Zinc, copper, or cerium accumulation from metal oxide nanoparticles or ions in sweet potato: Yield effects and projected dietary intake from consumption

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Running head: Accumulation of metals from metallic oxide nanoparticles and ions in sweet potato tubers

Abstract

The potential release of metallic oxide engineered nanoparticles (ENP) into agricultural systems has created the need to evaluate the impact of these materials on crop yield and food safety. The study here grew sweet potato (*Ipomoea batatas*) to maturity in field microcosms using substrate amended with three concentrations (100, 500 or 1,000 mg kg DW⁻¹) of either *n*ZnO, *n*CuO, or *n*CeO₂ or equivalent amounts of Zn²⁺, Cu²⁺, or Ce⁴⁺. Adverse effects on tuber biomass were observed only for the highest concentration of Zn or Cu applied. Exposure to both forms of Ce had no adverse effect on yield and a slight positive benefit at higher concentrations on tuber diameter. The three metals accumulated in both the peel and flesh of the sweet potato tubers, with concentrations higher in the peel than the flesh for each element. For Zn, >70% of the metal was in the flesh and for Cu >50%. The peels retained 75 to 95% of Ce in the tubers. The projected dietary intake of each metal by seven age-mass classes from child to adult only exceeded the oral reference dose for chronic toxicity in a scenario where children consumed tubers grown at the highest metal concentration. The results throughout were generally not different between the ENP- and ionic-treatments, suggesting that the added ENPs underwent dissolution to release their component ions prior to accumulation. The results offer insight into the fate and impact of these ENPs in soils.

Author keywords: Nanoparticles, Nanomaterials, Metals, ZnO, CuO, CeO₂, Sweet potato

1.0 Introduction

Nanotechnology is developing rapidly into a trillion-dollar industry [1]. One facet of this industry involves the production of engineered nanoparticles (ENPs), a material with at least two dimensions between 1 and 100 nm. Nanoparticles often display properties different than their bulk (>100 nm in at least two dimensions) or ionic counterparts due to their composition, surface properties, and high surface to volume ratio. Metallic nanoparticles such as ZnO, CuO, and CeO₂ are among the most common materials in commercial use due to their numerous applications. Nanoparticles of ZnO (*n*ZnO) have uses in personal care products like sunscreens as well as applications in catalysis, chemical manufacturing, optical devices, and biosensors [2]. For CuO nanoparticles (*n*CuO), there are uses as an antimicrobial agent, in chemical catalysis, and in a variety of products such as inks, ceramics, lubricants, and electronics [3]. Nanoparticles of CeO₂ (*n*CeO₂) have applications in fuel catalysis, paints and UV-protectant coatings, or electrolytic processes [4].

The rapid expansion of nanoparticle use has raised concerns about their prospective release into agricultural systems through application of biosolids or nano-functionalized agrochemicals and the potential impact of these engineered materials on crop plants and food safety. A wide variety of studies have been conducted with crop plants to examine the toxicity of metallic nanoparticles [for reviews, see 5, 6-9]. Many of these studies have been conducted under idealized conditions, such as in hydroponics or soilless media, and over relatively short periods of time. The primary goals of these studies have been to assess the physiological effects of the nanomaterials and determine their inherent toxicity. Additional studies have considered the genotoxicity of ENPs to plants [10-13] or used -omic technologies to determine how ENPs influence gene and protein expression [14-18]. More recently, some studies have examined the

influence of exposure during the entire life cycle [4, 19-22]. Such studies provide a much needed perspective on the impact on yield of agricultural crops and not just the short-term physiological effect on growth. The resulting accumulation of metals from metallic oxide ENPs such as *n*ZnO, *n*CuO, and *n*CeO₂ have also been reported in these studies, providing information useful in determining the potential impact of ENPs on the safety of the edible plant tissues.

To provide additional information on the influence of metallic oxide nanoparticles on the yield of an agricultural crop, the present research compared the impact of three materials in nanoparticle (*n*ZnO, *n*CuO, or *n*CeO₂) or ionic form (Zn²⁺, Cu²⁺, or Ce⁴⁺) on the yield of sweet potato (*Ipomoea batatas* (L.) Lam). These nanomaterials were selected because they represent three of the ten most commonly used nanomaterials [23]. The ionic forms of each metallic nanoparticle, added in parallel pots at the equivalent concentration of Zn, Cu, or Ce, were included for comparison. Sweet potato was the crop species examined because it is the third most common starchy root crop and the seventh most important crop globally [24]. There have been no prior studies with nanomaterials and this plant species. The sweet potato tuber has an outer “peel” that can be consumed hence the potential accumulation of metals in this layer has implications for food preparation and food safety. For example, the outer cell layer of carrot (*Daucus carota*) was shown recently to retain a large fraction of metals from a similar exposure regime of nanoparticle or ionic Zn, Cu, or Ce [25]. Removal of this layer greatly reduced the projected dietary exposure to these metals that might result from consumption [26]. A similar question was addressed here, estimating dietary intake of Zn, Cu, or Ce that might occur from the consumption of sweet potato (with or without the tuber peel) grown under each metal exposure regime. The overarching goal of this study was to provide insight into the potential fate, effect, and risk of these metal oxide nanomaterials in agricultural systems.

2.0 Materials and Methods

2.1 Microcosm experiment

Sweet potato (*Ipomoea batatas* var. Georgia Jet) plants were obtained as slips (30-35 cm in length) from New Hope Seed Company (Bon Aqua, TN). The slips were separated and planted temporarily in pots of Fafard 3B mix (Hummerts International, St. Louis, MO) until the nanoparticle-treated soils were prepared. During this growth period, the pots with the sweet potato slips were maintained under natural outdoor conditions (ambient light, natural precipitation with supplementary tap water as needed to maintain hydration). Plants for the Zn-, Cu-, and Ce-treated pots were grown under these conditions for 7, 9, and 16 d respectively. A minimum of one week of growth in the potting mix was allowed for the plants to acclimate and resume root growth. However, as it was not possible to establish all the microcosms for all three elements on the same day, the experiment was set up at intervals by metal in the order listed above. That is, the microcosms for the Zn treatment were established 7 d after the slips were planted in the potting mix, those for Cu 2 d later, and those for Ce one week later. These intervals were needed to allow sufficient time to establish all the treatments and replicates for each metal. The experiment design was not intended to make comparisons between the metal treatments so the logistical necessity of this planting schedule was utilized.

Fafard 3B potting mix, perlite, and sand were mixed in a 3:3:1 ratio (v/v/v) to provide a substrate suitable for sweet potato propagation. After mixing, 3 kg of the substrate mix was placed into a plastic pot and amended with 10-30-20 fertilizer along with supplemental KCl to provide final NPK application rates of 67.3, 201.8, and 201.8 kg ha⁻¹, respectively. Each plastic pot was seated on top of a 18.9 L bucket, providing a microcosm structure that allowed free flow of soil water through the pot to collect in the bucket. This approach was used to prevent

accumulation of excess water in the rhizosphere that would adversely affect sweet potato growth. Throughout the experiment, any discharge into the bucket was watered back into the pot to preclude losses of fertilizer or the amended treatments.

