



UNIVERSITÀ DI PARMA

ARCHIVIO DELLA RICERCA

University of Parma Research Repository

Nanomaterials biotransformation: In planta mechanisms of action.

This is the peer reviewed version of the following article:

Original

Nanomaterials biotransformation: In planta mechanisms of action / Pagano, Luca; Rossi, Riccardo; White Jason, C; Marmioli, Nelson; Marmioli, Marta. - In: ENVIRONMENTAL POLLUTION. - ISSN 0269-7491. - (2022). [10.1016/j.envpol.2022.120834]

Availability:

This version is available at: 11381/2935391 since: 2024-12-16T15:46:35Z

Publisher:

Published

DOI:10.1016/j.envpol.2022.120834

Terms of use:

Anyone can freely access the full text of works made available as "Open Access". Works made available

Publisher copyright

note finali coverpage

(Article begins on next page)

Environmental Pollution

Nanomaterials biotransformation: In planta mechanisms of action

--Manuscript Draft--

Manuscript Number:	ENVPOL-D-22-05734R1
Article Type:	VSI:Pollutants and plants
Section/Category:	Special Issues
Keywords:	biotransformation; plant; Nanomaterials; synchrotron-based analyses; molecular response
Corresponding Author:	Marta Marmiroli University of Parma Parma, Parma ITALY
First Author:	Luca Pagano
Order of Authors:	Luca Pagano Riccardo Rossi Jason C. White Nelson Marmiroli Marta Marmiroli
Abstract:	<p>Research on engineered nanomaterials (ENMs) exposure has continued to expand rapidly, with a focus on uncovering the underlying mechanisms. The EU largely limits the number and the type of organisms that can be used for experimental testing through the 3R normative. There are different routes through which ENMs can enter the soil-plant system: this includes the agricultural application of sewage sludges, and the distribution of nano-enabled agrochemicals. However, a thorough understanding of the physiological and molecular implications of ENMs dispersion and chronic low-dose exposure remains elusive, thus requiring new evidence and a more mechanistic overview of pathways and major effectors involved in plants. Plants can offer a reliable alternative to conventional model systems to elucidate the concept of ENM biotransformation within tissues and organs, as a crucial step in understanding the mechanisms of ENM-organism interaction. To facilitate the understanding of the physico-chemical forms involved in plant response, synchrotron-based techniques have added new potential perspectives in studying the interactions between ENMs and biota. These techniques are providing new insights on the interactions between ENMs and biomolecules. The present review discusses the principal outcomes for ENMs after intake by plants, including possible routes of biotransformation which make their final fate less uncertain, and therefore require further investigation.</p>
Suggested Reviewers:	<p>Nubia Zuverza-Mena Nubia.Zuverza@ct.gov expert in the field</p> <p>Luca Marchiol luca.marchiol@uniud.it Expert in the field</p> <p>Sanghamitra Majumdar majumdarsm@gmail.com Expert in the field</p> <p>Tomas Vanek vanek@ueb.cas.cz Expert in the field</p> <p>Alan Baker a.baker4@uq.edu.au Expert in the field</p> <p>Alessandra Gianoncelli</p>

alessandra.gianoncelli@elettra.eu
Expert in the field

Response to Reviewers:

Please see the attached file for complete Revisions.

Editor and Reviewer comments:

Reviewer #1: Manuscript review of ENVPOL-D-22-05734 "Nanomaterials biotransformation: In planta mechanisms of action"

This review article summarized recent works on the nanomaterial biotransformation within plant tissues using synchrotron-based techniques. The impacts of engineered nanomaterials (ENMs) on plant gene expression were also included. Specifically, studies regarding the biointeraction between plants and ENMs (nanoscale CeO₂, La₂O₃, TiO₂, Au, FeOx, ZVI, ZnO, CuO, and CdS QDs) were listed and discussed in detail. This review paper contributes to the deep understanding of the fate of ENMs in plant tissues, and the related genetic regulation of plants induced by ENM exposures. The contents are within the scope of the Environmental Pollution. However, I have a number of general concerns, followed by a range of specific comments, which prevent me from recommending this paper for publication in its current form.

General Comments-

Some parts of the abstract and introduction are not quite relevant to the main topic, and need to be revised. The main topic, synchrotron-based analysis of ENMs biotransformation, should be more emphasized and discussed in more detail. In part 1, there was only one sub-title 1.1. None of the figures or the tables provided any information related to the in planta biotransformation. Several figures with summarized information instead of only one figure are better for a review article. Some references are too old or not representative. They should be up to date.

We thank Rev1 for the constructive comments and suggestions given. The manuscript has been thoroughly improved in order to answer to all comments reported. Paragraph subdivision has been updated. Several new references and an additional Figure have been included. All the edited sections have been reported in the answer to each specific question.

Specific Comments-

The abstract used a large amount of space describing exposure pathways and low-dose effects. However, they were not well discussed in the main text. Similarly, the "potential applicability" was not given in the main text. The abstract should contain the most significant findings, critical comments on the current studies, or perspectives for future research.

The abstract has been modified in order to be more informative on the points discussed in the main text. The abstract now reads as follows:

"Research on engineered nanomaterials (ENMs) exposure has continued to expand rapidly, with a focus on uncovering the underlying mechanisms. The EU largely limits the number and the type of organisms that can be used for experimental testing through the 3R normative. There are different routes through which ENMs can enter the soil-plant system: this includes the agricultural application of sewage sludges, and the distribution of nano-enabled agrochemicals. However, a thorough understanding of the physiological and molecular implications of ENMs dispersion and chronic low-dose exposure remains elusive, thus requiring new evidence and a more mechanistic overview of pathways and major effectors involved in plants. Plants can offer a reliable alternative to conventional model systems to elucidate the concept of ENM biotransformation within tissues and organs, as a crucial step in understanding the mechanisms of ENM-organism interaction. To facilitate the understanding of the physico-chemical forms involved in plant response, synchrotron-based techniques have added new potential perspectives in studying the interactions between ENMs and biota. These techniques are providing new insights on the interactions between ENMs and biomolecules. The present review discusses the principal outcomes for ENMs after intake by plants, including possible routes of biotransformation which make their final

fate less uncertain, and therefore require further investigation.”

The Paragraph 3 has been also modified to give a more timely reference to potential applicability:

Lines 558-567: “This information is highly relevant with regard to potential applicability: ENMs can interact with sensitive ecosystem components within trophic food chains, affect microbial populations in soil, enter into the plant and where they can be translocated to different tissues and organs, including the edible tissues or organs (Holden et al., 2013; Liu et al., 2015). Biotransformation can occur at each step within these processes, modifying and/or amplifying ENM effects at organism level. These interactions from the level of ecosystem, organism, tissue, cell, and organelles become key factors when applying “ENM biotransformation” as a concept for a safer design, when considering applications for agriculture and food production, and for minimizing the adverse biological impact (Burello & Worth, 2015; Pagano et al., 2018; Lowry et al., 2019; Kah et al., 2019; Zulfiqar et al., 2019; Ma et al., 2020; Hameed et al., 2022; Xu et al., 2022).”

A new reference has been also included:

Xu T., Wang Y., Aytac Z., Zuverza-Mena N., Zhao Z., Hu X., Ng K.W., White J.C., Demokritou P.
Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli Responsive Core–Shell Nanostructures. *ACS Nano* 2022, 16, 4, 6034–6048.

You mentioned that three billion tons of crops are produced per year and cost a lot of source and energy in L41-47. What is the purpose? Please consider deleting them.

The new paragraph 1.1 entitled “from ENM exposure to biotransformation” has been reduced and thoroughly edited in order to be more focused on the main topic of the review:

Lines 47-99: “Although global food production has generally increased over time, the distribution has been far from equitable, with more than 820 million people having insufficient food and many more consuming low-quality diets leading directly to micronutrient deficiencies (Willett et al 2019; Zhong et al., 2020). In the past 20 years, and particularly in the past decade, engineered nanomaterials (ENMs) have seen dramatically increasing use and had an equally significant impact on ecosystems services and human society. As such, studies focused on the implications associated with their use are critical. In fact, it is known that ENMs exert important, but not completely understood, effects on biota; a particular topic of concern include the effect on crops, food production, and trophic transfer (Gardea-Torresdey et al., 2014; Ma et al., 2018; White et al., 2022).

The interplay between plant growth, dissolution, evaporation, and aggregation are key aspects of the dynamic behavior of ENMs in the environment. Directional aggregation can result in the formation of larger particles with a more complex morphology. However, given the complexity of natural environments, most nanomaterials can be found in hetero-aggregated composites of different inorganic and organic materials (Judy et al., 2012; Ma et al 2018). These aggregates can be very different from original simple pristine morphologies and may even form highly branched structures similar to fractals, all of which subsequently dramatically affect their reactivity and transport (Ma et al., 2018; Huangfu et al., 2019).

Sectors with a large nanomaterial application such as medicine and food production may experience greater risks of ENMs exposure due to their uses, with thousands of tons of ENMs that are eventually discarded into the three main environmental matrices: soil, water, and air (Keller et al., 2013; Zuverza Mena et al., 2017). ENMs with the greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015). Considering the long-time span of use of select materials (1950 to 2050), efforts to estimate ENMs release through commercial and associated activity into the environment have been undertaken. For example, for nSiO₂, a global production between 100,000 and three million tons per year has been estimated, while

for nCeO₂, levels likely reach the upper limit of 10,000 tons per year, and for nAg, the literature reflects a production volume below 1,000 tons per year (Giese et al., 2018). The use of nTiO₂ for the inhibition of microbial proliferation in food is one of the most important ways to prolong the shelf life of packaged products (Abutalib & Rajeh., 2020). However, a panel from EFSA concluded that E171 (TiO₂) can no longer be considered as safe when used as a food additive (EFSA Journal 2021). Gottschalk et al., (2009) calculated environmental concentrations using a probabilistic LCA (life cycle analysis) of ENM containing products. The authors modelled nTiO₂, nZnO, nAg, carbon nanotubes (CNT), and fullerenes for the U.S., Europe, and Switzerland. The concentrations in the environment were calculated through probabilistic density functions and compared to ecotoxicology data. In the simulations, the values ranged from 0.003 ng L⁻¹ (fullerenes) to 21 ng L⁻¹ (nTiO₂) for surface waters and from 4 ng L⁻¹ for fullerenes to 4 µg L⁻¹ for nTiO₂ for sewage treatment effluents. In Europe and the U.S., ENMs increased annually in sludge treated soil, and ranged from 1 ng kg⁻¹ for fullerenes to 89 µg kg⁻¹ for nTiO₂ (Gottschalk et al. 2009; Keller et al., 2013; Keller & Lazareva, 2014; Rincon, 2019). Importantly, quantum dots (QDs), as well as many carbon- and metal-based ENMs, have been shown to produce negative effects on animals and plants as a function of dose, including accumulation, alteration of physiological and biochemical parameters, and reduced growth or yield (Oh et al., 2016; Zuverza-Mena et al., 2017). Quantum dots have shown to enter plant roots and to damage the cell wall, dysregulating metabolism (Marmioli et al., 2020). While mercaptoacetic acid (MAA)-coated CdSe/ZnS QDs induced minimal toxicity on maize seedlings, pristine cadmium/tellurium (Cd/Te) QDs induced chromatin stress, mitochondrial damage and inhibition on green gram sprouts (*Phaseolus radiatus* L.) growth (Song et al., 2013). There are several routes by which ENM can enter the soil-plant system. These include agricultural application of sewage sludges which often contain nSiO₂, nTiO₂, nZnO, and nAg; as well as the application of nano-enabled agrochemicals, resulting in the direct entry of nSiO₂, nTiO₂, nZnO, nFeOx, nCuO, CeO₂ and nAg into agricultural soils (Lv et al., 2019; Verma et al., 2022)."

Additional references included:

Gardea-Torresdey J.L., Rico C.M., White J.C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* 2014, 48, 2526–2540.

Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* 15, 1692–1709.

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. *Environ. Sci. Technol. Lett.* 2014, 1, 1, 65–70.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, *Environment International*, 2015, 77, 132-147.

Rincon A.M. 2019. Chapter 6 - Presence of nanomaterials on consumer products: food, cosmetics, and drugs. pp. 165-181. Marmioli N., White J.C., Song J., Eds: In *Micro and Nano Technologies, Exposure to Engineered Nanomaterials in the Environment*, Elsevier, Amsterdam, The Netherlands.

Song U., Jun H., Waldman B., Roh J., Kim Y., Yi J., Lee E.J. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*) *Ecotoxicol. Environ. Saf.*, 2013. 93, 60-67.

White J.C., Zuverza-Mena N., Elmer W.H. From nanotoxicology to nano-enabled agriculture: Following the science at the Connecticut Agricultural Experiment Station (CAES). *Plant Nano Biology*, 2022, 1, 100007. Doi: 10.1016/j.plana.2022.100007.

L50-55 Please focus on plants, especially agricultural crops.

We agree with Rev1 give more relevance to the ENMs in agricultural practices. The sentence has been modified and two new references have been introduced.

Lines 52-55: "In fact, it is known that ENMs exert important, but not completely understood, effects on biota; a particular topic of concern include the effect on crops, food production, and trophic transfer (Gardea-Torresdey et al., 2014; Ma et al., 2018; White et al., 2022)."

References added:

Gardea-Torresdey J.L., Rico C.M., White J.C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* 2014, 48, 2526–2540.

White J.C., Zuverza-Mena N., Elmer W.H. From nanotoxicology to nano-enabled agriculture: Following the science at the Connecticut Agricultural Experiment Station (CAES). *Plant Nano Biology*, 2022, 1, 100007. Doi: 10.1016/j.plana.2022.100007.

