with carboxyl compounds to form complexes such as Ce(III)-acetate and finally translocated to the leaves via water transport.[28] The poly(acrylic acid) coated CeO_2 NPs could be transported into chloroplast by nonendocytic pathway,[4] and distributed mainly in the plant leaves. Therefore, the CeO_2 NPs transported to the leaves could be further transformed to Ce(III) species. This is another explanation for the high percentage of Ce(III) species in the leaves in the present work.

In the case of the hornworms, the Ce species were CeO_2 (36%), Ce(III)-cysteine (52.1%), $CePO_4$ (6%) and Ce(III)-acetate (5.9%). The proportion of Ce(III) species contributed to 64% of the total Ce in the hornworms. Additionally, the guts of the hornworms have diverse enzymes and metalloproteins, containing high amounts of thiol groups,[29, 30] which should be one reason for the observed Ce(IV) reduction and the formation of stable Ce(III)-cysteine complexes. Ce(III)-cysteine could immobilize Ce by reducing the bioavailability and mobility of dissolved Ce(III). In the intestines of chickens under leaf and hornworm dietaries, Ce(IV) measured out as 85.1% and 87.1%, respectively. Interestingly, Ce(III) was partially oxidized to Ce(IV) in the intestines of chickens. It is reported that nitrate and nitrite in the chicken intestines could be transformed to NO• radical, a source of radical peroxynitrite ($ONOO^-$) with high oxidizing capacity.[31, 32] Under the interaction with NO• radical, Ce(III) could be oxidized into Ce(IV). In addition, diet, intestine bacteria and body metabolites work together to the physicochemical/redox environment of intestine.[32] The contribution of the specific substances to the transformation of Ce(III) to Ce(IV) in chickens is complicated, and

requires further study.

4. Conclusions

The present study investigated the transfer and transformation of CeO_2 NPs through lettuce-hornworm-chicken food chain. CeO_2 NPs were taken up by lettuce roots and transferred to the leaves in a dose-dependent manner. The protein, vitamin C and Fe contents of lettuce leaves were increased upon exposure to CeO_2 NPs at low nominal concentrations (2-100 mg/L), but decreased at high nominal concentrations (500 and 1000 mg/L). Hornworms accumulated more CeO_2 NPs upon direct exposure in comparison with trophic exposure. After transferred to chickens, Ce was mainly distributed in the digestive organs including intestine and stomach under both hornworm and leaf dietaries. Subsequently, a large amount of Ce fraction was detected in the feces, showing that CeO_2 NPs (and/or other Ce species) could be excreted along food chains. However, biomagnification of CeO_2 was not observed along lettuce-hornworm, lettuce-chicken, and lettuce-hornworm-chicken food chains. Based on XANES analysis, CeO_2 was the predominated species in the roots (83.4%) and leaves (72.4%) of lettuce upon root exposure, while 64% of CeO_2 was reduced to Ce(III) species in the hornworms after trophic exposure. When transferred to the chickens, Ce was transformed back to CeO_2 in the intestines (85.1 and 87.1%) of chickens after leaf and hornworm dietaries. These results provide direct evidence that CeO_2 NPs could be transferred along the bi- and tri-trophic terrestrial food chains, whereas the biomagnification and biotransformation depended on the specific organisms within food chain rather than the number of trophic levels. It should be noted that CeO_2 NPs (or their transformed products) could be partly excreted from hornworms and chickens via feces. Given that Ce in the feces could be further taken up by decomposers and re-cycled in the food chains, possible environmental impacts of CeO_2 NPs and their transformed products deserve further investigation. These findings are useful for understanding the transfer of CeO_2 NPs along different terrestrial food chains and the fate of CeO_2 NPs during interacting with biological systems, thus providing important information for their potential impact on food safety and human health.