Three nanoparticle suspensions were obtained from US Research Nanomaterials, Inc. (Houston, TX): ZnO (30-40 nm), CuO (25-55 nm), and CeO₂ (30-50 nm). All suspensions were at least 99% purity. Aliquots were withdrawn from the nanoparticle stock suspension and diluted in deionized water to a total volume of 2 L. These solutions were opaque due to the high concentration of nanoparticles, limiting the utility of dynamic light scattering to collect data on the nanoparticles. These working stock suspensions were used to immediately treat the microcosms so the characteristics of the nanoparticles would have immediately changed upon addition to the soil. The ENPs were introduced to the 3 kg of soil by mixing the 2 L of the respective working stock suspension with the soil until evenly wetted. The corresponding solutions of ionic Zn, Cu, and Ce were prepared by dissolving the required mass of the sulfate salts in deionized water and amending them in the same manner as the ENP solutions. The final treatment concentrations in the substrate were 100, 500, and 1,000 mg kg DW⁻¹ of Zn, Cu, or Ce. The volume of water added brought the soil to ~80% of field capacity. The soils were allowed to equilibrate indoors under ambient conditions for 48 hr. Sweet potato slips were transplanted from the temporary pots to an ENP or ionic treated pot (one plant per pot) by placing the bottom of the slip 10.5 cm into the soil and then lightly packing the soil around the plant stem. Plants were grown under conditions of natural sunlight, temperature, and precipitation for 130 days. Supplemental tap water was added as needed to maintain soil hydration. Any water flowing through the pots was irrigated back into the pot during this time.

At harvest, the tubers were recovered from the pots and immediately brushed to remove adhering soil particles. The tubers were allowed to cure by being placed in brown paper bags and kept in a phytotron at 18-22°C. Once the potatoes had cured for at least 15 d, the tubers were rinsed with deionized water and lightly scrubbed with a brush to clean the tuber surface. While this may not have achieved complete removal of adhering soil particles, the goal was to emulate a typical harvesting procedure. One tuber free from cracks or defects of at least 9 cm in length, 5 cm in diameter, and 118 cm³ in volume, corresponding to U.S. Extra No. 1 grade, was selected from each harvested pot for analysis. Each of these tubers was cut into quarters along the horizontal axis. Two of the quarters were peeled while the other two were not peeled. All of the quarters were then cut into pieces no bigger than 0.5 cm. Half of the tuber (one quarter peeled and one quarter not peeled) were dried to constant mass at 65°C. The other half of the tuber was placed in a -80°C freezer for storage and subsequent study. Samples for elemental analysis were subjected to acid digestion according to USEPA method 3050b [27] and analysed for Zn or Cu using a SpectrAA 220FS Atomic Absorbance Spectrometer (Varian Inc., Palo Alto, CA) or for Ce using inductively coupled plasma mass spectroscopy (ICP-MS, Agilent 7500ce, Santa Clara, CA).

2.2 Dietary intake modeling for Zn, Cu, or Ce

The modeling of dietary intake utilized the fresh weight tissue concentration of Zn, Cu, or Ce. The fresh weight concentration was back-calculated using the dry weight concentration and the percent water content of the tuber tissue. Human dietary intake was calculated using the following formula [28, 29], where dietary intake is expressed in units of $\mu\text{g kg}^{-1} \text{ d}^{-1}$, tissue

concentration is in mg kg FW^{-1} , mass consumed and body mass are both in kilograms, and a conversion factor of 1,000 is included to adjust the units from milligrams to micrograms.

$$\text{dietary intake} = \frac{\text{tissue concentration} \times \text{mass per serving} \times \text{\#of servings} \times 1,000}{\text{body mass}}$$

Dietary intake was calculated for consumption of unpeeled or peeled sweet potato exposed either to the nanoparticle or the corresponding ion. The mass per serving of sweet potato (0.114 kg) was derived from the USDA Food and Nutrition Service (http://www.fns.usda.gov/sites/default/files/HHFS_SWEET%20POTATOES_FRESH_Dec%202012.pdf, November 2, 2015). The standard body mass values for age groups from 1-3 years through adult females and males was based on values used by the U.S. National Academy of Sciences [30]. The specific age-mass groups used were child 1-3 years (13 kg), child 4-8 years (22 kg), children 9-13 years (40 kg), adolescent female 14-18 years (57 kg), adolescent male 14-18 years (64 kg), adult female 19+ years (61 kg), and adult male 19+ years (76 kg). To provide a frame of reference for the interpreting the calculated dietary intake rates for Zn and Cu were compared to oral reference doses (oral RfD) for the elements. The oral RfD represents the daily exposure to a potential hazard that is likely to be without risk of deleterious effects over a lifetime [31]. The oral RfD values for Zn ($300 \mu\text{g kg}^{-1} \text{d}^{-1}$) and Cu ($40 \mu\text{g kg}^{-1} \text{d}^{-1}$) were obtained from the U.S. EPA Health Effects Assessment Summary Tables (HEAST)[32]. There is no established oral RfD for Ce. To provide surrogate parameters for comparison, the oral RfD values for nine other rare earth elements (Eu, La, Ly, Nd, Pr, Sc, Sm, U, Y) were obtained.[33] As these values range from 0.9 to $500 \mu\text{g kg}^{-1} \text{d}^{-1}$, the mean ($171.9 \mu\text{g kg}^{-1} \text{d}^{-1}$) and median ($5 \mu\text{g kg}^{-1} \text{d}^{-1}$) values were calculated and used here as general indicators of the oral RfD for Ce.

169

170 2.3 Data analysis

171 The soil to tuber transfer factor (TF) was calculated as the ratio of the concentration of
172 metal in the unpeeled or peeled tuber divided by the metal concentration (on a dry weight basis)
173 in the soil. The percent of Zn, Cu, or Ce element in either the tuber peel or tuber flesh was
174 calculated by dividing the mass of the element in that tissue (peel or flesh) by the total mass of
175 that metal in the entire unpeeled tuber. Each tuber yield parameter and the tuber metal
176 concentration was analyzed using a two-way ANOVA with concentration and chemical form
177 (ENP or ionic) as the main effects. For the transfer factor, a three-way ANOVA was used with
178 concentration, chemical form, and peeling (i.e., unpeeled or peeled tubers) as the main effects for
179 a given element. If interactions between main effects were observed, the data were reanalyzed
180 treating interacting factors as independent treatments. Analyses were carried out in SPSS (ver.
181 22) [34] with Duncan's test used for a post hoc comparison of the means.