L53-54 It is too broad to conclude that the impacts of nanomaterials on biota are poorly understood. There are hundreds of publications investigating this topic.

The sentence, as suggested by Rev1, have been modified in order to be more topic oriented.

Lines 52-55: "In fact, it is known that ENMs exert important, but not completely understood, effects on biota; a particular topic of concern include the effect on crops, food production, and trophic transfer (Gardea-Torresdey et al., 2014; Ma et al., 2018; White et al., 2022)."

L67-68 Please add nCuO, nTiO₂, and nZnO. nTiO₂ is the most used one among all the others.

Please check

[Ref]

[Ref]

The sentence has been modified as requested.

Lines 67-70: "ENMs with the greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015)."

The references suggested have been also added:

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. *Environ. Sci. Technol. Lett.* 2014, 1, 1, 65–70.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, *Environment International*, 2015, 77, 132-147.

L77 Can you find any latest references? 2009 is more than ten years ago.

Unfortunately, we have not found any new significant update on permissible concentrations, especially for the European side. From this point of view the cited paper is still maintained as an EU standards. Thus, we prefer to maintain this reference. To provide additional perspective, we included two additional references:

Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* 15, 1692–1709.

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. *Environ. Sci. Technol. Lett.* 2014, 1, 1, 65–70.

L92 Please refer to the right article.

We thank the Rev1 for the comment, the reference has been properly corrected and added to the references.

Song U., Jun H., Waldman B., Roh J., Kim Y., Yi J., Lee E.J. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*)
Ecotoxicol. Environ. Saf., 2013. 93, 60-67.

L99 In 1.1, "Once accumulated by plants" You haven't given any background regarding the uptake of ENMs by plants. At least add a few sentences to describe the possibility and give a few examples.

The beginning of the Paragraph, now 1.2, has been edited in order to give more information about the phases before plant accumulation. A new Figure 1 has been also included.

Lines 102-110: "Environmental and soil physico-chemical characteristics may significantly impact on ENMs aggregation, and dissolution, which may modify ENM bioavailability, uptake, translocation, and accumulation into terrestrial plants. In fact, light and temperature may induce potential changes on the ENM structure, such as during foliar spray (Hong et al., 2021). Once within plant tissues, ENM biotransformation may alter particle stability and behaviour in terms of interactions with biomolecules, triggering differential plant defense mechanisms (Ma et al., 2018; Rawat et al., 2018). ENMs are subject to a range of processes that may lead to their partial dissolution or result in structural modifications (Milosevic et al., 2020; Marmiroli et al., 2020). A schematic representation is reported in Figure 1."

New reference added:

Hong J., Wang C., Wagner D.C., Gardea-Torresdey J.L., He F., Rico C.M. 2021. Foliar application of nanoparticles: mechanisms of absorption, transfer, and multiple impacts. *Environ. Sci.: Nano*, 8, 1196-1210.

Rawat S., Pullagurula V.L.R., Adisa I.O., Wang Y., Peralta-Videa J.R., Gardea-Torresdey J.L. Factors affecting fate and transport of engineered nanomaterials in terrestrial environments, *COESH*, 2018, 6, 47-53.

L105-107 "biotransformation of nanomaterials lead to the attachment of biological molecules", or does the attachment of biological molecules lead to NPs biotransformation? Or both? Any examples?

The paragraph has been modified in order to avoid misinterpretation. A new Figure 1 has been also introduced.

Lines 110-129: "Nanoparticle biotransformation is a highly complex and poorly understood series of events and has been shown to occur during weathering in the soil, trophic transfer, and translocation within plant tissues. These reactions are highly dynamic and alter the original pristine structure of the nanoparticles in a number of ways, potentially causing the release of ions, but also the consequent restructuring (or destructuring) of the nanoparticle (Servin et al., 2017a). Biotransformation of nanomaterials may rest on the interaction with biological molecules that stabilize their external reactivity, such as peptides including those involved in detoxification, (e.g. glutathione), fatty acids, secondary metabolites, and even components of cell

membranes (Marmioli et al., 2020). Particle properties such as size, stability, charge, and dissolution may strongly influence other biotransformation mechanisms, potentially promoting enzymatic modification and functionalization with proteins (e.g., corona protein) present in the cytoplasm and organelles (Ma et al., 2018; Marmioli et al., 2020). ENMs may maintain crystal structure when internalized by cells or may be disassembled and converted into less complex structures (by biological modification or chelation), thus reducing toxicity, and the risk of their accumulation and translocation (Wang et al., 2022). These post-uptake structural modifications involve specific parameters such as bond distance with other atoms or nature of the ligand atoms. In consideration of this, one objective in biotransformation studies is to investigate the physico-chemical forms (e.g., nanocrystal structure) within exposed tissues and to characterize the structural differences within the new biotransformed molecules, including identification of the biomolecules interacting with the ENMs (Castillo-Michel et al., 2017; Marmioli et al., 2020).”

L110 More citations are needed to support the statement of "properties such as size, stability, charge, and dissolution influence the biotransformation". Besides, plant cultivars can also affect ENMs in planta biodistribution
[Ref]

The paragraph has been modified in order to include new references and also the effect of the plant cultivars.

Lines 115-131: “Biotransformation of nanomaterials may rest on the interaction with biological molecules that stabilize their external reactivity, such as peptides including those involved in detoxification, (e.g. glutathione), fatty acids, secondary metabolites, and even components of cell membranes (Marmioli et al., 2020). Particle properties such as size, stability, charge, and dissolution may strongly influence other biotransformation mechanisms, potentially promoting enzymatic modification and functionalization with proteins (e.g., corona protein) present in the cytoplasm and organelles (Ma et al., 2018; Marmioli et al., 2020). ENMs may maintain crystal structure when internalized by cells or may be disassembled and converted into less complex structures (by biological modification or chelation), thus reducing toxicity, and the risk of their accumulation and translocation (Wang et al., 2022). These post-uptake structural modifications involve specific parameters such as bond distance with other atoms or nature of the ligand atoms. In consideration of this, one objective in biotransformation studies is to investigate the physico-chemical forms (e.g., nanocrystal structure) within exposed tissues and to characterize the structural differences within the new biotransformed molecules, including identification of the biomolecules interacting with the ENMs (Castillo-Michel et al., 2017; Marmioli et al., 2020). It has furthermore to consider how genetic diversity across different plant species and within the same plant species (in different cultivars) may influence the ENM uptake and translocation (Deng et al., 2020).”

New references included:

Deng C., Wang Y., Cota-Ruiz K., Reyes A., Sun Y., Peralta-Videa J.R., Hernandez-Viezcas J.A., Turley R.S., Niu G., Li C., Gardea-Torresdey J.L. Bok choy (Brassica rapa) grown in copper oxide nanoparticles-amended soils exhibits toxicity in a phenotype-dependent manner: Translocation, biodistribution and nutritional disturbance. *J Haz Mater*, 2020, 398, 122978.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, *Environment International*, 2015, 77, 132-147.

Rawat S., Pullagurala V.L.R., Adisa I.O., Wang Y., Peralta-Videa J.R., Gardea-Torresdey J.L. Factors affecting fate and transport of engineered nanomaterials in terrestrial environments, *COESH*, 2018, 6, 47-53.

Xu T., Wang Y., Aytac Z., Zuverza-Mena N., Zhao Z., Hu X., Ng K.W., White J.C., Demokritou P.

Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli Responsive Core–Shell Nanostructures. ACS Nano 2022, 16, 4, 6034–6048.

L125-147 Please add a few sentences to compare the differences between these synchrotron-based techniques and non-synchrotron-supported ones, For example, u-XRF vs. TEM-EDS. What are the advantages?

According to the Abbe's Law the resolution power of a particle is d.

$d = \lambda / (2n \sin \alpha)$ In optical microscopy we can approximate d with λ .

In electron microscopy like TEM or SEM we use electrons that are accelerated passing through a potential difference they become thus equivalent to electromagnetic wave, as reported in the scheme.

Therefore, thanks to the De Broglie law we have $\lambda(\text{nm}) = 1.22 / \sqrt{V}$, where V is the acceleration voltage of the electrons.

In TEM $V =$ around 30 to 100, thus λ varies between 0.007 nm and 0.004 nm respectively. The smallest of these resolutions are enough to see the electron lattice. With the synchrotron we use photon that do not have mass, therefore the factor 1.22 is substituted which is the non-relativistic mass of the electron which is $m = 5.48579909065(16) \times 10^{-4}$ Da (according to The NIST Reference on Constants, Units, and Uncertainty. NIST. 20 May 2019. Retrieved 2020-06-21), this makes the resolution even smaller and increases the penetration depth into the sample.

The EDX is another thing because it depends on the acceleration voltage of the particle or of the photon. Every Element in the periodic table has its own orbitals energies, and the acceleration voltage allows to excite one or more of these, independently if it comes from a TEM or from a synchrotron. Hence in this respect the two techniques are alike. (Goldstein, Newbury et al Editors. Scanning Electron Microscopy and X-Ray Microanalysis, Third Edition. 2003 Springer Science+Business Media LLC, 233 Spring Street, New York, NY 10013 USA).

The text below has been added as a shortened version of these concepts.

Lines 160-165: "Synchrotron-based techniques use photons, which do not have mass; therefore the factor 1.22 is substituted by the non-relativistic mass of the electron which is $m = 5.485 \times 10^{-4}$ Da. This makes the resolution even smaller and increases penetration depth into the sample. On the other hand, EDX depends on the acceleration voltage of the particle or of the photon. Every element has its own orbital energies, and the acceleration voltage allows excitation one or more of these, independently if it comes from a TEM or from a synchrotron (Goldstein et al., 2003)."

A new reference has been included:

Goldstein J.I., Newbury D.E., Echlin P., Joy D.C., Lyman C.E., Lifshin E., Sawyer L., Michael J.R. Editors. Scanning Electron Microscopy and X-Ray Microanalysis, Third Edition. 2003 Springer Science+Business Media LLC, 233 Spring Street, New York, NY 10013 USA.

L195 Add nano delivery system for smart release
[Ref]

We thank Rev1 for the suggestion. The reference has been included and the text has been edited accordingly:

Lines 210-215: "Beyond the potential adverse effects upon bioaccumulation from soil or other exposure routes, there is an increasing interest in exploiting the potential positive effects of ENMs on plants, aiming to improve crop yields and quality. A range of mechanisms, including direct use as nanofertilizers (Verma et al., 2022), nanocarriers (Karny et al., 2018), smart delivery systems (Xu et al., 2022) or when in

association to plant growth-promoting bacteria, are considered (Prado de Moraes et al., 2021).”

Xu T., Wang Y., Aytac Z., Zuverza-Mena N., Zhao Z., Hu X., Ng K.W., White J.C., Demokritou P.
Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli Responsive Core–Shell Nanostructures. *ACS Nano* 2022, 16, 4, 6034–6048.

L251-267 Only one sentence was describing the synchrotron-based analysis. All the others were biomass or plant physiological responses. What does "limited stability of nLa2O3" mean? What are the results of the u-XRF analysis? Why is it unique compared to the other related analysis?

We thank Rev1 for the comment. The sentence has been clarified.

Lines 272-275:“The limited stability of nLa2O3, as compared to nCeO2, has been confirmed by -XRF analysis in *Cucumis sativus* L. through element speciation, dissolution studies in aqueous solution and in planta. After 14d treatment, the nCeO2 structure in the roots remains mostly preserved (more than 80%) while pristine nLa2O3 structure was observed at levels below 10% (Ma et al., 2015).”

Considering how Ce and La elemental properties play a fundamental role in their biotransformation, but also how their similarity in term of effects when analyzing the respective bulk forms, we believe that the direct comparison of the two nanoforms by XRF, as supported by data published in literature, could be an aspect to be described, and an interesting explanation for the physiological and molecular outcomes. Information about comparisons with other nanoforms has been reported at level of physiological and molecular analyses, both in terms of individual treatments, but also as a function of binary co-exposure (see Pagano et al., 2016, Pagano et al. 2017), which are reported in the text. This have been done with the aim to combine the potential results from physical and chemical analyses with results from physiological and molecular evidence, thereby providing a more mechanistic overview of the biotransformation processes.

L269 Please add more recently works:

[Ref]

[Ref]weathering effect in soil

The references indicated have been included and discussed.

Lines 312-318: “Interestingly, when either considering the utilization of pristine and coated nTiO2 (hydrophilic or hydrophobic) in carrot (*Daucus carota* L.), responses observed depended mainly on the nTiO2 surface coating, concentration and in soil weathering (Wang et al., 2021a; 2021b). Taproot and leaf fresh biomass and plant height were all increased with exposure, as well as nutrient uptake (Fe in leaves; Mg in taproots; Ca, Zn, K in roots). Conversely, sugar and starch contents were negatively affected, compromising the nutritional quality (Wang et al., 2021b).”

References included:

Wang Y., Deng C., Cota-Ruiz K., Tan W., Reyes A., Peralta-Videa J.R., Hernandez-Viezcas J.A., Li C., Gardea-Torresdey J.L. Effects of different surface-coated nTiO2 on full-grown carrot plants: Impacts on root splitting, essential elements, and Ti uptake. *J Haz Mater*, 2021a, 402, 123768.

Wang Y., Deng C., Cota-Ruiz K., Peralta-Videa J.R., Hernandez-Viezcas J.A., Gardea-Torresdey J.L. Soil-aged nano titanium dioxide effects on full-grown carrot: Dose and surface-coating dependent improvements on growth and nutrient quality. *Scie Tot Environ*, 2021b, 774, 145699.

L395-398 Please add more recent works regarding the nCuO-plant interactions.

Please check:

[Ref]

[Ref](in planta biodistribution of nCuO in the particle form and growth related gene expression)

The references indicated have been included and discussed.