Supplementary Information

Five figures and two tables. This material is available free of charge via the Internet at http://pubs.rsc.org/.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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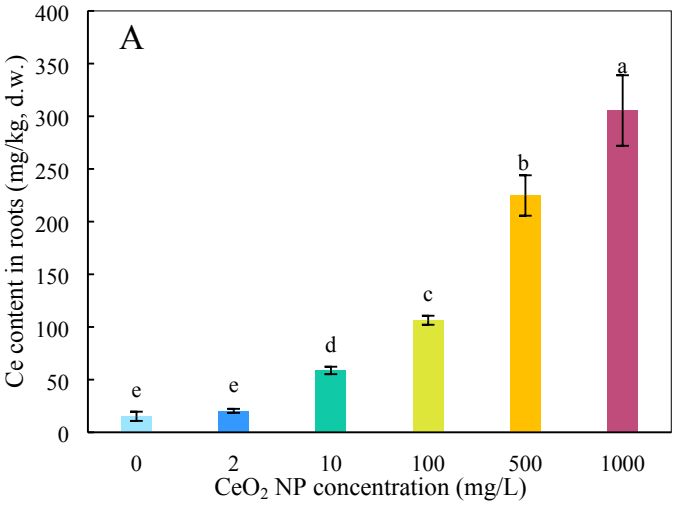
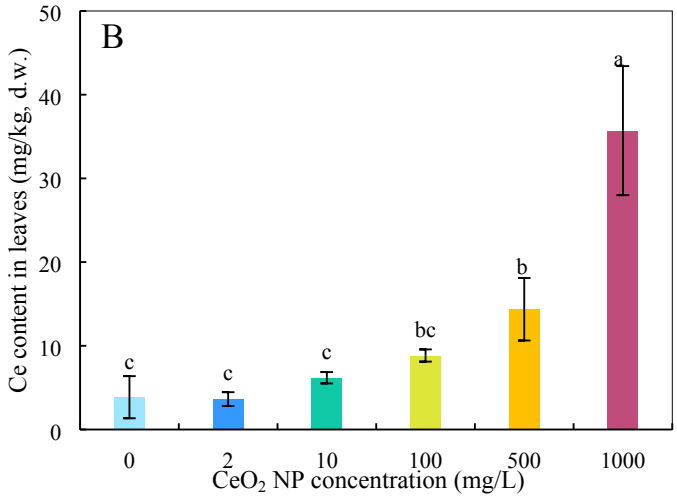
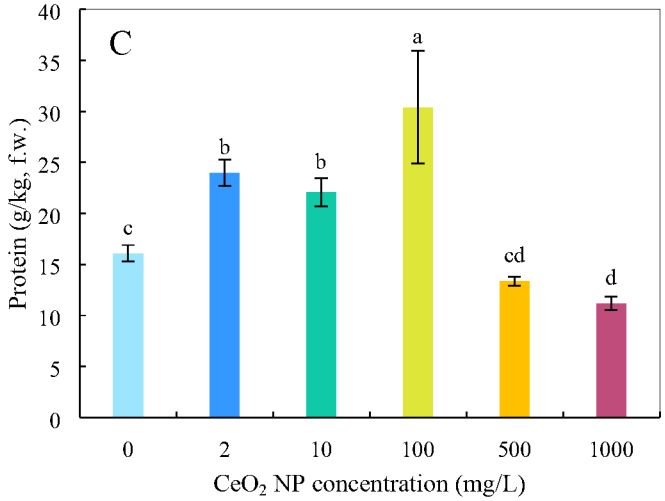
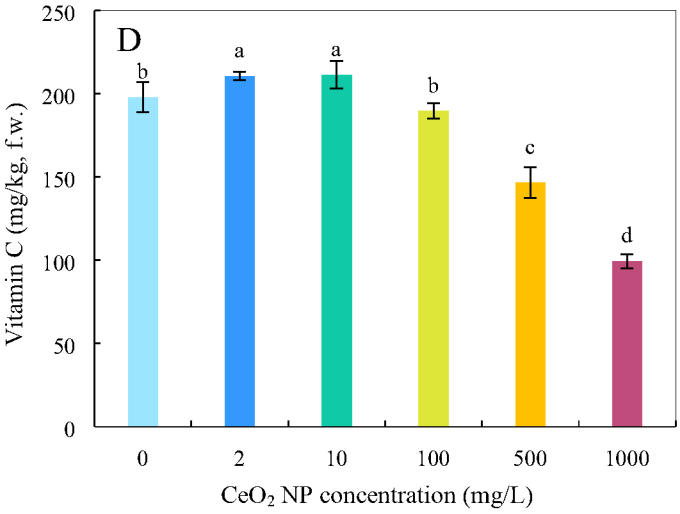
Figures:


A
Ce content in roots (mg/kg, d.w.)
400
350
300
250
200
150
100
50
0
e
e
d
c
b
a
0
2
10
100
500
1000
CeO2 NP concentration (mg/L)


B
Ce content in leaves (mg/kg, d.w.)
50
40
30
20
10
0
c
c
c
bc
b
a
0
2
10
100
500
1000
CeO2 NP concentration (mg/L)


C
Protein (g/kg, f.w.)
40
35
30
25
20
15
10
5
0
c
b
b
a
cd
d
0
2
10
100
500
1000
CeO2 NP concentration (mg/L)


D
Vitamin C (mg/kg, f.w.)
250
200
150
100
50
0
b
a
a
b
c
d
0
2
10
100
500
1000
CeO2 NP concentration (mg/L)

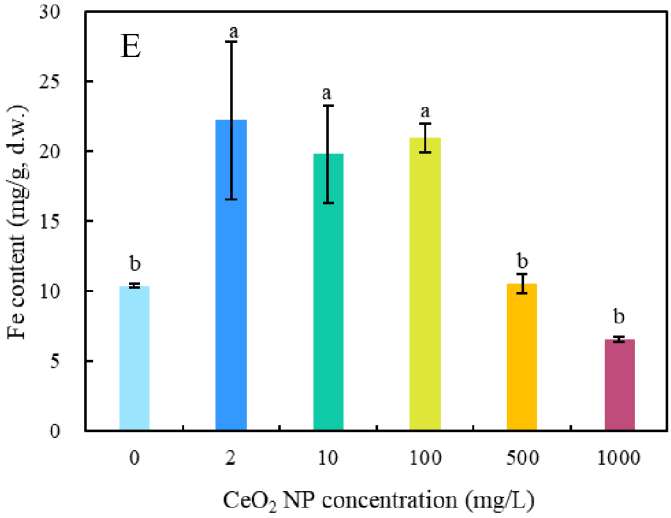

E
Fe content (mg/g, d.w.)
30
25
20
15
10
5
0
b
a
a
a
b
b
0
2
10
100
500
1000
CeO2 NP concentration (mg/L)

Figure 1. The Ce contents in lettuce plants and nutritional quality of lettuce leaves. (A), (B): Ce contents in lettuce roots and leaves, respectively. (C)-(E): protein, vitamin C and Fe contents in lettuce leaves, respectively. The lettuce was treated with CeO_2 NPs (nominal concentrations: 0-1000 mg/L) under root exposure for 7 days. Values are expressed as mean ± SE (n = 3). Different letters (a-e) denote significant difference. f.w.: fresh weight, d.w.: dry weight.