182

183 3.0 Results

184 3.1 Tuber formation and biomass

185 There were no apparent signs of stress or toxicity in the aboveground tissues for any of
186 the plants throughout the growth period. By the time of harvest, most of the leaves had naturally
187 abscised from the stems, precluding an accurate measurement of aboveground biomass. There
188 were some pots in all treatments, including the control that did not produce commercial grade
189 tubers. These pots produced sweet potato culls (soft, poorly shaped, cracked, or undersized
190 tubers) that are not marketable yield or retained for human consumption. These pots were
191 excluded from further analysis. As a whole, the control pots showed 80% success in tuber

formation (i.e., 4 out of 5 pots formed commercial grade tubers, Table S1). Results from the metal nanoparticle and ionic treatments were similar to the control with the exception of those receiving the highest ionic Zn concentration (40%, 2 out of 5 pots formed tubers). Yield parameters for pots that successfully formed tubers are summarized in Table 1. None of the parameters showed a significant difference between the ENP and ionic forms. There were no significant differences as a function of concentration for most parameters. Some differences observed for tuber biomass or number of tubers occurred for the highest treatment concentration relative to the other concentrations. There was a significant effect of Cu concentration on tuber diameter and length and for tuber diameter in response to Ce.

3.2 Metal concentration in the tubers

There was a two-way interaction between the tissue type and concentration for Zn and Ce concentration in the tubers while for Cu there was three-way interaction between tissue type, concentration, and form (Figure 1). A general concentration-dependent increase was observed for metal concentrations of each element with peels showing the highest concentration. The magnitude of the difference in peel concentration relative to that of unpeeled or peeled tubers was greatest for Ce and least for Zn. The concentration of Cu and Ce in unpeeled tubers was typically significantly less as compared to unpeeled tubers.

3.3 Contribution of the tuber peel to metal retention

The contribution of the tuber peel to the retention of Zn, Cu or Ce was examined in two ways – using the soil to tuber transfer factor (ratio of tuber concentration to soil concentration) and by expressing the metal accumulation results on a mass basis in terms of the total percent of

tissue metal present in the tuber peel relative to the entire tuber. The transfer factor (TF) values were determined for unpeeled or peeled tubers with ENP or ionic treated sweet potatoes (Figure 2). There was no significant difference in TF for Zn between metal form (ENP or ionic) and for unpeeled and peeled tubers. The TF for the 100 mg Zn kg DW⁻¹ concentration was significantly higher than the other two treatments, although the magnitude of the difference was no more than two- to three-fold. There was a significant two-way interaction between concentration and peeling for Cu. For Ce there were no significant differences as a function of form or concentration. There was however a highly significant difference between unpeeled and peeled tubers. The metal accumulation and the TF values for Zn indicated that the peels represented a consistent but small percentage of the total metal accumulated by the tuber (data not shown). The tuber peel accounted for 30 to 70% of the total metal associated with Cu-treated plants (Figure 3). The percentage values for Ce were higher, ranging from >75% to >95% of the total metal.

3.4 Projected dietary intake of Zn, Cu, or Ce from consumption of treated tubers

Dietary intake modeling was performed for all three elements and using unpeeled or peeled tubers from the two highest ENP or ionic exposures (Figure 4). There was little difference in projected dietary intake of Zn from unpeeled or peeled tubers from ENP-treated plants and none of the values for any age-mass class approached the oral RfD for Zn. For tubers from the ionic Zn-treated plants, the projected dietary exposures were similar to the values for the ENP form for the 500 mg Zn kg DW⁻¹ treatment but higher for the 1,000 mg Zn kg DW⁻¹ exposure level. The oral RfD for Zn was exceeded only in one instance, namely for the 1-3 year old child age-mass class and the 1,000 mg Zn kg⁻¹ ionic treatment. The projected dietary

exposure for Cu exceeded the oral RfD only for the highest ENP concentration, unpeeled tubers, and the smallest of the age-mass classes. The projected dietary exposures from consumption of unpeeled, ENP-treated tubers at 500 mg Cu kg DW⁻¹ were similar to those for the 1,000 mg Cu kg DW⁻¹ ionic exposure, although the values were higher than the corresponding projected intakes for unpeeled tubers from the corresponding ionic Cu treated plants. The projected dietary intake for peeled tubers at 500 mg Cu kg DW⁻¹ were similar to those for the 1,000 mg Cu kg DW⁻¹ treatment. The same general trends observed for unpeeled tubers from Cu-treated plants were also observed for unpeeled tubers from Ce-treated plants when the tubers were compared to the median oral RfD value of 5 µg kg⁻¹ d⁻¹ for nine rare earth elements (REEs). However if the data are compared to the mean oral RfD value of 171.9 µg kg⁻¹ d⁻¹ for these same nine elements, all projected dietary exposures fell more than 10-fold below this value. The projected dietary exposures for peeled tubers were much lower than that for the unpeeled tubers (data not shown), consistent with the results above showing that the majority of Ce from each treatment was associated with the peel tissue. Neither oral RfD values would be exceeded for any of the dietary projections made for peeled, ENP- or ionic-treated sweet potato tubers.

4.0 Discussion

The discharge of engineered nanoparticles into the environment, both intentionally and unintentionally, is anticipated based on their growing use. As metal oxide ENPs are expected to be released from consumer products into wastewaters and retained in the sewage sludge, land application of biosolids represents a potential source of ENPs in agricultural systems [35-37]. Additional potential sources of ENPs in agricultural soils include irrigation water, aerial deposition, or the application of nano-enabled products [38-40]. This potential for nanoparticle

release has created the need for studies examining the impact on the growth and yield of agricultural crops as well as the possible accumulation of ENP-derived metals in the edible plant tissues.

Studies examining the impact of ENPs on crops have reported contrasting effects of ENP treatment on biomass or yield. Individual studies with soybean (*Glycine max*) and ZnO, wheat (*Triticum aestivum*) and CeO₂, or carrot and CuO reported no negative impacts of ENP treatment on biomass or yield [4, 19, 25]. In contrast, some species have displayed negative yield effects in response to ENPs. Examples include a reduction in pod biomass for soybean exposed to CeO₂ [19], reduction in the number of developed cobs for corn (*Zea mays*) in the presence of ZnO [20], and a reduction in fruit yield for cucumber (*Cucumis sativus*) following exposure to CeO₂ [21]. Not surprisingly, all these studies have reported a significant increase in the concentration of the metal in vegetative tissues upon ENP exposure and in some cases, also in the edible tissues. In the study here there were no specific benefits to sweet potato yield from any of the treatments aside from modest effects on tuber diameter or length observed in some instances.