Lines 452-464: "The grain of weedy and cultivated rice were differentially impacted by nCuO, bulk or ionic forms, showing also a cultivar-specific and concentration-dependent response. Cu translocation directly influenced plant yield, sugar production, starch content, protein content, and expression of auxin associated genes in grain (Deng et al., 2022b). Analyzing the effect of citric acid (CA) coated copper oxide (CA-nCuO) and its application (foliar spray or soil exposure) on the growth and physiology of soybean (*Glycine max* L.), nCuO appeared to be more accessible for plant uptake, as compared to CA-nCuO, decreasing the protein content, and inhibiting plant growth. CA reduced CuO NPs toxicity, demonstrating that surface modification may change the toxic properties of NPs (Deng et al., 2022c).

Treatment of *Lactuca sativa* L. with nCuO significantly increased biomass as compared to CuO microparticles (Wang et al., 2019). In addition, plants can benefit from nCuO treatment through enhanced defensive pathways, and through direct antimicrobial and antifungal activities (Elmer et al., 2018)."

References included:

Deng C., Wang Y., Navarro G., Sun Y., Cota-Ruiz K., Hernandez-Viezcas J.A., Niu G., Li C., White J.C., Gardea-Torresdey J. 2022b. Copper oxide (CuO) nanoparticles affect yield, nutritional quality, and auxin associated gene expression in weedy and cultivated rice (*Oryza sativa* L.) grains, *Scie Tot Environ*, 810, 152260.

Deng C., Wang Y., Cantu J.M., Valdes C., Navarro G., Cota-Ruiz K., Hernandez-Viezcas J.A., Li C., Elmer W.H., Dimkpa C.O., White J.C., Gardea-Torresdey, J.L. 2022c. Soil and foliar exposure of soybean (*Glycine max*) to Cu: Nanoparticle coating-dependent plant responses, *NanoImpact*, 26,100406.

Reviewer #2:

1. Please rewrite highlights 1 and 3. The highlight should point out the most critical findings, conclusions, or perspectives in this review.

Highlights have been modified in order to be more representative of the review text:

- Biotransformation is a fundamental phenomenon for understanding ENM-organism response mechanisms
- Synchrotron-based methodological analyses are critical for investigating ENM biotransformation
- Biotransformation of ENMs may have positive or negative effects when considering the agri-food application

2. Half of the text in this review are talking about the synchrotron-based analyses of ENM treated plant samples. However, it was not mentioned in the title, and the abstract only mentioned it once of its "increased use".

We thank Rev2 for the suggestions. Synchrotron-based techniques are becoming more important to comprehend the real physico-chemical forms of ENMs within plants tissues and organs and can give the missing information that we do not have from the physiological and molecular analyses, as suggested by the relevance of some of the most influential publications on this topic cited in the text (Castillo et al., 2017; Hameed et al., 2022). However, synchrotron-based techniques are not the main focus of the review. The mechanism of biotransformation behind the plant response to ENMs is the fundamental point. Synchrotron based techniques are certainly instrumental for shedding light on those mechanisms and the combination of results from physical analyses with physiological and molecular observations can give a more

comprehensive picture of what happens during ENMs treatment in planta. We are aware of the relevance of Synchrotron based techniques and we decided to give them an appropriate space in the introduction, in the (new) paragraph 1.2.

For these reasons we believe that title represents our work in the proper manner, and we would like to maintain it in the present form, where the emphasis is more on the mechanisms, while the techniques are tools to clarify those mechanisms.

The abstract, on the other hand, has been modified on order to be more informative on the points discussed in the main text:

“Research on engineered nanomaterials (ENMs) exposure has continued to expand rapidly, with a focus on uncovering the underlying mechanisms. The EU largely limits the number and the type of organisms that can be used for experimental testing through the 3R normative. There are different routes through which ENMs can enter the soil-plant system: this includes the agricultural application of sewage sludges, and the distribution of nano-enabled agrochemicals. However, a thorough understanding of the physiological and molecular implications of ENMs dispersion and chronic low-dose exposure remains elusive, thus requiring new evidence and a more mechanistic overview of pathways and major effectors involved in plants. Plants can offer a reliable alternative to conventional model systems to elucidate the concept of ENM biotransformation within tissues and organs, as a crucial step in understanding the mechanisms of ENM-organism interaction. To facilitate the understanding of the physico-chemical forms involved in plant response, synchrotron-based techniques have added new potential perspectives in studying the interactions between ENMs and biota. These techniques are providing new insights on the interactions between ENMs and biomolecules. The present review discusses the principal outcomes for ENMs after intake by plants, including possible routes of biotransformation which make their final fate less uncertain, and therefore require further investigation.”

3. The whole introduction (from L41-L96) is talking about the reported environmental exposure of ENM and the effects of quantum dots on plant growth. Please revise this part and be more focused on your main topic. This review aims to summarize the synchrotron-based analysis of the ENM biotransformation in plants and some molecular effects.

We thank Rev2 for the comment. Introduction has been modified in order to be more topic oriented.

Lines 47-99: “Although global food production has generally increased over time, the distribution has been far from equitable, with more than 820 million people having insufficient food and many more consuming low-quality diets leading directly to micronutrient deficiencies (Willett et al 2019; Zhong et al., 2020). In the past 20 years, and particularly in the past decade, engineered nanomaterials (ENMs) have seen dramatically increasing use and had an equally significant impact on ecosystems services and human society. As such, studies focused on the implications associated with their use are critical. In fact, it is known that ENMs exert important, but not completely understood, effects on biota; a particular topic of concern include the effect on crops, food production, and trophic transfer (Gardea-Torresdey et al., 2014; Ma et al., 2018; White et al., 2022).

The interplay between plant growth, dissolution, evaporation, and aggregation are key aspects of the dynamic behavior of ENMs in the environment. Directional aggregation can result in the formation of larger particles with a more complex morphology. However, given the complexity of natural environments, most nanomaterials can be found in hetero-aggregated composites of different inorganic and organic materials (Judy et al., 2012; Ma et al 2018). These aggregates can be very different from original simple pristine morphologies and may even form highly branched structures similar to fractals, all of which subsequently dramatically affect their reactivity and transport (Ma et al., 2018; Huangfu et al., 2019).

Sectors with a large nanomaterial application such as medicine and food production may experience greater risks of ENMs exposure due to their uses, with thousands of tons of ENMs that are eventually discarded into the three main environmental matrices: soil, water, and air (Keller et al., 2013; Zuverza Mena et al., 2017). ENMs with the

greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015). Considering the long-time span of use of select materials (1950 to 2050), efforts to estimate ENMs release through commercial and associated activity into the environment have been undertaken. For example, for nSiO₂, a global production between 100,000 and three million tons per year has been estimated, while for nCeO₂, levels likely reach the upper limit of 10,000 tons per year, and for nAg, the literature reflects a production volume below 1,000 tons per year (Giese et al., 2018). The use of nTiO₂ for the inhibition of microbial proliferation in food is one of the most important ways to prolong the shelf life of packaged products (Abutalib & Rajeh., 2020). However, a panel from EFSA concluded that E171 (TiO₂) can no longer be considered as safe when used as a food additive (EFSA Journal 2021). Gottschalk et al., (2009) calculated environmental concentrations using a probabilistic LCA (life cycle analysis) of ENM containing products. The authors modelled nTiO₂, nZnO, nAg, carbon nanotubes (CNT), and fullerenes for the U.S., Europe, and Switzerland. The concentrations in the environment were calculated through probabilistic density functions and compared to ecotoxicology data. In the simulations, the values ranged from 0.003 ng L⁻¹ (fullerenes) to 21 ng L⁻¹ (nTiO₂) for surface waters and from 4 ng L⁻¹ for fullerenes to 4 µg L⁻¹ for nTiO₂ for sewage treatment effluents. In Europe and the U.S., ENMs increased annually in sludge treated soil, and ranged from 1 ng kg⁻¹ for fullerenes to 89 µg kg⁻¹ for nTiO₂ (Gottschalk et al. 2009; Keller et al., 2013; Keller & Lazareva, 2014; Rincon, 2019). Importantly, quantum dots (QDs), as well as many carbon- and metal-based ENMs, have been shown to produce negative effects on animals and plants as a function of dose, including accumulation, alteration of physiological and biochemical parameters, and reduced growth or yield (Oh et al., 2016; Zuverza-Mena et al., 2017). Quantum dots have shown to enter plant roots and to damage the cell wall, dysregulating metabolism (Marmioli et al., 2020). While mercaptoacetic acid (MAA)-coated CdSe/ZnS QDs induced minimal toxicity on maize seedlings, pristine cadmium/tellurium (Cd/Te) QDs induced chromatin stress, mitochondrial damage and inhibition on green gram sprouts (*Phaseolus radiatus* L.) growth (Song et al., 2013). There are several routes by which ENM can enter the soil-plant system. These include agricultural application of sewage sludges which often contain nSiO₂, nTiO₂, nZnO, and nAg; as well as the application of nano-enabled agrochemicals, resulting in the direct entry of nSiO₂, nTiO₂, nZnO, nFeOx, nCuO, CeO₂ and nAg into agricultural soils (Lv et al., 2019; Verma et al., 2022)."

Additional references included:

Gardea-Torresdey J.L., Rico C.M., White J.C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* 2014, 48, 2526–2540.

Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* 15, 1692–1709.

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. *Environ. Sci. Technol. Lett.* 2014, 1, 1, 65–70.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, *Environment International*, 2015, 77, 132-147.

Rincon A.M. 2019. Chapter 6 - Presence of nanomaterials on consumer products: food, cosmetics, and drugs. pp. 165-181. Marmioli N., White J.C., Song J., Eds: In *Micro and Nano Technologies, Exposure to Engineered Nanomaterials in the Environment*, Elsevier, Amsterdam, The Netherlands.

Song U., Jun H., Waldman B., Roh J., Kim Y., Yi J., Lee E.J. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*) *Ecotoxicol. Environ. Saf.*, 2013. 93, 60-67.

White J.C., Zuverza-Mena N., Elmer W.H. From nanotoxicology to nano-enabled agriculture: Following the science at the Connecticut Agricultural Experiment Station (CAES). *Plant Nano Biology*, 2022, 1, 100007. Doi: 10.1016/j.plana.2022.100007.

4. L66 Please add more appropriate citations. Can you provide any related numbers?

New references have been added in order to give more quantitative information:

Lines 64-70: "Sectors with a large nanomaterial application such as medicine and food production may experience greater risks of ENMs exposure due to their uses, with thousands of tons of ENMs that are eventually discarded into the three main environmental matrices: soil, water, and air (Keller et al., 2013; Zuverza Mena et al., 2017). ENMs with the greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015)."

New references included:

Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* 15, 1692–1709.

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. *Environ. Sci. Technol. Lett.* 2014, 1, 1, 65–70.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, *Environment International*, 2015, 77, 132-147.

5. L67 and L78 Please define nSiO₂, nAg, nZnO, and so forth.

We thank Rev2 for the comment. We included the definition of each ENM cited:

Lines 67-70: "ENMs with the greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015)."

6. L71 Please add a citation.

The sentence has been fixed in order to give the proper citation.

Lines 72-75: "For example, for nSiO₂, a global production between 100,000 and three million tons per year has been estimated, while for nCeO₂, levels likely reach the upper limit of 10,000 tons per year, and for nAg, the literature reflects a production volume below 1,000 tons per year (Giese et al., 2018)."

7. L73 TiO₂ or nanoscale TiO₂?

The typo has been fixed.

8. You only have one subsection "1.1" in part 1. Please consider merging it into part 1. Or change it to 1.2, and put all the content before it into 1.1.

Paragraph subdivision has been updated, including an initial subsection called "1. Engineered nanomaterial (ENM) biotransformation". The first section is now "1.1. ENMs: from exposure to biotransformation", while the previous section 1.1 is now shifted to 1.2.

9. L101-105 Please check

[Ref]

[Ref] for factors and mechanisms of the NPs biotransformation, especially the effect of soil weathering on plant responses.

We thank Rev2 for the suggestions. The text has been updated, as reported:

Lines 102-121: "Environmental and soil physico-chemical characteristics may significantly impact on ENMs aggregation, and dissolution, which may modify ENM bioavailability, uptake, translocation, and accumulation into terrestrial plants. In fact, light and temperature may induce potential changes on the ENM structure, such as during foliar spray (Hong et al., 2021). Once within plant tissues, ENM biotransformation may alter particle stability and behaviour in terms of interactions with biomolecules, triggering differential plant defense mechanisms (Ma et al., 2018; Rawat et al., 2018). ENMs are subject to a range of processes that may lead to their partial dissolution or result in structural modifications (Milosevic et al., 2020; Marmioli et al., 2020). A schematic representation is reported in Figure 1. Nanoparticle biotransformation is a highly complex and poorly understood series of events and has been shown to occur during weathering in the soil, trophic transfer, and translocation within plant tissues. These reactions are highly dynamic and alter the original pristine structure of the nanoparticles in a number of ways, potentially causing the release of ions, but also the consequent restructuring (or destructuring) of the nanoparticle (Servin et al., 2017a). Biotransformation of nanomaterials may rest on the interaction with biological molecules that stabilize their external reactivity, such as peptides including those involved in detoxification, (e.g. glutathione), fatty acids, secondary metabolites, and even components of cell membranes (Marmioli et al., 2020). Particle properties such as size, stability, charge, and dissolution may strongly influence other biotransformation mechanisms, potentially promoting enzymatic modification and functionalization with proteins (e.g., corona protein) present in the cytoplasm and organelles (Ma et al., 2018; Marmioli et al., 2020)."