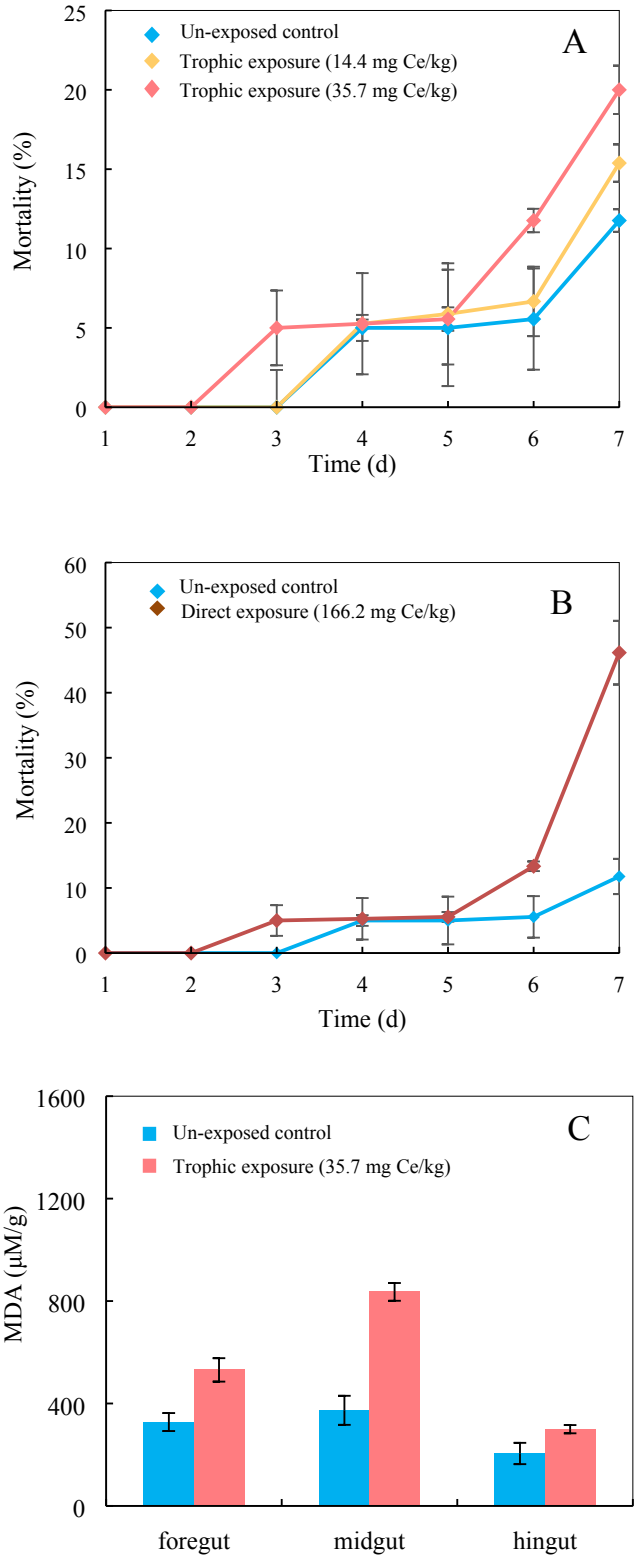


Figure 2. Mortality of hornworms during trophic and direct exposures, and MDA contents of gut tissues in hornworms under trophic exposure. (A): Mortality of hornworms under trophic exposure.

Hornworms were fed with lettuce leaves under root exposure (14.4 and 35.7 mg Ce/kg dry leaves, respectively). (B): Mortality of hornworms under direct exposure. Hornworms consumed leaves of lettuce under foliar exposure (166.2 mg Ce/kg dry leaves). (C): MDA contents in gut tissues of hornworms under trophic exposure (35.7 mg Ce/kg dry leaves). Error bars indicate standard deviations (n = 20).