Several characteristics of soil systems, such as the pH, mineralogy (particularly the clay content), organic matter content, and ionic strength, are known to influence the solubility, bioavailability, and toxicity of ENPs to biota [37, 41]. Organic matter has a notable effect on the solubility, dissolution, and uptake of ENPs [37, 42-46]. Organic matter can also affect the solubility of heavy metals, particularly Cu [47, 48]. Most of the life cycle studies cited above [19-21] used predominantly sandy soils (e.g., 66-84% sand) with low organic matter content (<5%). The Fafard potting mix used in this experiment was 50% sphagnum moss by mass and also included composted pine bark in the formulation. This material provided an organic fraction that likely interacted with both the added nanomaterials and ionic metals, effectively

mitigating their toxicity. Analyses were not conducted to assess the solubility or speciation of the metals in the ENP- and ionic-amended substrates, but the tuber metal concentrations illustrated a dose-dependent relationship, indicative of the relative plant bioavailability of each metal at each concentration. Biological transport mechanisms for these three elements differ in plants and this likely contributes to these trends. However, if one considers the concentration for unpeeled tubers or the tuber peels, this could reflect adsorption of the elements as opposed to biological transport and could therefore be used to make inferences about the solubility and bioavailability of these three metals. One important limitation to this assumption is that there may have been residual soil particles adhering to the tuber peel that were not removed after harvest. As the concentration of the metal treatments increased in the soil, residual soil particles may have inflated the peel concentrations. This contention would need to be evaluated using tubers that had been more thoroughly cleaned to minimize or preclude the effects of soil retention. Nevertheless, overall for this substrate and possibly for other high organic content substrates, adverse effects for sweet potato yield would likely occur only at concentrations that exceed 1,000 mg Zn kg DW⁻¹ and perhaps higher for Cu or Ce. These concentrations are most likely well above those that would be encountered under current exposure regimes (excluding a spill scenario), which may argue against significant concerns of adverse effects on this crop from these ENPs.

An unexpected result observed throughout this study was that there were few instances of significant differences in effect or accumulation as a function of chemical form (ENP or ionic). A meta-analysis of ecotoxicity studies for *n*ZnO and *n*CuO found that the vast majority of studies reported that ionic Zn²⁺ or Cu²⁺ was more toxic than *n*ZnO or *n*CuO when the ENP and ionic forms are presented at equivalent concentrations [49]. Several nanotoxicology studies with

plants similarly found that the ENP form was either less toxic or no more toxic than the ionic form [25, 50-54]. Both *n*ZnO and *n*CuO can undergo rapid dissolution to release component ions [55, 56], with the rate of *n*ZnO dissolution generally more rapid than that of *n*CuO [57]. The stability of *n*CeO₂ is higher [13, 58] but dissolution does occur with the rate dependent on the chemical conditions in the media [46, 59] and biological effects mediated by plant roots [59]. Over the 130 d treatment period there was considerable time for these nanomaterials to undergo dissolution. The similarity in the results throughout this study suggest that the bioavailable fraction in this substrate did not differ substantially between the two chemical forms of these three metals. Had the speciation of the metals been examined in the substrate during or after the treatment period, it may have been possible to confirm the dissolution of the nanomaterials and the plant bioavailability of Zn, Cu, or Ce. Techniques such as uXRF and XANES have been used in some studies to demonstrate the speciation and chemical form of the target metals [60, 61]. The results underscore the importance of soil characteristics and processes in assessing the impact and accumulation of nanomaterials in plants.

Anatomically speaking, the “peel” of a sweet potato tuber is composed largely of periderm, a tissue composed of an outer phellem (or cork layer), a layer of dividing cork cambium cells (phellogen), and a layer of parenchymal cells (phelloderm) inside the phellogen. The phellem layer replaces the epidermis as the outer protective cell layer and plays a key role in the resistance to pests [62]. Phellem cells are dead at maturity and have thin cell walls that are partially lignified [63]. Other root and tuberous vegetables have a similar structure to their belowground storage organ. This layer of dead cells in the vegetable surface has in studies with radish (*Raphanus sativus*) and carrot been shown to restrict the penetration of ENPs more so than ionic forms of metals [25, 53]. Such results suggest that for these vegetables, the periderm (i.e.,

the peel) acts as a screen to restrict the inward radial transfer of ENPs. Presumably then, if there is no difference for the roots or tubers of a plant species in accumulation between the ENP and ionic forms, then there are at least two possibilities. One is that the species may have less selectivity than that for carrot and radish, therefore allowing the ENP to penetrate as readily as the ion. In contrast, perhaps the more plausible explanation is that tissues were in contact with ionic Zn, Cu, or Ce in both the ENP and ionic treatments. The latter possibility could be investigated if soil-based studies of ENP uptake by plants provided data characterizing the dissolution, speciation, and or bioavailability of the ENPs or their metals in the soils.

The goal of the dietary intake projections was to relate the accumulation of Zn, Cu, or Ce in unpeeled and peeled tuber from the two forms (ENP or ionic) to a potential impact on human consumers. The oral RfD for chronic dietary intake was selected as a parameter to provide context for those dietary intake values. This is difficult for Ce since there is no oral RfD for this element. The lack of a value was compensated for through the use of a median and a mean value calculated from the oral RfD values for nine other REEs. These two values for the REEs varied by more than 20-fold and the more conservative of the two values was used to evaluate the dietary intake of Ce. There was little difference in the dietary intake trends with the exception of the Zn. Overall there was little indication that there would be dietary exposure in excess of the oral RfD except if the tubers were consumed unpeeled by small children. It is doubtful that children of that age would consume a full serving so the dietary intake would likely be substantially lower. In addition, dietary intake in excess of the oral RfD was projected primarily for plants grown at the highest treatment concentration (1,000 mg kg DW⁻¹). Whether this concentration of ENP would be reached under environmentally realistic exposure scenarios is unknown but is either unlikely or would require considerable time (absent a spill situation or

other unintended massive release) to reach through repeated introduction of ENPs. As illustrated here, projecting dietary intake based on the ionic metal produced very similar results to those for the ENP form, indicating that the potential risks posed by ENPs in soil grown plants are likely no worse than those for the comparable ionic treatment. This assumes however that effects of metal oxide ENPs even if intact in plant foods on consumers are no different than those that might be caused by the same amount of the ionic metal. Specific nano-effects that manifest after consumption still need to be investigated to provide a more complete picture of the potential impacts of ENPs in plant foods. The value of peeling the tubers in terms of dietary intake was clearly illustrated by the data, even when the highly conservative median oral RfD for the REEs was employed for Ce. The same interpretations were made for dietary intake of carrot exposed to *n*ZnO, *n*CuO, or *n*CeO₂ or the ionic counterpart [25]. Peeling root and tuberous vegetables therefore represents a simple step to ameliorate potential exposure to ENPs or ionic metals. One other means of mitigating potential dietary intake would be to more thoroughly clean the surface of the tubers. The study here did not use an aggressive strategy to clean the tuber surface. A more vigorous abrasion of the surface, perhaps with the addition of surfactants, could remove a larger fraction of metals associated with the peels, creating a commensurate decrease in the potential dietary intake.