A new reference has been included, as well as an additional Figure (new Figure 1):

Hong J., Wang C., Wagner D.C., Gardea-Torresdey J.L., He F., Rico C.M. 2021. Foliar application of nanoparticles: mechanisms of absorption, transfer, and multiple impacts. *Environ. Sci.: Nano*, 8, 1196-1210.

Rawat S., Pullagurala V.L.R., Adisa I.O., Wang Y., Peralta-Videa J.R., Gardea-Torresdey J.L. Factors affecting fate and transport of engineered nanomaterials in terrestrial environments, *COESH*, 2018, 6, 47-53.

The suggested reference related to "Soil-aged nano titanium dioxide effects on full-grown carrot" has been included in the main text in the case studies reported in the paragraph 2.2 as Wang et al., 2021b.

10. L110-113 Besides the listed possibilities, ENMs can also remain as nanoscale particles with/without the loss of surface coatings in plant tissues after being uptaken. Please check

[ref]

We thank Rev 2 for the suggestion. The reference has been cited, as reported.

Lines 121-124: "ENMs may maintain crystal structure when internalized by cells or may be disassembled and converted into less complex structures (by biological modification or chelation), thus reducing toxicity, and the risk of their accumulation and translocation (Wang et al., 2022)."

11. L178-181 Add more latest references. Please check

[ref]

[ref]

We thank Rev2 for the suggestions. The text has been updated, as reported:

Lines 196-199: "Studies have added significant molecular data on the effects of ENMs exposure in plants (Schwab et al., 2016; Ma et al., 2018). Different results are often observed for the same element as a function of its form or size, i.e. nanostructured, bulk, or ionic species (Pagano et al., 2016; Wang et al., 2022)."

The suggested reference related to "Copper oxide (CuO) nanoparticles affect yield, nutritional quality, and auxin associated gene expression in weedy and cultivated rice" has been included in the text in the case studies reported in the paragraph 2.6 as Deng et al., 2022b.

12. L202 Can you briefly describe Table 1? What is Table 1 for and why are genes listed? Were they significantly affected by the ENM exposure? All the citations listed in Table 1 are related to the author themselves. Please include some works from the others.

Genes reported in Figure 2 (former Figure 1) and Table 1 are some of those that are modulated by the different type of ENMs as reported in the cited studies. Other genes also resulted responsiveness in a ENM-specific manner during exposure and are considered for this reason as potential biomarker of exposure/effect. Moreover, these specific biomarkers are also able to testify how the modulation of the genes in the different organs is in some cases convergent between differential forms (nano, bulk, ion) of the same element. Considering that the concept of nano-specific biomarker has been introduced in some of our research groups papers, we thought to include these references in the Table 1. This was not the case of Table 2, in which papers not related to our research group on the principal evidence of the ENM biotransformation in plant observed by physiological, molecular and synchrotron-based analyses have been included.

Table 1 description has b...

To Co-Editors-in-Chief, Environmental Pollution Journal

Prof. Jörg Rinklebe,

Prof. Christian Sonne,

Prof. Eddy Zeng

To Special Issue Editor,

Prof. Da Chen

To Guest Editor,

Prof. M.H. Siddiqui

Special Issue “Emerging Pollutants and their effects on plants: present and future challenges, and their solutions”

Dear Editor,

We thank the Editor and Reviewers for their comments and suggestions which gave the opportunity to improve our Review manuscript “Nanomaterials biotransformation: In planta mechanisms of action” in all its aspects.

As requested, the manuscript has been modified, including the abstract, highlights and paragraph subdivisions (in the introduction). In addition, the introduction has been thoroughly edited. A list of new references has been included. A new Figure 1 has been included in order to give more explanations to the biotransformation mechanisms.

Below are the point-to-point answers to Reviewers queries.

We believe the manuscript has been strongly improved and hope it is now acceptable for publication.

Kind Regards

Marta Marmiroli

To Co-Editors-in-Chief, Environmental Pollution Journal

Prof. Jörg Rinklebe,
Prof. Christian Sonne,
Prof. Eddy Zeng

To Special Issue Editor,

Prof. Da Chen

To Guest Editor,

Prof. M.H. Siddiqui

Special Issue “Emerging Pollutants and their effects on plants: present and future challenges, and their solutions”

Dear Editor,

We thank the Editor and Reviewers for their comments and suggestions which gave the opportunity to improve our Review manuscript “Nanomaterials biotransformation: In planta mechanisms of action” in all its aspects.

As requested, the manuscript has been modified, including the abstract, highlights and paragraph subdivisions (in the introduction). In addition, the introduction has been thoroughly edited. A list of new references has been included. A new Figure 1 has been included in order to give more explanations to the biotransformation mechanisms.

Below are the point-to-point answers to Reviewers queries.

We believe the manuscript has been strongly improved and hope it is now acceptable for publication.

Kind Regards

Marta Marmiroli

Editor and Reviewer comments:

Reviewer #1: *Manuscript review of ENVPOL-D-22-05734 "Nanomaterials biotransformation: In planta mechanisms of action"*

This review article summarized recent works on the nanomaterial biotransformation within plant tissues using synchrotron-based techniques. The impacts of engineered nanomaterials (ENMs) on plant gene expression were also included. Specifically, studies regarding the biointeraction between plants and ENMs (nanoscale CeO₂, La₂O₃, TiO₂, Au, FeO_x, ZVI, ZnO, CuO, and CdS QDs) were listed and discussed in detail. This review paper contributes to the deep understanding of the fate of ENMs in plant tissues, and the related genetic regulation of plants induced by ENM exposures. The contents are within the scope of the Environmental Pollution. However, I have a number of general concerns, followed by a range of specific comments, which prevent me from recommending this paper for publication in its current form.

General Comments-

Some parts of the abstract and introduction are not quite relevant to the main topic, and need to be revised. The main topic, synchrotron-based analysis of ENMs biotransformation, should be more emphasized and discussed in more detail. In part 1, there was only one sub-title 1.1. None of the figures or the tables provided any information related to the in planta biotransformation. Several figures with summarized information instead of only one figure are better for a review article. Some references are too old or not representative. They should be up to date.

We thank Rev1 for the constructive comments and suggestions given. The manuscript has been thoroughly improved in order to answer to all comments reported. Paragraph subdivision has been updated. Several new references and an additional Figure have been included. All the edited sections have been reported in the answer to each specific question.

Specific Comments-

The abstract used a large amount of space describing exposure pathways and low-dose effects. However, they were not well discussed in the main text. Similarly, the "potential applicability" was not given in the main text. The abstract should contain the most significant findings, critical comments on the current studies, or perspectives for future research.

The abstract has been modified in order to be more informative on the points discussed in the main text. The abstract now reads as follows:

“Research on engineered nanomaterials (ENMs) exposure has continued to expand rapidly, with a focus on uncovering the underlying mechanisms. The EU largely limits the number and the type of organisms that can be used for experimental testing through the 3R normative. There are different routes through which ENMs can enter the soil-plant system: this includes the agricultural application of sewage sludges, and the distribution of nano-enabled agrochemicals. However, a thorough understanding of the physiological and molecular implications of ENMs dispersion and chronic low-dose exposure remains elusive, thus requiring new evidence and a more mechanistic overview of pathways and major effectors involved in plants. Plants can offer a reliable alternative to conventional model systems to elucidate the concept of ENM biotransformation within tissues and organs, as a crucial step in understanding the mechanisms of ENM-organism interaction. To facilitate the understanding of the physico-chemical forms involved in plant response, synchrotron-based techniques have added new potential perspectives in studying the interactions between ENMs and

biota. These techniques are providing new insights on the interactions between ENMs and biomolecules. The present review discusses the principal outcomes for ENMs after intake by plants, including possible routes of biotransformation which make their final fate less uncertain, and therefore require further investigation.”

The Paragraph 3 has been also modified to give a more timely reference to potential applicability:

Lines 558-567: “This information is highly relevant with regard to potential applicability: ENMs can interact with sensitive ecosystem components within trophic food chains, affect microbial populations in soil, enter into the plant and where they can be translocated to different tissues and organs, including the edible tissues or organs (Holden et al., 2013; Liu et al., 2015). Biotransformation can occur at each step within these processes, modifying and/or amplifying ENM effects at organism level. These interactions from the level of ecosystem, organism, tissue, cell, and organelles become key factors when applying “ENM biotransformation” as a concept for a safer design, when considering applications for agriculture and food production, and for minimizing the adverse biological impact (Burello & Worth, 2015; Pagano et al., 2018; Lowry et al., 2019; Kah et al., 2019; Zulfiqar et al., 2019; Ma et al., 2020; Hameed et al., 2022; Xu et al., 2022).”

A new reference has been also included:

Xu T., Wang Y., Aytac Z., Zuverza-Mena N., Zhao Z., Hu X., Ng K.W., White J.C., Demokritou P. Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli Responsive Core–Shell Nanostructures. ACS Nano 2022, 16, 4, 6034–6048.

You mentioned that three billion tons of crops are produced per year and cost a lot of source and energy in L41-47. What is the purpose? Please consider deleting them.

The new paragraph 1.1 entitled “from ENM exposure to biotransformation” has been reduced and thoroughly edited in order to be more focused on the main topic of the review:

Lines 47-99: “Although global food production has generally increased over time, the distribution has been far from equitable, with more than 820 million people having insufficient food and many more consuming low-quality diets leading directly to micronutrient deficiencies (Willett et al 2019; Zhong et al., 2020). In the past 20 years, and particularly in the past decade, engineered nanomaterials (ENMs) have seen dramatically increasing use and had an equally significant impact on ecosystems services and human society. As such, studies focused on the implications associated with their use are critical. In fact, it is known that ENMs exert important, but not completely understood, effects on biota; a particular topic of concern include the effect on crops, food production, and trophic transfer (Gardea-Torresdey et al., 2014; Ma et al., 2018; White et al., 2022).

The interplay between plant growth, dissolution, evaporation, and aggregation are key aspects of the dynamic behavior of ENMs in the environment. Directional aggregation can result in the formation of larger particles with a more complex morphology. However, given the complexity of natural environments, most nanomaterials can be found in hetero-aggregated composites of different inorganic and organic materials (Judy et al., 2012; Ma et al 2018). These aggregates can be very different from original simple pristine morphologies and may even form highly branched structures similar to fractals, all of which subsequently dramatically affect their reactivity and transport (Ma et al., 2018; Huangfu et al., 2019).

Sectors with a large nanomaterial application such as medicine and food production may experience greater risks of ENMs exposure due to their uses, with thousands of tons of ENMs that are eventually discarded into the three main environmental matrices: soil, water, and air (Keller et al., 2013; Zuverza Mena et al., 2017). ENMs with the greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015). Considering the long-time span of use of select materials (1950 to 2050), efforts to estimate ENMs release through commercial and associated activity into the environment have been undertaken. For example, for nSiO₂, a global production between 100,000 and three million tons per year has been estimated, while for nCeO₂, levels likely reach the upper limit of 10,000 tons per year, and for nAg, the literature reflects a production volume below 1,000 tons per year (Giese et al., 2018). The use of nTiO₂ for the inhibition of microbial proliferation in food is one of the most important ways to prolong the shelf life of packaged products (Abutalib & Rajeh., 2020). However, a panel from EFSA concluded that E171 (TiO₂) can no longer be considered as safe when used as a food additive (EFSA Journal 2021).

Gottschalk et al., (2009) calculated environmental concentrations using a probabilistic LCA (life cycle analysis) of ENM containing products. The authors modelled nTiO₂, nZnO, nAg, carbon nanotubes (CNT), and fullerenes for the U.S., Europe, and Switzerland. The concentrations in the environment were calculated through probabilistic density functions and compared to ecotoxicology data. In the simulations, the values ranged from 0.003 ng L⁻¹ (fullerenes) to 21 ng L⁻¹ (nTiO₂) for surface waters and from 4 ng L⁻¹ for fullerenes to 4 µg L⁻¹ for nTiO₂ for sewage treatment effluents. In Europe and the U.S., ENMs increased annually in sludge treated soil, and ranged from 1 ng kg⁻¹ for fullerenes to 89 µg kg⁻¹ for nTiO₂ (Gottschalk et al. 2009; Keller et al., 2013; Keller & Lazareva, 2014; Rincon, 2019).

Importantly, quantum dots (QDs), as well as many carbon- and metal-based ENMs, have been shown to produce negative effects on animals and plants as a function of dose, including accumulation, alteration of physiological and biochemical parameters, and reduced growth or yield (Oh et al., 2016; Zuverza-Mena et al., 2017). Quantum dots have shown to enter plant roots and to damage the cell wall, dysregulating metabolism (Marmioli et al., 2020). While mercaptoacetic acid (MAA)-coated CdSe/ZnS QDs induced minimal toxicity on maize seedlings, pristine cadmium/tellurium (Cd/Te) QDs induced chromatin stress, mitochondrial damage and inhibition on green gram sprouts (*Phaseolus radiatus* L.) growth (Song et al., 2013). There are several routes by which ENM can enter the soil-plant system. These include agricultural application of sewage sludges which often contain nSiO₂, nTiO₂, nZnO, and nAg; as well as the application of nano-enabled agrochemicals, resulting in the direct entry of nSiO₂, nTiO₂, nZnO, nFeOx, nCuO, CeO₂ and nAg into agricultural soils (Lv et al., 2019; Verma et al., 2022).”

Additional references included:

Gardea-Torresdey J.L., Rico C.M., White J.C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* 2014, 48, 2526–2540.

Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* 15, 1692–1709.

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. *Environ. Sci. Technol. Lett.* 2014, 1, 1, 65–70.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, *Environment International*, 2015,

77, 132-147.

Rincon A.M. 2019. Chapter 6 - Presence of nanomaterials on consumer products: food, cosmetics, and drugs. pp. 165-181. Marmiroli N., White J.C., Song J., Eds: In *Micro and Nano Technologies, Exposure to Engineered Nanomaterials in the Environment*, Elsevier, Amsterdam, The Netherlands.