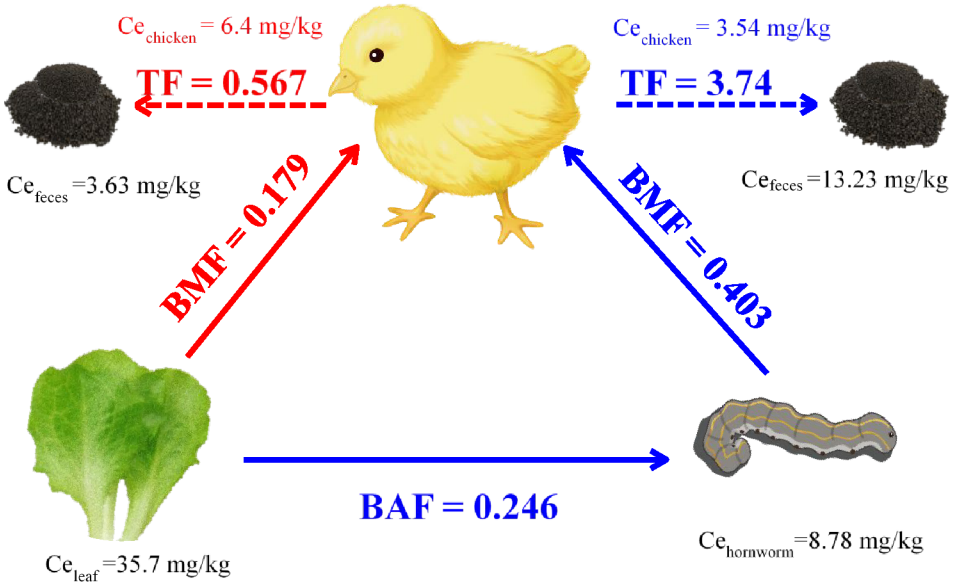
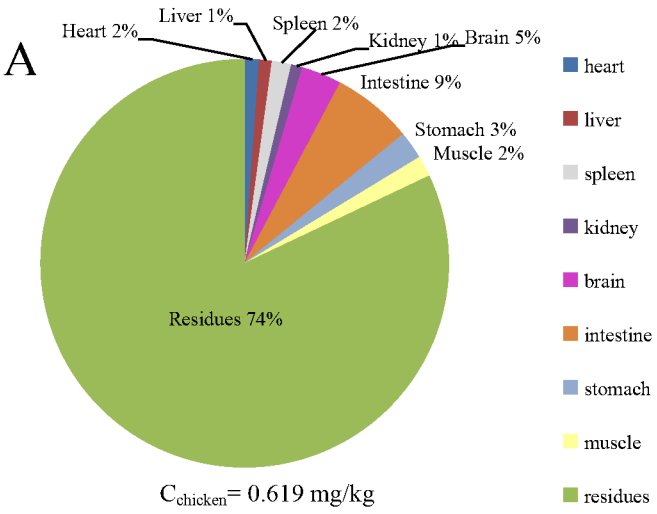
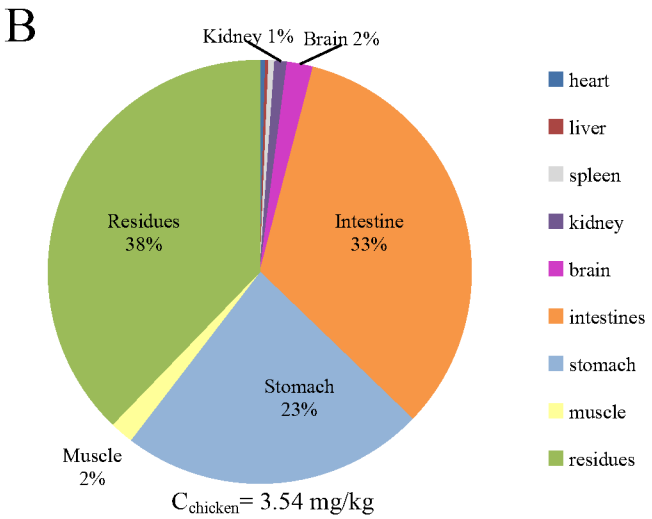


Figure 3. Bioaccumulation of Ce along different food chains. The red line represented lettuce-chicken food chain. The blue line indicated lettuce-hornworm-chicken food chain. The consumed leaves were collected from lettuce under root exposure (35.7 mg Ce/kg dry leaves). $Ce_{chicken}$ in red and blue color indicate the Ce contents in chickens transferred from lettuce leaves and hornworms, respectively. BMF: biomagnification factor, TF: translocation factor.


A
Heart 2%
Liver 1%
Spleen 2%
Kidney 1%
Brain 5%
Intestine 9%
Stomach 3%
Muscle 2%
Residues 74%
heart
liver
spleen
kidney
brain
intestine
stomach
muscle
residues
$C_{chicken}$= 0.619 mg/kg


B
Kidney 1%
Brain 2%
Residues 38%
Intestine 33%
Stomach 23%
Muscle 2%
heart
liver
spleen
kidney
brain
intestines
stomach
muscle
residues
$C_{chicken}$= 3.54 mg/kg