5.0 Conclusions

The results from this sweet potato life cycle microcosm study with *n*ZnO, *n*CuO, and *n*CeO₂ demonstrated that in this soil system, adverse effects on yield (if present) were only observed at the higher exposure concentrations used and were independent of the chemical form of the metal. In comparing the tuber concentration and transfer factor data between the three

376 metals, the results imply that Zn was more bioavailable than Cu, which was in turn more
377 bioavailable than Ce. The lack of a difference in sweet potato tuber yield or metal concentration
378 between the chemical forms could suggest that the plants were not actually exposed to the ENP
379 but were instead exposed predominantly to the ionic forms. This is further supported by the
380 observation that the peels of sweet potato had higher concentrations of metals than the inner
381 tuber flesh, but there was no consistent evidence of differential accumulation between the ENP-
382 and ionic-treated plants. With respect to the associated of metals with the peels, one trend that
383 was clear from the results is that regardless of chemical form (ENP versus ionic), sweet potato
384 peel screened considerably more Ce than Zn or Cu. Overall, adverse outcomes in terms of
385 reductions in sweet potato yield, metal accumulation, and dietary intake occurred regardless of
386 the chemical form of the metal added to the substrate but were predominantly associated with
387 exposure concentrations unlikely to occur in the environment. These results imply that under
388 such conditions, ENPs pose no greater risk to agricultural productivity or food safety than do the
389 ionic metals. The results also highlight several research areas for future study with regard to
390 nanomaterials and food safety. More realistic estimates of the likely concentrations of ENPs that
391 would occur in the environment are needed. Investigation of the subsequent accumulation of
392 metal oxide ENPs by agricultural crops needs to be linked to studies of ENP chemical fate and
393 speciation in soil, as well as in biota. Several recent studies have successfully used uXRF and/or
394 XANES to characterize the *in vivo* speciation of metals in plant tissues originating from metallic
395 oxide ENPs [20, 21, 58, 64-66]. Determining the *in vivo* speciation and conformation of the
396 metals is necessary then to assess internal distribution and potential impact to plants. Such
397 information is paramount to the subsequent assessment of their biological effects and the
398 mechanism of accumulation in food crops. Investigation of the impact of consumption on

animal consumers have largely been limited to projected intake, as was done in this study [19, 67]. There is a pressing need for studies that provide information on the actual form and concentration of dietary exposure that results from the consumption of plant foods grown in the presence of metallic oxide ENPs. With such data, a more conclusive determination of the risks of ENPs to food safety and human health would be possible.

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REFERENCES

- [1] A. Nel, T. Xia, L. Madler, N. Li, Toxic potential of materials at the nanolevel, *Sci. Total Environ.*, 311 (2006) 622-627.
- [2] P. Boonyanitipong, B. Kositup, P. Kumar, S. Baruah, J. Dutta, Toxicity of ZnO and TiO₂ nanoparticles on germinating rice seed *Oryza sativa* L., *International Journal of Bioscience, Biochemistry, and Bioinformatics*, 1 (2011) 282-285.
- [3] W.-M. Lee, Y.-J. An, H. Yoon, H.-S. Kweon, Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): Plant agar test for water-insoluble nanoparticles, *Environ. Toxicol. Chem.*, 27 (2008) 1915-1921.
- [4] W. Du, J.L. Gardea-Torresdey, R. Ji, Y. Yin, J. Zhu, J.R. Peralta-Videa, H. Guo, Physiological and biochemical changes imposed by CeO₂ nanoparticles on wheat: A life cycle field study, *Environ. Sci. Technol.*, 49 (2015) 11884-11893.
- [5] C.O. Dimkpa, J.E. McLean, D.W. Britt, A.J. Anderson, Bioactivity and biomodification of Ag, ZnO, and CuO nanoparticles with relevance to plant performance in agriculture, *Indust. Biotechnol.*, 8 (2012) 344-357.
- [6] J.L. Gardea-Torresdey, C.M. Rico, J.C. White, Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments, *Environ. Sci. Technol.*, 48 (2014) 2526-2540.
- [7] P. Miralles, T.L. Church, A.T. Harris, Toxicity, uptake, and translocation of engineered nanomaterials in vascular plants, *Environ. Sci. Technol.*, 46 (2012) 9224-9239.
- [8] C. Ma, J.C. White, O.P. Dhankher, B. Xing, Metal-based nanotoxicity and detoxification pathways in higher plants, *Environ. Sci. Technol.*, 49 (2015) 7109-7122.

432 [9] J.D. Judy, P.M. Bertsch, Bioavailability, toxicity, and fate of manufactured nanomaterials in terrestrial
433 ecosystems, in: L.S. Donald (Ed.) *Advances in Agronomy*, Academic Press, 2014, pp. 1-64.

434 [10] M. Ghosh, M. Bandyopadhyay, A. Mukherjee, Genotoxicity of titanium dioxide (TiO₂)
435 nanoparticles at two trophic levels: Plant and human lymphocytes, *Chemosphere*, 81 (2010) 1253-1262.

436 [11] M. Kumari, S.S. Khan, S. Pakrashi, A. Mukherjee, N. Chandrasekaran, Cytogenetic and genotoxic
437 effects of zinc oxide nanoparticles on root cells of *Allium cepa*, *J. Hazard. Mater.*, 190 (2011) 613-621.

438 [12] M. Ruffini Castiglione, L. Giorgetti, C. Geri, R. Cremonini, The effects of nano-TiO₂ on seed
439 germination, development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L, *J. Nano.*
440 *Res.*, 13 (2011) 2443-2449.

441 [13] M.L. López-Moreno, G. de la Rosa, J.Á. Hernández-Viezcás, H. Castillo-Michel, C.E. Botez, J.R.
442 Peralta-Videa, J.L. Gardea-Torresdey, Evidence of the differential biotransformation and genotoxicity of
443 ZnO and CeO₂ nanoparticles on soybean (*Glycine max*) plants, *Environ. Sci. Technol.*, 44 (2010) 7315-
444 7320.

445 [14] S. Majumdar, I.C. Almeida, E.A. Arigi, H. Choi, N.C. VerBerkmoes, J. Trujillo-Reyes, J.P. Flores-
446 Margez, J.C. White, J.R. Peralta-Videa, J.L. Gardea-Torresdey, Environmental effects of nanocerium on
447 seed production of common bean (*Phaseolus vulgaris*): A proteomic analysis, *Environ. Sci. Technol.*, 49
448 (2015) 13283-13293.

449 [15] C. Vannini, G. Domingo, E. Onelli, B. Prinsi, M. Marsoni, L. Espen, M. Bracale, Morphological and
450 proteomic responses of *Eruca sativa* exposed to silver nanoparticles or silver nitrate, *PloS one*, 8 (2013)
451 e68752.