Song U., Jun H., Waldman B., Roh J., Kim Y., Yi J., Lee E.J. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*) *Ecotoxicol. Environ. Saf.*, 2013. 93, 60-67.

White J.C., Zuverza-Mena N., Elmer W.H. From nanotoxicology to nano-enabled agriculture: Following the science at the Connecticut Agricultural Experiment Station (CAES). *Plant Nano Biology*, 2022, 1, 100007. Doi: 10.1016/j.plana.2022.100007.

L50-55 Please focus on plants, especially agricultural crops.

We agree with Rev1 give more relevance to the ENMs in agricultural practices. The sentence has been modified and two new references have been introduced.

Lines 52-55: “In fact, it is known that ENMs exert important, but not completely understood, effects on biota; a particular topic of concern include the effect on crops, food production, and trophic transfer (Gardea-Torresdey et al., 2014; Ma et al., 2018; White et al., 2022).”

References added:

Gardea-Torresdey J.L., Rico C.M., White J.C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* 2014, 48, 2526–2540.

White J.C., Zuverza-Mena N., Elmer W.H. From nanotoxicology to nano-enabled agriculture: Following the science at the Connecticut Agricultural Experiment Station (CAES). *Plant Nano Biology*, 2022, 1, 100007. Doi: 10.1016/j.plana.2022.100007.

L53-54 It is too broad to conclude that the impacts of nanomaterials on biota are poorly understood. There are hundreds of publications investigating this topic.

The sentence, as suggested by Rev1, have been modified in order to be more topic oriented.

Lines 52-55: “In fact, it is known that ENMs exert important, but not completely understood, effects on biota; a particular topic of concern include the effect on crops, food production, and trophic transfer (Gardea-Torresdey et al., 2014; Ma et al., 2018; White et al., 2022).”

L67-68 Please add nCuO, nTiO₂, and nZnO. nTiO₂ is the most used one among all the others.

Please check

[Ref]

[Ref]

The sentence has been modified as requested.

Lines 67-70: “ENMs with the greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015).”

The references suggested have been also added:

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. Environ. Sci. Technol. Lett. 2014, 1, 1, 65–70.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, Environment International, 2015, 77, 132-147.

L77 Can you find any latest references? 2009 is more than ten years ago.

Unfortunately, we have not found any new significant update on permissible concentrations, especially for the European side. From this point of view the cited paper is still maintained as an EU standards. Thus, we prefer to maintain this reference. To provide additional perspective, we included two additional references:

Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered nanomaterials. J. Nanopart. Res. 15, 1692–1709.

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. Environ. Sci. Technol. Lett. 2014, 1, 1, 65–70.

L92 Please refer to the right article.

We thank the Rev1 for the comment, the reference has been properly corrected and added to the references.

Song U., Jun H., Waldman B., Roh J., Kim Y., Yi J., Lee E.J. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*) Ecotoxicol. Environ. Saf., 2013. 93, 60-67.

L99 In 1.1, "Once accumulated by plants" You haven't given any background regarding the uptake of ENMs by plants. At least add a few sentences to describe the possibility and give a few examples.

The beginning of the Paragraph, now 1.2, has been edited in order to give more information about the phases before plant accumulation. A new Figure 1 has been also included.

Lines 102-110: “Environmental and soil physico-chemical characteristics may significantly impact on ENMs aggregation, and dissolution, which may modify ENM bioavailability, uptake, translocation, and accumulation into terrestrial plants. In fact, light and temperature may induce potential changes on the ENM structure, such as during foliar spray (Hong et al., 2021). Once within plant tissues, ENM biotransformation may alter particle stability and behaviour in terms of interactions with biomolecules, triggering differential plant defense mechanisms (Ma et al., 2018; Rawat et al., 2018). ENMs are subject to a range of processes that may lead to their partial dissolution or result in structural modifications (Milosevic et al., 2020; Marmiroli et al., 2020). A schematic representation is reported in Figure 1.”

New reference added:

Hong J., Wang C., Wagner D.C., Gardea-Torresdey J.L., He F., Rico C.M. 2021. Foliar application of nanoparticles: mechanisms of absorption, transfer, and multiple impacts. *Environ. Sci.: Nano*, 8, 1196-1210.

Rawat S., Pullagurala V.L.R., Adisa I.O., Wang Y., Peralta-Videa J.R., Gardea-Torresdey J.L. Factors affecting fate and transport of engineered nanomaterials in terrestrial environments, *COESH*, 2018, 6, 47-53.

L105-107 "biotransformation of nanomaterials lead to the attachment of biological molecules", or does the attachment of biological molecules lead to NPs biotransformation? Or both? Any examples?

The paragraph has been modified in order to avoid misinterpretation. A new Figure 1 has been also introduced.

Lines 110-129: “Nanoparticle biotransformation is a highly complex and poorly understood series of events and has been shown to occur during weathering in the soil, trophic transfer, and translocation within plant tissues. These reactions are highly dynamic and alter the original pristine structure of the nanoparticles in a number of ways, potentially causing the release of ions, but also the consequent restructuring (or destructuring) of the nanoparticle (Servin et al., 2017a). Biotransformation of nanomaterials may rest on the interaction with biological molecules that stabilize their external reactivity, such as peptides including those involved in detoxification, (*e.g.* glutathione), fatty acids, secondary metabolites, and even components of cell membranes (Marmiroli et al., 2020). Particle properties such as size, stability, charge, and dissolution may strongly influence other biotransformation mechanisms, potentially promoting enzymatic modification and functionalization with proteins (*e.g.*, corona protein) present in the cytoplasm and organelles (Ma et al., 2018; Marmiroli et al., 2020). ENMs may maintain crystal structure when internalized by cells or may be disassembled and converted into less complex structures (by biological modification or chelation), thus reducing toxicity, and the risk of their accumulation and translocation (Wang et al., 2022). These post-uptake structural modifications involve specific parameters such as bond distance with other atoms or nature of the ligand atoms. In consideration of this, one objective in biotransformation studies is to investigate the physico-chemical forms (*e.g.*, nanocrystal structure) within exposed tissues and to characterize the structural differences within the new biotransformed molecules, including identification of the biomolecules interacting with the ENMs (Castillo-Michel et al., 2017; Marmiroli et al., 2020).”

L110 More citations are needed to support the statement of "properties such as size, stability, charge, and dissolution influence the biotransformation". Besides, plant cultivars can also affect ENMs in planta biodistribution
[Ref]

The paragraph has been modified in order to include new references and also the effect of the plant cultivars.

Lines 115-131: “Biotransformation of nanomaterials may rest on the interaction with biological molecules that stabilize their external reactivity, such as peptides including those involved in detoxification, (e.g. glutathione), fatty acids, secondary metabolites, and even components of cell membranes (Marmioli et al., 2020). Particle properties such as size, stability, charge, and dissolution may strongly influence other biotransformation mechanisms, potentially promoting enzymatic modification and functionalization with proteins (e.g., corona protein) present in the cytoplasm and organelles (Ma et al., 2018; Marmioli et al., 2020). ENMs may maintain crystal structure when internalized by cells or may be disassembled and converted into less complex structures (by biological modification or chelation), thus reducing toxicity, and the risk of their accumulation and translocation (Wang et al., 2022). These post-uptake structural modifications involve specific parameters such as bond distance with other atoms or nature of the ligand atoms. In consideration of this, one objective in biotransformation studies is to investigate the physico-chemical forms (e.g., nanocrystal structure) within exposed tissues and to characterize the structural differences within the new biotransformed molecules, including identification of the biomolecules interacting with the ENMs (Castillo-Michel et al., 2017; Marmioli et al., 2020). It has furthermore to consider how genetic diversity across different plant species and within the same plant species (in different cultivars) may influence the ENM uptake and translocation (Deng et al., 2020).”

New references included:

Deng C., Wang Y., Cota-Ruiz K., Reyes A., Sun Y., Peralta-Videa J.R., Hernandez-Viezcas J.A., Turley R.S., Niu G., Li C., Gardea-Torresdey J.L. Bok choy (*Brassica rapa*) grown in copper oxide nanoparticles-amended soils exhibits toxicity in a phenotype-dependent manner: Translocation, biodistribution and nutritional disturbance. *J Haz Mater*, 2020, 398, 122978.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, *Environment International*, 2015, 77, 132-147.

Rawat S., Pullagurala V.L.R., Adisa I.O., Wang Y., Peralta-Videa J.R., Gardea-Torresdey J.L. Factors affecting fate and transport of engineered nanomaterials in terrestrial environments, *COESH*, 2018, 6, 47-53.

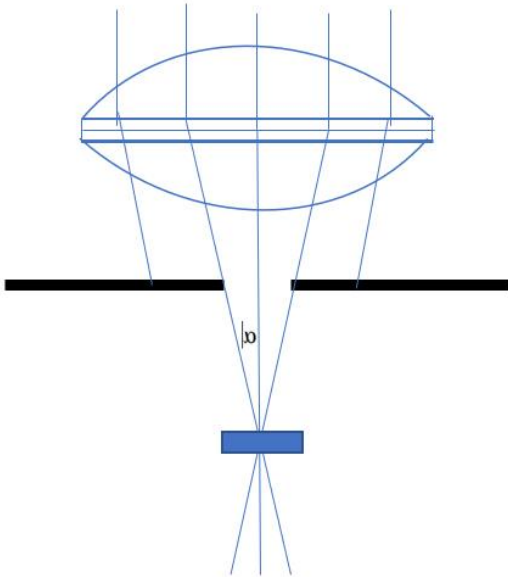
Xu T., Wang Y., Aytac Z., Zuverza-Mena N., Zhao Z., Hu X., Ng K.W., White J.C., Demokritou P. Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli Responsive Core–Shell Nanostructures. *ACS Nano* 2022, 16, 4, 6034–6048.

L125-147 Please add a few sentences to compare the differences between these synchrotron-based techniques and non-synchrotron-supported ones, For example, u-XRF vs. TEM-EDS. What are the advantages?

According to the Abbe's Law the resolution power of a particle is d .

$d = \lambda / (2n \sin \alpha)$ In optical microscopy we can approximate d with λ .

In electron microscopy like TEM or SEM we use electrons that are accelerated passing through a potential difference they become thus equivalent to electromagnetic wave, as reported in the scheme.



Therefore, thanks to the De Broglie law we have $\lambda(\text{nm}) = 1.22 / \sqrt{V}$, where V is the acceleration voltage of the electrons.

In TEM V is around 30 to 100, thus λ varies between 0.007 nm and 0.004 nm respectively. The smallest of these resolutions are enough to see the electron lattice. With the synchrotron we use photon that do not have mass, therefore the factor 1.22 is substituted by the non-relativistic mass of the electron which is $m = 5.485799065(16) \times 10^{-4}$ Da (according to *The NIST Reference on Constants, Units, and Uncertainty*. NIST. 20 May 2019. Retrieved 2020-06-21), this makes the resolution even smaller and increases the penetration depth into the sample.

The EDX is another thing because it depends on the acceleration voltage of the particle or of the photon. Every Element in the periodic table has its own orbitals energies, and the acceleration voltage allows to excite one or more of these, independently if it comes from a TEM or from a synchrotron. Hence in this respect the two techniques are alike. (Goldstein, Newbury et al Editors. *Scanning Electron Microscopy and X-Ray Microanalysis*, Third Edition. 2003 Springer Science+Business Media LLC, 233 Spring Street, New York, NY 10013 USA).

The text below has been added as a shortened version of these concepts.

Lines 160-165: "Synchrotron-based techniques use photons, which do not have mass; therefore the factor 1.22 is substituted by the non-relativistic mass of the electron which is $m = 5.485 \times 10^{-4}$ Da. This makes the resolution even smaller and increases penetration depth into the sample. On the other hand, EDX depends on the acceleration voltage of the particle or of the photon. Every element has its own orbital energies, and the acceleration voltage allows excitation one or more of these, independently if it comes from a TEM or from a synchrotron (Goldstein et al., 2003)."

A new reference has been included:

Goldstein J.I., Newbury D.E., Echlin P., Joy D.C., Lyman C.E., Lifshin E., Sawyer L., Michael J.R. Editors. Scanning Electron Microscopy and X-Ray Microanalysis, Third Edition. 2003 Springer Science+Business Media LLC, 233 Spring Street, New York, NY 10013 USA.

*L195 Add nano delivery system for smart release
[Ref]*

We thank Rev1 for the suggestion. The reference has been included and the text has been edited accordingly:

Lines 210-215: “Beyond the potential adverse effects upon bioaccumulation from soil or other exposure routes, there is an increasing interest in exploiting the potential positive effects of ENMs on plants, aiming to improve crop yields and quality. A range of mechanisms, including direct use as nanofertilizers (Verma et al., 2022), nanocarriers (Karny et al., 2018), smart delivery systems (Xu et al., 2022) or when in association to plant growth-promoting bacteria, are considered (Prado de Moraes et al., 2021).”

Xu T., Wang Y., Aytac Z., Zuverza-Mena N., Zhao Z., Hu X., Ng K.W., White J.C., Demokritou P. Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli Responsive Core–Shell Nanostructures. ACS Nano 2022, 16, 4, 6034–6048.

L251-267 Only one sentence was describing the synchrotron-based analysis. All the others were biomass or plant physiological responses. What does "limited stability of nLa2O3" mean? What are the results of the u-XRF analysis? Why is it unique compared to the other related analysis?

We thank Rev1 for the comment. The sentence has been clarified.