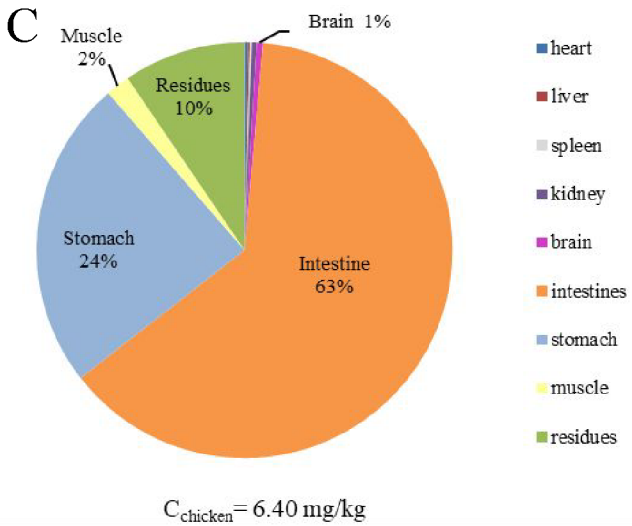

C
Muscle 2%
Brain 1%
Residues 10%
Stomach 24%
Intestine 63%
heart
liver
spleen
kidney
brain
intestines
stomach
muscle
residues
$C_{chicken}$= 6.40 mg/kg

Figure 4. Ce distribution in chicken organs under hornworm and leaf dietaries over 10 days. (A): Un-exposed control; (B): Hornworm dietary: chickens were fed with hornworms that consumed the leaves of lettuce under root exposure (35.7 mg Ce/kg dry leaves); (C): Leaf dietary: chickens were fed with the leaves of lettuce under root exposure (35.7 mg Ce/kg dry leaves). “Residues” include all the rest parts except for feather and bones.