452 [16] F. Mirzajani, H. Askari, S. Hamzelou, Y. Schober, A. Römpp, A. Ghassempour, B. Spengler,
453 Proteomics study of silver nanoparticles toxicity on *Oryza sativa* L, Ecotoxicology and environmental
454 safety, 108 (2014) 335-339.

455 [17] P. Landa, R. Vankova, J. Andrlouva, J. Hodek, P. Marsik, H. Storchova, J.C. White, T. Vanek,
456 Nanoparticle-specific changes in *Arabidopsis thaliana* gene expression after exposure to ZnO, TiO₂, and
457 fullerene soot, J. Hazard. Mater., 241-242 (2012) 55-62.

458 [18] C. Ma, S. Chhikara, B. Xing, C. Musante, J.C. White, O.P. Dhankher, Physiological and molecular
459 response of *Arabidopsis thaliana* (L.) to nanoparticle cerium and indium oxide exposure, ACS Sustain.
460 Chem. Engr., 1 (2013) 768-778.

461 [19] J.H. Priester, Y. Ge, R.E. Mielke, A.M. Horst, S.C. Moritz, K. Espinosa, J. Gelb, S.L. Walker, R.M.
462 Nisbet, Y.-J. An, J.P. Schimel, R.G. Palmer, J.A. Hernandez-Viezcas, L. Zhao, J.L. Gardea-Torresdey,
463 P.A. Holden, Soybean susceptibility to manufactured nanomaterials with evidence for food quality and
464 soil fertility interruption, P. Natl. Acad. Sci. USA, 109 (2012) E2451–E2456.

465 [20] L. Zhao, Y. Sun, J.A. Hernandez-Viezcas, J. Hong, S. Majumdar, G. Niu, M. Duarte-Gardea, J.R.
466 Peralta-Videa, J.L. Gardea-Torresdey, Monitoring the environmental effects of CeO₂ and ZnO
467 nanoparticles through the life cycle of corn (*Zea mays*) plants and in situ μ -XRF mapping of nutrients in
468 kernels, Environ. Sci. Technol., 49 (2015) 2921-2928.

469 [21] L. Zhao, Y. Sun, J.A. Hernandez-Viezcas, A.D. Servin, J. Hong, G. Niu, J.R. Peralta-Videa, M.
470 Duarte-Gardea, J.L. Gardea-Torresdey, Influence of CeO₂ and ZnO nanoparticles on cucumber
471 physiological markers and bioaccumulation of Ce and Zn: A life cycle study, J. Agric. Food Chem., 61
472 (2013) 11945-11951.

473 [22] S. Arora, P. Sharma, S. Kumar, R. Nayan, P. Khanna, M. Zaidi, Gold-nanoparticle induced
474 enhancement in growth and seed yield of *Brassica juncea*, Plant Growth Regulation, 66 (2012) 303-310

475 [23] A.A. Keller, S. McFerran, A. Lazareva, S. Suh, Global life cycle releases of engineered
 476 nanomaterials, *J. Nano. Res.*, 15 (2013) 1692.

477 [24] G. Antonious, S. Dennis, J. Unrine, J. Syder, Heavy metals uptake in plant parts of sweet potato
 478 grown in soil fertilized with municipal sewage sludge, *International Journal of Geology*, 5 (2011) 14-20.

479 [25] S.D. Ebbs, S. Bradfield, P. Kumar, C. Musante, J.C. White, X. Ma, Accumulation of zinc, copper, or
 480 cerium in carrot (*Daucus carota*) exposed to metal oxide nanoparticles and metal ions, *Environ. Sci.:*
 481 *Nano*, 3 (2016) 114-126.

482 [26] S.D. Ebbs, S.J. Bradfield, P. Kumar, J.C. White, X. Ma, Projected dietary intake of zinc, copper, and
 483 cerium from consumption of carrot (*Daucus carota*) exposed to metal oxide nanoparticles or metal ions
 484 *Frontiers in Plant Science*, (2016) In press.

485 [27] USEPA, Method 3050b: Acid Digestion of Sediments, Sludges, and Soils in: *Test Methods for*
 486 *Evaluating Solid Waste Physical/Chemical Methods*, SW-846 Environmental Protection Agency, Office
 487 of Solid Waste, Washington, DC, 1996, pp. 12 pp.

488 [28] S.D. Ebbs, J. Talbott, R. Sankaran, Cultivation of garden vegetables in Peoria pool sediments from
 489 the Illinois River: A case study in trace element accumulation and dietary exposures, *Environ. Int.*, 32
 490 (2006) 766-774.

491 [29] S. Ebbs, S. Hatfield, V. Nagarajan, M. Blaylock, A comparison of the dietary arsenic exposures from
 492 ingestion of contaminated soil and hyperaccumulating *Pteris* ferns used in a residential phytoremediation
 493 project, *Int. J. Phytoremediat.*, 12 (2010) 121-132.

494 [30] National Academy of Sciences, *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic,*
 495 *Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc,*
 496 *National Academy Press, Washington, D.C., 2001.*

497 [31] Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological profile for copper, U.S.
498 Department of Health and Human Services, Public Health Service, Atlanta, GA, 2004.

499 [32] U.S. EPA, Health Effects Assessment Summary Tables (HEAST), in, U.S. Environmental Protection
500 Agency, Washington, DC, 1997.

501 [33] USEPA, Rare earth elements: A review of production, processing, recycling, and associated
502 environmental issues, in, Office of Research and Development, Cincinnati, OH, 2012.

503 [34] IBM Corp., IBM SPSS Statistics for Windows, Version 19.0., IBM Corp. , Armonk, NY 2010.

504 [35] F. Gottschalk, T. Sonderer, R.W. Scholz, B. Nowack, Modeled environmental concentrations of
505 engineered nanomaterials (TiO₂, ZnO, Ag, CNT, Fullerenes) for different regions, Environ. Sci. Technol.,
506 43 (2009) 9216-9222.

507 [36] N.C. Mueller, B. Nowack, Exposure modeling of engineered nanoparticles in the environment,
508 Environ. Sci. Technol., 42 (2008) 4447-4453.

509 [37] P.S. Tourinho, C.A. Van Gestel, S. Lofts, C. Svendsen, A.M. Soares, S. Loureiro, Metal-based
510 nanoparticles in soil: Fate, behavior, and effects on soil invertebrates, Environ. Toxicol. Chem., 31 (2012)
511 1679-1692.

512 [38] A. Gogos, K. Knauer, T.D. Bucheli, Nanomaterials in plant protection and fertilization: current state,
513 foreseen applications, and research priorities, J. Agric. Food Chem., 60 (2012) 9781-9792.