Lines 272-275: “The limited stability of nLa₂O₃, as compared to nCeO₂, has been confirmed by μ -XRF analysis in *Cucumis sativus* L. through element speciation, dissolution studies in aqueous solution and *in planta*. After 14d treatment, the nCeO₂ structure in the roots remains mostly preserved (more than 80%) while pristine nLa₂O₃ structure was observed at levels below 10% (Ma et al., 2015).”

Considering how Ce and La elemental properties play a fundamental role in their biotransformation, but also how their similarity in term of effects when analyzing the respective bulk forms, we believe that the direct comparison of the two nanoforms by XRF, as supported by data published in literature, could be an aspect to be described, and an interesting explanation for the physiological and molecular outcomes. Information about comparisons with other nanoforms has been reported at level of physiological and molecular analyses, both in terms of individual treatments, but also as a function of binary co-exposure (see Pagano et al., 2016, Pagano et al. 2017), which are reported in the text. This have been done with the aim to combine the potential results from physical and chemical analyses with results from physiological and molecular evidence, thereby providing a more mechanistic overview of the biotransformation processes.

*L269 Please add more recently works:
[Ref]
[Ref]weathering effect in soil*

The references indicated have been included and discussed.

Lines 312-318: “Interestingly, when either considering the utilization of pristine and coated nTiO₂ (hydrophilic or hydrophobic) in carrot (*Daucus carota* L.), responses observed depended mainly on the nTiO₂ surface coating, concentration and in soil weathering (Wang et al., 2021a; 2021b). Taproot and leaf fresh biomass and plant height were all increased with exposure, as well as nutrient uptake (Fe in leaves; Mg in taproots; Ca, Zn, K in roots). Conversely, sugar and starch contents were negatively affected, compromising the nutritional quality (Wang et al., 2021b).”

References included:

Wang Y., Deng C., Cota-Ruiz K., Tan W., Reyes A., Peralta-Videa J.R., Hernandez-Viezcas J.A., Li C., Gardea-Torresdey J.L. Effects of different surface-coated nTiO₂ on full-grown carrot plants: Impacts on root splitting, essential elements, and Ti uptake. *J Haz Mater*, 2021a, 402, 123768.

Wang Y., Deng C., Cota-Ruiz K., Peralta-Videa J.R., Hernandez-Viezcas J.A., Gardea-Torresdey J.L. Soil-aged nano titanium dioxide effects on full-grown carrot: Dose and surface-coating dependent improvements on growth and nutrient quality. *Scie Tot Environ*, 2021b, 774, 145699.

L395-398 Please add more recent works regarding the nCuO-plant interactions. Please check:

[Ref]

[Ref](in planta biodistribution of nCuO in the particle form and growth related gene expression)

The references indicated have been included and discussed.

Lines 452-464: “The grain of weedy and cultivated rice were differentially impacted by nCuO, bulk or ionic forms, showing also a cultivar-specific and concentration-dependent response. Cu translocation directly influenced plant yield, sugar production, starch content, protein content, and expression of auxin associated genes in grain (Deng et al., 2022b). Analyzing the effect of citric acid (CA) coated copper oxide (CA-nCuO) and its application (foliar spray or soil exposure) on the growth and physiology of soybean (*Glycine max* L.), nCuO appeared to be more accessible for plant uptake, as compared to CA-nCuO, decreasing the protein content, and inhibiting plant growth. CA reduced CuO NPs toxicity, demonstrating that surface modification may change the toxic properties of NPs (Deng et al., 2022c).

Treatment of *Lactuca sativa* L. with nCuO significantly increased biomass as compared to CuO microparticles (Wang et al., 2019). In addition, plants can benefit from nCuO treatment through enhanced defensive pathways, and through direct antimicrobial and antifungal activities (Elmer et al., 2018).”

References included:

Deng C., Wang Y., Navarro G., Sun Y., Cota-Ruiz K., Hernandez-Viezcas J.A., Niu G., Li C., White J.C., Gardea-Torresdey J. 2022b. Copper oxide (CuO) nanoparticles affect yield, nutritional quality, and auxin associated gene expression in weedy and cultivated rice (*Oryza sativa* L.) grains, *Scie Tot Environ*, 810, 152260.

Deng C., Wang Y., Cantu J.M., Valdes C., Navarro G., Cota-Ruiz K., Hernandez-Viezcas J.A., Li C., Elmer W.H., Dimkpa C.O., White J.C., Gardea-Torresdey, J.L. 2022c. Soil and foliar exposure of soybean (*Glycine max*) to Cu: Nanoparticle coating-dependent plant responses, *NanoImpact*, 26,100406.

Reviewer #2:

1. Please rewrite highlights 1 and 3. The highlight should point out the most critical findings, conclusions, or perspectives in this review.

Highlights have been modified in order to be more representative of the review text:

- Biotransformation is a fundamental phenomenon for understanding ENM-organism response mechanisms
- Synchrotron-based methodological analyses are critical for investigating ENM biotransformation
- Biotransformation of ENMs may have positive or negative effects when considering the agri-food application

2. Half of the text in this review are talking about the synchrotron-based analyses of ENM treated plant samples. However, it was not mentioned in the title, and the abstract only mentioned it once of its "increased use".

We thank Rev2 for the suggestions. Synchrotron-based techniques are becoming more important to comprehend the real physico-chemical forms of ENMs within plants tissues and organs and can give the missing information that we do not have from the physiological and molecular analyses, as suggested by the relevance of some of the most influential publications on this topic cited in the text (Castillo et al., 2017; Hameed et al., 2022). However, synchrotron-based techniques are not the main focus of the review. The mechanism of biotransformation behind the plant response to ENMs is the fundamental point. Synchrotron based techniques are certainly instrumental for shedding light on those mechanisms and the combination of results from physical analyses with physiological and molecular observations can give a more comprehensive picture of what happens during ENMs treatment *in planta*. We are aware of the relevance of Synchrotron based techniques and we decided to give them an appropriate space in the introduction, in the (new) paragraph 1.2.

For these reasons we believe that title represents our work in the proper manner, and we would like to maintain it in the present form, where the emphasis is more on the mechanisms, while the techniques are tools to clarify those mechanisms.

The abstract, on the other hand, has been modified on order to be more informative on the points discussed in the main text:

“Research on engineered nanomaterials (ENMs) exposure has continued to expand rapidly, with a focus on uncovering the underlying mechanisms. The EU largely limits the number and the type of organisms that can be used for experimental testing through the 3R normative. There are different routes through which ENMs can enter the soil-plant system: this includes the agricultural application of sewage sludges, and the distribution of nano-enabled agrochemicals. However, a thorough understanding of the physiological and molecular implications of ENMs dispersion and chronic low-dose exposure remains elusive, thus requiring new evidence and a more mechanistic overview of pathways and major effectors involved in plants. Plants can offer a reliable alternative to conventional model systems to elucidate the concept of ENM biotransformation within tissues and organs, as a crucial step in understanding the mechanisms of ENM-organism interaction. To facilitate the understanding of the physico-chemical forms involved in plant response, synchrotron-based techniques have added new potential perspectives in studying the interactions between ENMs and biota. These techniques are providing new insights on the interactions between ENMs and biomolecules. The present review discusses the principal outcomes for ENMs after intake by plants, including possible routes of biotransformation which make their final fate less uncertain, and therefore require further investigation.”

3. The whole introduction (from L41-L96) is talking about the reported environmental exposure of ENM and the effects of quantum dots on plant growth. Please revise this part and be more focused on your main topic. This review aims to summarize the synchrotron-based analysis of the ENM biotransformation in plants and some molecular effects.

We thank Rev2 for the comment. Introduction has been modified in order to be more topic oriented.

Lines 47-99: “Although global food production has generally increased over time, the distribution has been far from equitable, with more than 820 million people having insufficient food and many more consuming low-quality diets leading directly to micronutrient deficiencies (Willett et al 2019; Zhong et al., 2020). In the past 20 years, and particularly in the past decade, engineered nanomaterials (ENMs) have seen dramatically increasing use and had an equally significant impact on ecosystems services and human society. As such, studies focused on the implications associated with their use are critical. In fact, it is known that ENMs exert important, but not completely understood, effects on biota; a particular topic of concern include the effect on crops, food production, and trophic transfer (Gardea-Torresdey et al., 2014; Ma et al., 2018; White et al., 2022).

The interplay between plant growth, dissolution, evaporation, and aggregation are key aspects of the dynamic behavior of ENMs in the environment. Directional aggregation can result in the formation of larger particles with a more complex morphology. However, given the complexity of natural environments, most nanomaterials can be found in hetero-aggregated composites of different inorganic and organic materials (Judy et al., 2012; Ma et al 2018). These aggregates can be very different from original simple pristine morphologies and may even form highly branched structures similar to fractals, all of which subsequently dramatically affect their reactivity and transport (Ma et al., 2018; Huangfu et al., 2019).

Sectors with a large nanomaterial application such as medicine and food production may experience greater risks of ENMs exposure due to their uses, with thousands of tons of ENMs that are eventually discarded into the three main environmental matrices: soil, water, and air (Keller et al., 2013; Zuverza Mena et al., 2017). ENMs with the greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015). Considering the long-time span of use of select materials (1950 to 2050),

efforts to estimate ENMs release through commercial and associated activity into the environment have been undertaken. For example, for nSiO₂, a global production between 100,000 and three million tons per year has been estimated, while for nCeO₂, levels likely reach the upper limit of 10,000 tons per year, and for nAg, the literature reflects a production volume below 1,000 tons per year (Giese et al., 2018). The use of nTiO₂ for the inhibition of microbial proliferation in food is one of the most important ways to prolong the shelf life of packaged products (Abutalib & Rajeh., 2020). However, a panel from EFSA concluded that E171 (TiO₂) can no longer be considered as safe when used as a food additive (EFSA Journal 2021).

Gottschalk et al., (2009) calculated environmental concentrations using a probabilistic LCA (life cycle analysis) of ENM containing products. The authors modelled nTiO₂, nZnO, nAg, carbon nanotubes (CNT), and fullerenes for the U.S., Europe, and Switzerland. The concentrations in the environment were calculated through probabilistic density functions and compared to ecotoxicology data. In the simulations, the values ranged from 0.003 ng L⁻¹ (fullerenes) to 21 ng L⁻¹ (nTiO₂) for surface waters and from 4 ng L⁻¹ for fullerenes to 4 µg L⁻¹ for nTiO₂ for sewage treatment effluents. In Europe and the U.S., ENMs increased annually in sludge treated soil, and ranged from 1 ng kg⁻¹ for fullerenes to 89 µg kg⁻¹ for nTiO₂ (Gottschalk et al. 2009; Keller et al., 2013; Keller & Lazareva, 2014; Rincon, 2019).

Importantly, quantum dots (QDs), as well as many carbon- and metal-based ENMs, have been shown to produce negative effects on animals and plants as a function of dose, including accumulation, alteration of physiological and biochemical parameters, and reduced growth or yield (Oh et al., 2016; Zuverza-Mena et al., 2017). Quantum dots have shown to enter plant roots and to damage the cell wall, dysregulating metabolism (Marmioli et al., 2020). While mercaptoacetic acid (MAA)-coated CdSe/ZnS QDs induced minimal toxicity on maize seedlings, pristine cadmium/tellurium (Cd/Te) QDs induced chromatin stress, mitochondrial damage and inhibition on green gram sprouts (*Phaseolus radiatus* L.) growth (Song et al., 2013). There are several routes by which ENM can enter the soil-plant system. These include agricultural application of sewage sludges which often contain nSiO₂, nTiO₂, nZnO, and nAg; as well as the application of nano-enabled agrochemicals, resulting in the direct entry of nSiO₂, nTiO₂, nZnO, nFeOx, nCuO, CeO₂ and nAg into agricultural soils (Lv et al., 2019; Verma et al., 2022).”

Additional references included:

Gardea-Torresdey J.L., Rico C.M., White J.C. Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* 2014, 48, 2526–2540.

Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* 15, 1692–1709.

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. *Environ. Sci. Technol. Lett.* 2014, 1, 1, 65–70.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, *Environment International*, 2015, 77, 132-147.

Rincon A.M. 2019. Chapter 6 - Presence of nanomaterials on consumer products: food, cosmetics, and drugs. pp. 165-181. Marmioli N., White J.C., Song J., Eds: In *Micro and Nano Technologies, Exposure to Engineered Nanomaterials in the Environment*, Elsevier, Amsterdam, The Netherlands.

Song U., Jun H., Waldman B., Roh J., Kim Y., Yi J., Lee E.J. Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*) *Ecotoxicol. Environ. Saf.*, 2013. 93, 60-67.

White J.C., Zuverza-Mena N., Elmer W.H. From nanotoxicology to nano-enabled agriculture: Following the science at the Connecticut Agricultural Experiment Station (CAES). *Plant Nano Biology*, 2022, 1, 100007. Doi: 10.1016/j.plana.2022.100007.

4. L66 Please add more appropriate citations. Can you provide any related numbers?

New references have been added in order to give more quantitative information:

Lines 64-70: “Sectors with a large nanomaterial application such as medicine and food production may experience greater risks of ENMs exposure due to their uses, with thousands of tons of ENMs that are eventually discarded into the three main environmental matrices: soil, water, and air (Keller et al., 2013; Zuverza Mena et al., 2017). ENMs with the greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015).”

New references included:

Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* 15, 1692–1709.

Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional to Local. *Environ. Sci. Technol. Lett.* 2014, 1, 1, 65–70.

Mitrano D.M., Motellier S., Clavaguera S., Nowack B. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products, *Environment International*, 2015, 77, 132-147.

5. L67 and L78 Please define nSiO₂, nAg, nZnO, and so forth.

We thank Rev2 for the comment. We included the definition of each ENM cited:

Lines 67-70: “ENMs with the greatest historical use include nanoscale ceria (nCeO₂), silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva, 2014; Mitrano et al., 2015).”