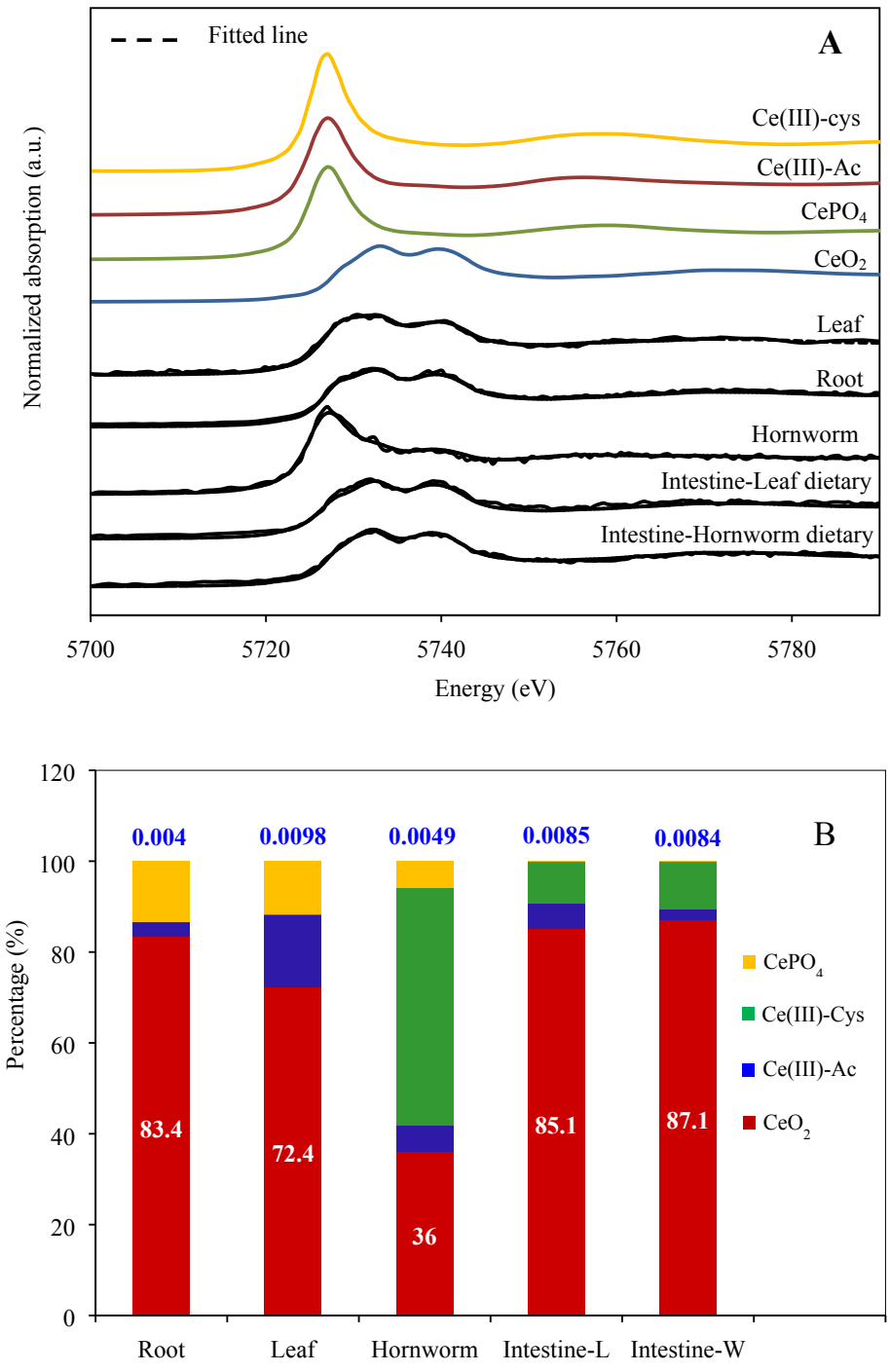
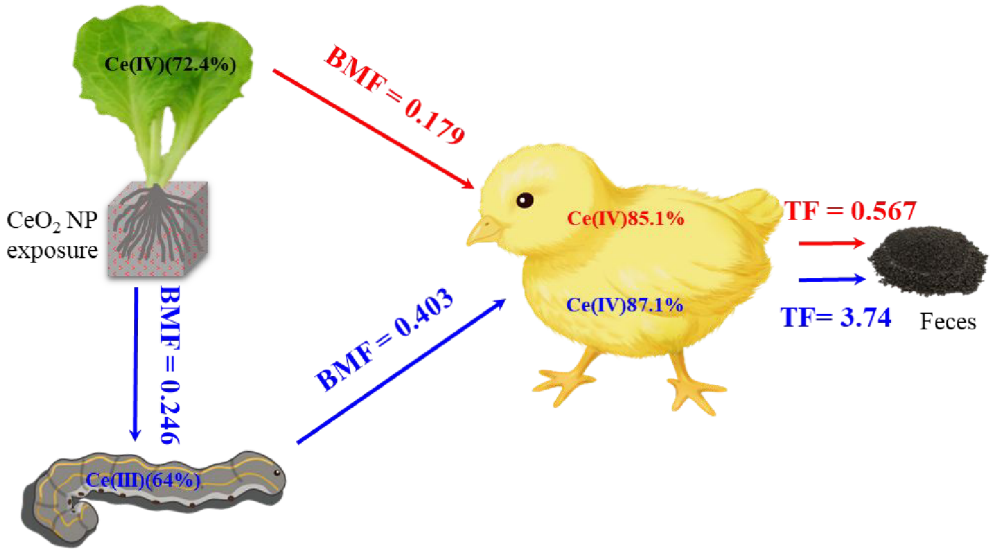


Figure 5. Ce L_{III}-edge XANES spectra (A) and percentages of Ce species (B) in different plant tissues, hornworms and chicken intestines. The black dashed line is the fitted line. The blue number is the R-factor of each sample. The root and leaf samples were collected from the lettuce plants after roots exposure to CeO_2 NPs (35.7 mg Ce/kg dry leaves). Hornworm samples were obtained after

consuming the above lettuce leaves for 7 days. Intestine-L: intestines of chickens after leaf dietary. Intestine-W: intestines of chickens after hornworm dietary. Ce(III)-cys: Ce(III)-cysteine; Ce(III)-Ac: Ce(III)-acetate.

Graphic Abstract



CeO_2 NPs could be transferred along a lettuce-hornworm-chicken terrestrial food chain, and the transformation of CeO_2 NPs was depended on the organisms within food chains.