514 [39] S. Mura, G. Seddaiu, F. Bacchini, P.P. Roggero, Advances of nanotechnology in agro-environmental
515 studies, Ital. J. Agron., 8 (2013) e18.

516 [40] S. Suppan, Nanomaterials in Soil: Our Future Food Chain?, in, Institute for Agriculture and Trade
517 Policy, 2013, pp. 17 pp.

518 [41] B. Pan, B. Xing, Applications and implications of manufactured nanoparticles in soils: a review, Eur.
519 J. Soil Sci., 63 (2012) 437-456.

520 [42] R. Handy, R. Owen, E. Valsami-Jones, The ecotoxicology of nanoparticles and nanomaterials:
521 current status, knowledge gaps, challenges, and future needs, Ecotoxicology, 17 (2008) 315-325.

522 [43] X. Jiang, M. Tong, H. Li, K. Yang, Deposition kinetics of zinc oxide nanoparticles on natural
523 organic matter coated silica surfaces, Journal of Colloid and Interface Science, 350 (2010) 427-434.

524 [44] O.N. Mileyeva-Biebesheimer, A. Zaky, C.L. Gruden, Assessing the impact of titanium dioxide and
525 zinc oxide nanoparticles on bacteria using a fluorescent-based cell membrane integrity assay,
526 Environmental Engineering Science, 27 (2010) 329-335.

527 [45] Y. Zhang, Y. Chen, P. Westerhoff, J. Crittenden, Impact of natural organic matter and divalent
528 cations on the stability of aqueous nanoparticles, Water Research, 43 (2009) 4249-4257.

529 [46] F. Schwabe, R. Schulin, P. Rupper, A. Rotzetter, W. Stark, B. Nowack, Dissolution and
530 transformation of cerium oxide nanoparticles in plant growth media, J. Nano. Res., 16 (2014) 1-11.

531 [47] A. Kabata-Pendias, Trace Elements in Soils and Plants, 4th Ed., CRC Press, Boca Raton, FL, 2011.

532 [48] H. Marschner, Mineral Nutrition of Higher Plants, Academic Press, London, 2003.

533 [49] D.A. Notter, D.M. Mitrano, B. Nowack, Are nanosized or dissolved metals more toxic in the
534 environment? A meta-analysis, Environ. Toxicol. Chem., 33 (2014) 2733-2739.

535 [50] L.R. Pokhrel, B. Dubey, Evaluation of developmental responses of two crop plants exposed to silver
536 and zinc oxide nanoparticles, Sci. Total Environ., 452-453 (2013) 321-332.

537 [51] G. de la Rosa, M.L. López-Moreno, D. de Haro, C.E. Botez, J.R. Peralta-Videa, J.L. Gardea-
538 Torresdey, Effects of ZnO nanoparticles in alfalfa, tomato, and cucumber at the germination stage: Root

539 development and X-ray absorption spectroscopy studies, Pure & Applied Chemistry, 85 (2013) 1261-
540 2174.

541 [52] D. Lin, B. Xing, Root uptake and phytotoxicity of ZnO nanoparticles, Environ. Sci. Technol., 42
542 (2008) 5580-5585.

543 [53] W. Zhang, S. Ebbs, C. Musante, J. White, C. Gao, X. Ma, Uptake and accumulation of bulk and
544 nano-sized cerium oxide particles and ionic cerium by radish (*Raphanus sativus* L.), J Agric. Food Chem,
545 63 (2015) 382-390.

546 [54] Q. Wang, S. Ebbs, Y. Chen, X. Ma, Trans-generational impact of cerium oxide nanoparticles on
547 tomato plants Metallomics, 5 (2013) 753-759.

548 [55] N.M. Franklin, N.J. Rogers, S.C. Apte, G.E. Batley, G.E. Gadd, P.S. Casey, Comparative toxicity of
549 nanoparticulate ZnO, bulk ZnO, and ZnCl₂ to a freshwater microalga (*Pseudokirchneriella subcapitata*):
550 The importance of particle solubility, Environ. Sci. Technol., 41 (2007) 8484-8490.

551 [56] C.O. Dimkpa, J.E. McLean, D.E. Latta, E. Manangón, D.W. Britt, W.P. Johnson, M.I. Boyanov, A.J.
552 Anderson, CuO and ZnO nanoparticles: Phytotoxicity, metal speciation, and induction of oxidative stress
553 in sand-grown wheat, J. Nano. Res., 14 (2012) 1-15.

554 [57] C.O. Dimkpa, D.E. Latta, J.E. McLean, D.W. Britt, M.I. Boyanov, A.J. Anderson, Fate of CuO and
555 ZnO nano- and microparticles in the plant environment, Environ. Sci. Technol., 47 (2013) 4734-4742.

556 [58] S. Majumdar, J.R. Peralta-Videa, S. Bandyopadhyay, H. Castillo-Michel, J.-A. Hernandez-Viezcas,
557 S. Sahi, J.L. Gardea-Torresdey, Exposure of cerium oxide nanoparticles to kidney bean shows disturbance
558 in the plant defense mechanisms, J. Hazard. Mater., 278 (2014) 279-287.

559 [59] F. Schwabe, S. Tanner, R. Schulin, A. Rotzetter, W. Stark, A. von Quadt, B. Nowack, Dissolved
560 cerium contributes to uptake of Ce in the presence of differently sized CeO₂-nanoparticles by three crop
561 plants, *Metallomics*, 7 (2015) 466-477.

562 [60] A. Manceau, K.L. Nagy, M.A. Marcus, M. Lanson, N. Geoffroy, T. Jacquet, T. Kirpichtchikova,
563 Formation of metallic copper nanoparticles at the soil– root interface, *Environ. Sci. Technol.*, 42 (2008)
564 1766-1772.

565 [61] J.M. Unrine, O.V. Tsyusko, S.E. Hunyadi, J.D. Judy, P.M. Bertsch, Effects of particle size on
566 chemical speciation and bioavailability of copper to earthworms (*Eisenia fetida*) exposed to copper
567 nanoparticles, *Journal of Environmental Quality*, 39 (2010) 1942-1953.

568 [62] J. Schalk, J. Peterson, A. Jones, P. Dukes, W. Walter Jr, The anatomy of sweet potato periderm and
569 its relationship to wireworm, *Diabrotica*, *Systema resistance*, *J. Agric. Entomol.*, 3 (1986) 350-356.

570 [63] E. Artschwager, On the anatomy of the sweet potato root with notes on internal breakdown, *Journal*
571 *of Agricultural Research*, 27 (1924).

572 [64] J.D. Judy, J.M. Unrine, P.M. Bertsch, Evidence for biomagnifications of gold nanoparticles within a
573 terrestrial food chain, *Environ. Sci. Technol.*, 45 (2011) 776-781.

574 [65] C. Larue, H. Castillo-Michel, S. Sobanska, N. Trcera, S. Sorieul, L. Cécillon, L. Ouerdane, S.
575 Legros, G. Sarret, Fate of pristine TiO₂ nanoparticles and aged paint-containing TiO₂ nanoparticles in
576 lettuce crop after foliar exposure, *J. Hazard. Mater.*, 273 (2014) 17-26.

577 [66] A.D. Servin, M.I. Morales, H. Castillo-Michel, J.A. Hernandez-Viezcas, B. Munoz, L. Zhao, J.E.
578 Nunez, J.R. Peralta-Videa, J.L. Gardea-Torresdey, Synchrotron verification of TiO₂ accumulation in
579 cucumber fruit: A possible pathway of TiO₂ nanoparticle transfer from soil into the food chain, *Environ.*
580 *Sci. Technol.*, 47 (2013) 11592-11598.

581 [67] A. Weir, P. Westerhoff, L. Fabricius, K. Hristovski, N. von Goetz, Titanium dioxide nanoparticles in
582 food and personal care products, *Environ. Sci. Technol.*, 46 (2012) 2242-2250.

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Figure legends

Figure 1. Concentration of Zn, Cu or Ce in unpeeled, peeled tubers, and the peels of sweet potato grown for 130 d in field microcosms in the presence of three concentrations of each element presented as metal oxide engineered nanoparticles (ENP) or in the ionic form (ion). Data represent the mean and standard error ($n=3-5$). Within each panel, bars with different letters are significantly different from one another. l.o.d. = limit of detection.

Figure 2. Soil to tuber transfer factors (TF) for unpeeled or peeled sweet potatoes grown for 130 d in field microcosms in the presence of three concentrations of Zn, Cu, or Ce presented as either metal oxide nanoparticles or in the ionic form. As there was no significant effect of form within each metal, the data were pooled for the two forms and presented as a function of treatment concentration. Data represent the mean and standard error ($n=3-5$). Within each panel, bars with different letters are significantly different from one another.

Figure 3. Distribution of Cu or Ce across the peels (outer periderm) and tuber flesh of sweet potato grown for 130 d in field microcosms in the presence of three concentrations of either element presented as metal oxide engineered nanoparticles (ENP) or in the ionic form (Ion). Data represent the mean and standard error ($n=3-5$). Within each panel, the letters above the bars denote significant differences in the percentage of metal associated with the peel as a function of form and concentration.

Figure 4. Dietary intake models for Zn, Cu, or Ce based on consumption of a single serving of unpeeled or peeled sweet potato grown for 130 d in field microcosms in the presence of either

607 500 or 1,000 mg kg DW⁻¹ of each element presented as metal oxide engineered nanoparticles
608 (ENP, left panels) or in the ionic form (ion, right panels). Intake for each scenario is modeled for
609 seven age-mass classes ranging from child to adult females and males. For Zn and Cu, the solid
610 line represents the chronic oral RfD value. The solid line for the Ce panels represents the median
611 oral RfD calculated from nine other rare earth elements. Data represent the mean and standard
612 error ($n=3-5$).

Figure 1

Figure 2

Figure 3

Figure 4

Table 1. Yield data for tubers of sweet potato grown in field microcosms containing substrate amended with one of four concentrations of $n\text{ZnO}$, $n\text{CuO}$, or $n\text{CeO}_2$ or the same concentration of Zn^{2+} , Cu^{2+} , or Ce^{4+} . The results of the two-way ANOVA indicated no effect of form for any metal. There was a significant effect of concentration for some parameters so the results are presented only in terms of treatment concentration. Data illustrate the mean and standard error ($n=3-5$). Within a metal and an individual parameter, different letters are used to indicate significant differences.

Yield data for sweet potato tubers							
Element	Treatment	Tuber biomass, g FW	Number of tubers	Mass per tuber, g FW	Tuber water content, %	Tuber diameter, cm	Tuber length, cm
Zn	Control	249.6 $\pm$ (46.3) a	7.8 $\pm$ (2.0) a	38.3 $\pm$ (11.1)	72.4 $\pm$ (1.8)	2.0 $\pm$ (0.2)	8.5 $\pm$ (0.5)
	100	211.2 $\pm$ (24.7) a	6.2 $\pm$ (0.6) a	34.0 $\pm$ (2.8)	72.8 $\pm$ (1.8)	2.3 $\pm$ (0.1)	9.9 $\pm$ (0.1)
	500	220.6 $\pm$ (15.5) a	7.6 $\pm$ (1.0) a	31.8 $\pm$ (4.0)	74.0 $\pm$ (2.4)	2.2 $\pm$ (0.1)	9.7 $\pm$ (0.2)
	1,000	98.0 $\pm$ (16.8) b	3.0 $\pm$ (0.7) b	44.6 $\pm$ (13.2)	74.8 $\pm$ (1.5)	2.1 $\pm$ (0.2)	10.6 $\pm$ (0.8)
Cu	Control	256.3 $\pm$ (26.3) a	7.3 $\pm$ (1.8)	41.2 $\pm$ (8.9)	66.5 $\pm$ (2.5)	2.3 $\pm$ (0.2) b	9.4 $\pm$ (0.7) a
	100	265.0 $\pm$ (10.4) a	6.3 $\pm$ (0.8)	62.2 $\pm$ (23.0)	75.1 $\pm$ (0.8)	3.0 $\pm$ (0.1) a	10.0 $\pm$ (0.5) a
	500	255.7 $\pm$ (10.6) a	7.3 $\pm$ (0.6)	36.9 $\pm$ (3.1)	70.5 $\pm$ (2.1)	2.4 $\pm$ (0.1) b	8.9 $\pm$ (0.5) b
	1,000	179.4 $\pm$ (16.7) b	5.7 $\pm$ (1.0)	48.1 $\pm$ (12.6)	73.2 $\pm$ (1.4)	2.3 $\pm$ (0.1) b	7.1 $\pm$ (0.4) b
Ce	Control	139.2 $\pm$ (63.8)	5.0 $\pm$ (2.0)	23.6 $\pm$ (5.4)	65.0 $\pm$ (1.7)	2.0 $\pm$ (0.3) b	5.8 $\pm$ (1.0)
	100	199.3 $\pm$ (23.9)	6.0 $\pm$ (0.7)	34.7 $\pm$ (4.5)	71.8 $\pm$ (1.5)	1.9 $\pm$ (0.1) b	6.9 $\pm$ (0.5)
	500	212.9 $\pm$ (36.7)	4.6 $\pm$ (0.5)	51.0 $\pm$ (11.8)	72.3 $\pm$ (2.3)	2.6 $\pm$ (0.2) ab	8.2 $\pm$ (0.7)
	1,000	185.0 $\pm$ (23.6)	3.7 $\pm$ (0.8)	51.0 $\pm$ (9.3)	71.1 $\pm$ (2.4)	2.7 $\pm$ (0.2) a	6.7 $\pm$ (0.5)