6. L71 Please add a citation.

The sentence has been fixed in order to give the proper citation.

Lines 72-75: “For example, for nSiO₂, a global production between 100,000 and three million tons per year has been estimated, while for nCeO₂, levels likely reach the upper limit of 10,000 tons per year, and for nAg, the literature reflects a production volume below 1,000 tons per year (Giese et al., 2018).”

7. L73 TiO₂ or nanoscale TiO₂?

The typo has been fixed.

8. *You only have one subsection "1.1" in part 1. Please consider merging it into part 1. Or change it to 1.2, and put all the content before it into 1.1.*

Paragraph subdivision has been updated, including an initial subsection called “1. Engineered nanomaterial (ENM) biotransformation”. The first section is now “1.1. ENMs: from exposure to biotransformation”, while the previous section 1.1 is now shifted to 1.2.

9. L101-105 Please check

[Ref]

[Ref] for factors and mechanisms of the NPs biotransformation, especially the effect of soil weathering on plant responses.

We thank Rev2 for the suggestions. The text has been updated, as reported:

Lines 102-121: “Environmental and soil physico-chemical characteristics may significantly impact on ENMs aggregation, and dissolution, which may modify ENM bioavailability, uptake, translocation, and accumulation into terrestrial plants. In fact, light and temperature may induce potential changes on the ENM structure, such as during foliar spray (Hong et al., 2021). Once within plant tissues, ENM biotransformation may alter particle stability and behaviour in terms of interactions with biomolecules, triggering differential plant defense mechanisms (Ma et al., 2018; Rawat et al., 2018). ENMs are subject to a range of processes that may lead to their partial dissolution or result in structural modifications (Milosevic et al., 2020; Marmiroli et al., 2020). A schematic representation is reported in Figure 1. Nanoparticle biotransformation is a highly complex and poorly understood series of events and has been shown to occur during weathering in the soil, trophic transfer, and translocation within plant tissues. These reactions are highly dynamic and alter the original pristine structure of the nanoparticles in a number of ways, potentially causing the release of ions, but also the consequent restructuring (or destructuring) of the nanoparticle (Servin et al., 2017a). Biotransformation of nanomaterials may rest on the interaction with biological molecules that stabilize their external reactivity, such as peptides including those involved in detoxification, (*e.g.* glutathione), fatty acids, secondary metabolites, and even components of cell membranes (Marmiroli et al., 2020). Particle properties such as size, stability, charge, and dissolution may strongly influence other biotransformation mechanisms, potentially promoting enzymatic modification and functionalization with proteins (*e.g.*, corona protein) present in the cytoplasm and organelles (Ma et al., 2018; Marmiroli et al., 2020).”

A new reference has been included, as well as an additional Figure (new Figure 1):

Hong J., Wang C., Wagner D.C., Gardea-Torresdey J.L., He F., Rico C.M. 2021. Foliar application of nanoparticles: mechanisms of absorption, transfer, and multiple impacts. *Environ. Sci.: Nano*, 8, 1196-1210.

Rawat S., Pullagurala V.L.R., Adisa I.O., Wang Y., Peralta-Videa J.R., Gardea-Torresdey J.L. Factors affecting fate and transport of engineered nanomaterials in terrestrial environments, *COESH*, 2018, 6, 47-53.

The suggested reference related to “Soil-aged nano titanium dioxide effects on full-grown carrot” has been included in the main text in the case studies reported in the paragraph 2.2 as Wang et al., 2021b.

10. L110-113 Besides the listed possibilities, ENMs can also remain as nanoscale particles with/without the loss of surface coatings in plant tissues after being uptaken. Please check [ref]

We thank Rev 2 for the suggestion. The reference has been cited, as reported.

Lines 121-124: “ENMs may maintain crystal structure when internalized by cells or may be disassembled and converted into less complex structures (by biological modification or chelation), thus reducing toxicity, and the risk of their accumulation and translocation (Wang et al., 2022).”

*11. L178-181 Add more latest references. Please check [ref]
[ref]*

We thank Rev2 for the suggestions. The text has been updated, as reported:

Lines 196-199: “Studies have added significant molecular data on the effects of ENMs exposure in plants (Schwab et al., 2016; Ma et al., 2018). Different results are often observed for the same element as a function of its form or size, i.e. nanostructured, bulk, or ionic species (Pagano et al., 2016; Wang et al., 2022).”

The suggested reference related to “Copper oxide (CuO) nanoparticles affect yield, nutritional quality, and auxin associated gene expression in weedy and cultivated rice” has been included in the text in the case studies reported in the paragraph 2.6 as Deng et al., 2022b.

12. L202 Can you briefly describe Table 1? What is Table 1 for and why are genes listed? Were they significantly affected by the ENM exposure? All the citations listed in Table 1 are related to the author themselves. Please include some works from the others.

Genes reported in Figure 2 (former Figure 1) and Table 1 are some of those that are modulated by the different type of ENMs as reported in the cited studies. Other genes also resulted responsiveness in a ENM-specific manner during exposure and are considered for this reason as potential biomarker of exposure/effect. Moreover, these specific biomarkers are also able to testify how the modulation of the genes in the different organs is in some cases convergent between differential forms (nano, bulk, ion) of the same element. Considering that the concept of nano-specific biomarker has been introduced in some of our research groups papers, we thought to include these references in the Table

1. This was not the case of Table 2, in which papers not related to our research group on the principal evidence of the ENM biotransformation in plant observed by physiological, molecular and synchrotron-based analyses have been included.

Table 1 description has been improved as requested:

“Table 1. Genes as potential biomarkers of exposure/effect observed in roots, leaves and pollen, in different plant species (reported in Figure 2). Genes reported cannot be considered only as modulated in the different plant organs by the different type of ENMs, but also they showed a nano-specificity during the ENM response. It is also important to observe how, depending on the ENM type, biotransformation, and as indirect consequence, the transcriptomic response, can be convergent between different forms (nano, bulk, ion) of the same element (see Figure 2).”

13. L396 According to your reference, "nanofertilizer" should be "nanofungicide or nanofertilizer" Please consider to add more recently works [ref]

The manuscript has been modified and the citation has been included:

Lines 423-424: “Copper oxide nanomaterials (nCuO) are among the most utilized ENMs with plants, including use as a nanopesticide or nanofertilizer (Elmer et al., 2018; Lowry et al., 2019; Xu et al., 2022).”

New reference included:

Xu T., Wang Y., Aytac Z., Zuverza-Mena N., Zhao Z., Hu X., Ng K.W., White J.C., Demokritou P. Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli Responsive Core–Shell Nanostructures. ACS Nano 2022, 16, 4, 6034–6048.

14. L499-500 What kinds of "physical evidence" were obtained through synchrotron-based techniques? What is missing in the current studies?

The sentence has been improved in order to clarify those physical evidence that can be retrieved through synchrotron-based techniques:

Lines 536-541: “Integration of the information from physiological and molecular analyses with physical evidence (e.g., types and number of atoms surrounding the ENM, radial distance between atoms of the interactors and atoms constituting the ENM; ENM crystal structure) obtained through high energy X-ray spectroscopy platforms such as synchrotron-based techniques will enable a more realistic, mechanistic, and systems-level picture of plant response to ENM exposure.”

15. Figure: Only one figure was provided in the main text regarding the list of genes being affected by the ENMs. But the main focus of this review is not ENMs affected gene expressions. What happens with ENMs biotransformation and synchrotron analysis?

We thank Rev2 for the comment. We decided to include a new Figure 1, described in the new paragraph 1.2, which schematized the major modifications that may occur during ENMs-soil-plant

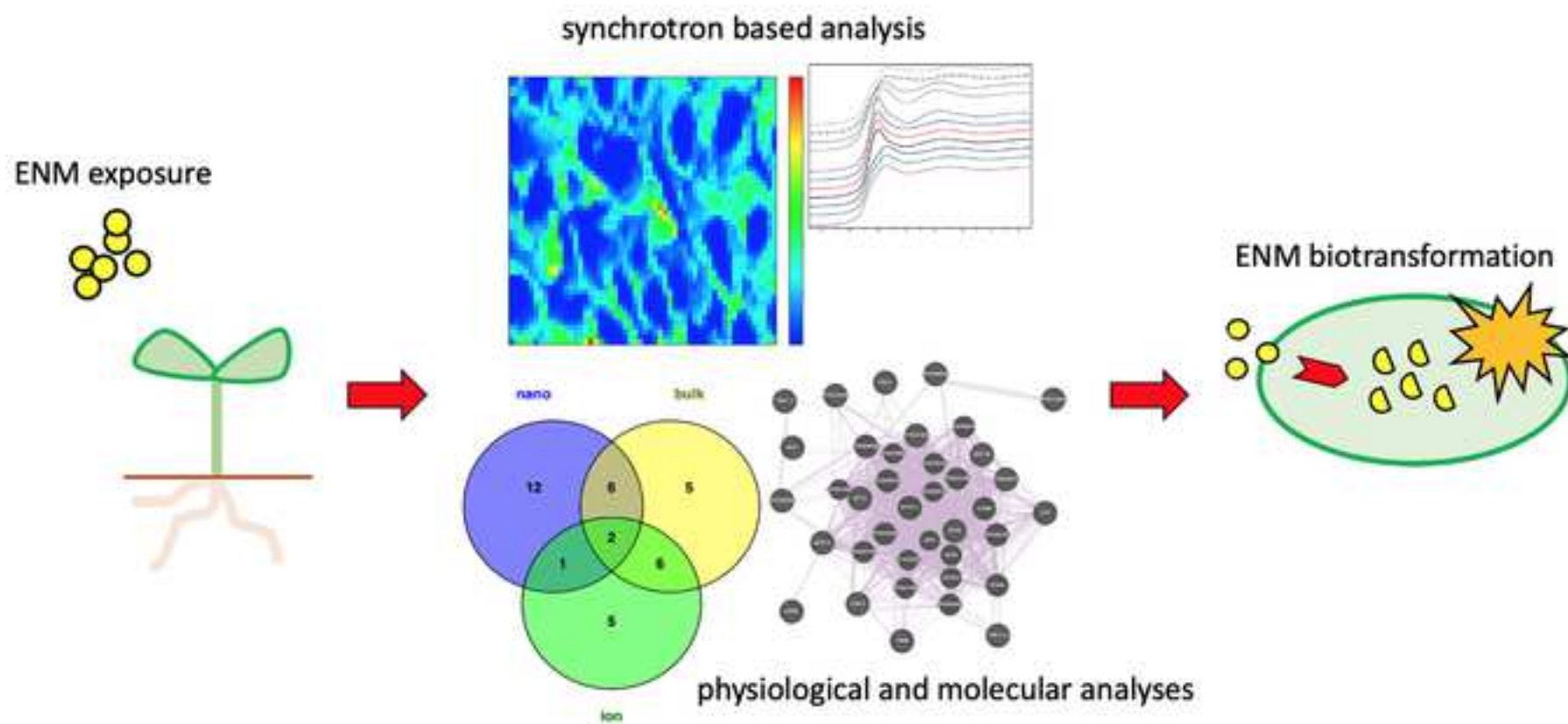
interaction. In order to maximize the comprehension of the new Figure 1, a new caption was constructed to explain in details of these modifications and their practical effects during each phase (soil, foliar surface, plant).

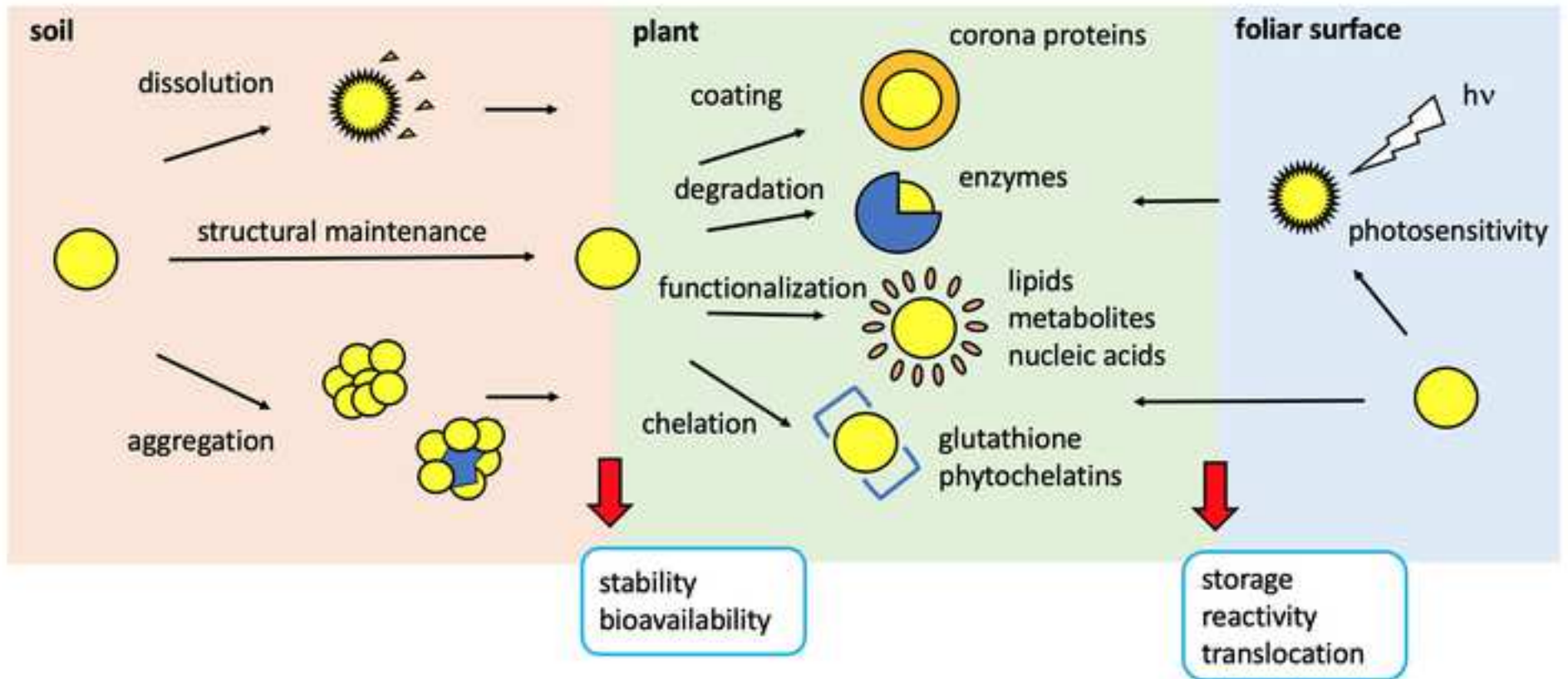
New Figure 1 caption:

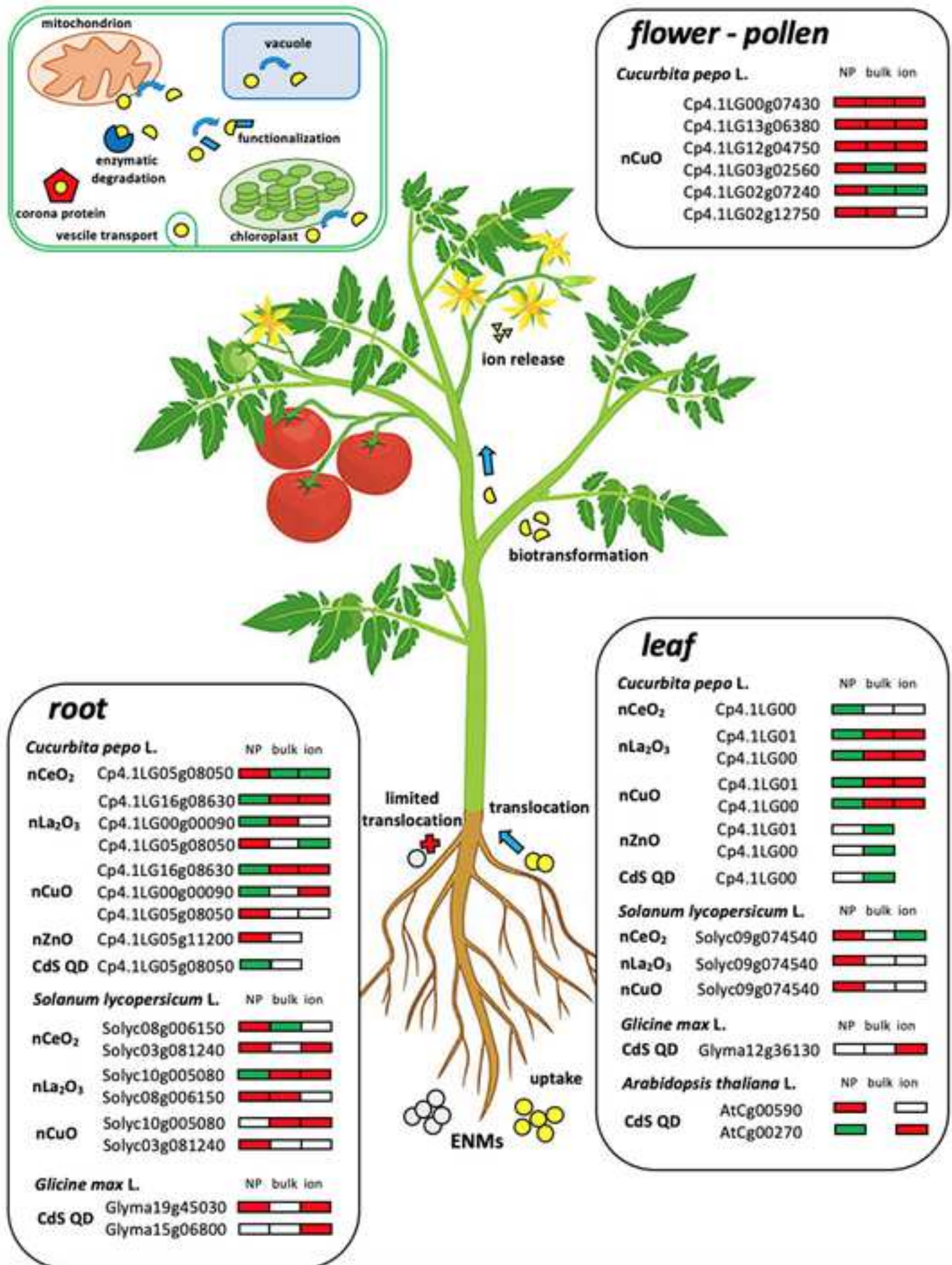
“Figure 1. Schematic representation of principal effects emerging after biotransformation of ENMs in soil, on foliar surface and within the plant tissues. ENMs in soil, due to particle and soil physico-chemical interactions, may remain unmodified, or may undergo to potential dissolution (with ions release), or undergo homo-/hetero-aggregation, which may highly influence particle stability and potential bioavailability (and consequently their uptake). On the foliar surface, temperature and light may also affect particle stability before uptake into the leaf tissues. Once within the plant, ENMs pristine, or modified, can interact with several biomolecule types (peptides, sugars, lipids, nucleic acids, secondary metabolites) leading to phenomena such as coating, enzymatic degradation, chelation or functionalization, which may influence the biotransformed particle at level of translocation, storage or reactivity. These parameters may also influence the interaction within the plant cell, triggering differential responses (*e.g.*, toxicity, oxidative stress, ROS production), which may be indirectly measured by physiological and molecular assays, but directly observed through physical strategies, including synchrotron-based methods.”

Highlights

- Biotransformation is a fundamental phenomenon for understanding ENM-organism response mechanisms
- Synchrotron-based methodological analyses are critical for investigating ENM biotransformation
- Biotransformation of ENMs may have positive or negative effects when considering the agri-food application







Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Nelson Marmiroli reports financial support was provided by European Union.

Conceptualization: LP, MM; original draft preparation: LP, MM, RR; review and editing: JCW, NM. All authors revised and agreed on the final version of the manuscript.

1 **Nanomaterials biotransformation: In planta mechanisms of action**

2

3 Luca Pagano,^{1,*} Riccardo Rossi,^{1,2} Jason C. White,³ Nelson Marmioli,^{1,4} Marta Marmioli^{1,5*}

4

5 ¹, Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma,
6 43124 Parma, Italy.

7 ², Centro Interdipartimentale per l'Energia e l'Ambiente (CIDEA), University of Parma, 43124 Parma,
8 Italy.

9 ³, The Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504, United States.

10 ⁴, Consorzio Interuniversitario Nazionale per le Scienze Ambientali (CINSA), University of Parma,
11 43124 Parma, Italy.

12 ⁵, Interdepartmental Centre for Food Safety, Technologies and Innovation for Agri-food
13 (SITEIA.PARMA), 43124 Parma, Italy.

14 *, corresponding authors: ~~luca.pagano@unipr.it~~; marta.marmioli@unipr.it.

15

16 **Abstract**

17 Research on engineered nanomaterials (ENMs) exposure has continued to expand rapidly, with a
18 focus on uncovering the underlying mechanisms. The EU largely limits the number and the type of
19 organisms that can be used for experimental testing through the 3R normative. There are different
20 routes through which ENMs can enter the soil-plant system: this includes the agricultural application
21 of sewage sludges, and the distribution of nano-enabled agrochemicals. However, a thorough
22 understanding of the physiological and molecular implications of ENMs dispersion and chronic low-
23 dose exposure remains elusive, thus requiring new evidence and a more mechanistic overview of
24 pathways and major effectors involved in plants. Plants can offer a reliable alternative to conventional
25 model systems to elucidate the concept of ENM biotransformation within tissues and organs, as a
26 crucial step in understanding the mechanisms of ENM-organism interaction. To facilitate the

27 understanding of the physico-chemical forms involved in plant response, synchrotron-based
28 techniques have added new potential perspectives in studying the interactions between ENMs and
29 biota. These techniques are providing new insights on the interactions between ENMs and
30 biomolecules. The present review discusses the principal outcomes for ENMs after intake by plants,
31 including possible routes of biotransformation which make their final fate less uncertain, and
32 therefore require further investigation.Research on engineered nanomaterials (ENMs) exposure has
33 continued to expand rapidly, and with studies focused on uncovering underlying mechanisms are
34 concerned, EU largely limits the number and the type of organisms that can be used so as to support
35 the 3R normative. There are different routes by which ENMs can enter the soil-plant system: this
36 includes the agricultural application of sewage sludges, and the application of nano enabled
37 agrochemicals, resulting in the direct entry of ENMs into agricultural soil. However, a thorough
38 understanding of the physiological and molecular implications of chronic low dose exposure to
39 engineered nanomaterials remains elusive. Plants can offer a reliable alternative and, in this context,
40 the concept of nanomaterial biotransformation within plant tissues and organs is one of increasing
41 interest. To facilitate understanding of the physico-chemical forms involved in plant response,
42 synchrotron based techniques have seen increasing use and have added new perspectives on the
43 interactions between ENMs and biota. The review will discuss the principal outcomes related to the
44 ENMs biotransformation in plants and the practical relevance of those findings, as well as the
45 potential applicability of those findings to other biotic species.

46
47 **Keywords:** biotransformation, plant, nanomaterials, synchrotron-based analyses, molecular response

48
49 **Highlights**

- 50 • Biotransformation is a fundamental phenomenon for understanding ENM-organism response
51 mechanisms

52 • Synchrotron-based methodological analyses are critical for investigating ENM
53 biotransformation

54 • Biotransformation of ENMs may have positive or negative effects when considering the agri-
55 food application

56 • Biotransformation is fundamental to understanding ENM-organism response

57 • Synchrotron-based analyses are critical to investigate ENM biotransformation

58 • Understanding ENM biotransformation is critical to safer by design applications

59

60

61 **1. Engineered nanomaterial (ENM) biotransformation**

62 ~~From ENM exposure to biotransformation~~ ENMs: from ~~From ENM exposure to~~
63 biotransformation

64 **1.1.1.**

65 ~~More than three billion metric tons of crops are produced globally each year in the world, requiring~~
66 ~~187 million metric tons of fertilizer, nearly 4 million tons of pesticides, 2.7 trillion cubic meters of~~
67 ~~water (about 70% of all freshwater consumptive use globally), and over two quadrillion British~~
68 ~~thermal units (BTU) of embodied energy (Lowry et al., 2019; Zhong et al., 2020). Although the~~
69 ~~benefits of the Green Revolution have enabled consumption of an average 2,884 kcal per capita per~~
70 ~~day in the WHO Countries, conventional agricultural practices are unsustainable, and have directly~~
71 ~~led to significant environmental damage (Willett et al., 2019). Although global food production of~~
72 ~~calories has generally kept pace with population growth, the distribution has been far from equitable,~~
73 ~~with more than 820 million people having insufficient food and many more consuming low quality~~
74 ~~diets leading directly micronutrient deficiencies (Willett et al 2019). In the past 20 years, and~~
75 ~~particularly in the past decade, engineered nanomaterials (ENMs) have seen dramatically increasing~~
76 ~~use and have had equally significant impact on ecosystems and human society; as such, studies~~
77 ~~focused on the implications associated with that use are critical. In fact, it is known that nanomaterials~~

Formatted: List Paragraph, Indent: Left: 0", Hanging: 0.3", Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 0.25" + Indent at: 0.5"

Formatted: Font: (Default) Times New Roman, Bold

Formatted: Font: (Default) Times New Roman, Bold, Italic

Formatted: Normal, Numbered + Level: 2 + Numbering Style: a, b, c, ... + Start at: 1 + Alignment: Left + Aligned at: 0.75" + Indent at: 1"

Formatted: Font: Italic

Formatted: Font: (Default) Times New Roman, Italic

Formatted: Font: (Default) Times New Roman, Bold, Italic

Formatted: Font: (Default) Times New Roman, Italic

Formatted: Outline numbered + Level: 2 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 0" + Indent at: 0.29"

78 exert important, but often poorly understood, impacts on biota; particular topics of concern include
79 human health, agriculture and food production (Ma et al., 2018).

80 The interplay between growth, dissolution, evaporation, and aggregation are key aspects of the
81 dynamic behavior of nanomaterials in the environment. Directional aggregation can result in the
82 formation of larger particles with complex morphologies. However, given the complexity of natural
83 environments, most nanomaterials are found in heteroaggregated composites of different inorganic
84 and organic materials (Judy et al., 2012; Ma et al 2018). These aggregates can be very different from
85 original simple pristine morphologies and may even form highly branched structures similar to
86 fractals, all of which subsequently dramatically affects reactivity and transport properties (Ma et al.,
87 2018; Huangfu et al., 2019).

88 Sectors with widespread nanomaterial application such as medicine and food production carry a
89 greater risk of ENMs exposure, and associated with these uses is the thousands of tons of ENMs that
90 are discarded into the three main environmental matrices of soil, water, and air (Zuverza Mena et al.,
91 2017). The ENMs with the greatest historical use include nanoscale CeO_2 (nCeO₂), nSiO₂, and nAg,
92 and as such, release in the environment has been investigated. Given the long time span of use of
93 select materials (1950 to 2050), efforts to estimate ENMs release through commercial and associated
94 activity into the environment have been undertaken. For example, for nSiO₂, global production
95 between 100,000 and about three million tons per year has been estimated. For nCeO₂, levels likely
96 reach the upper limit of 10,000 tons per year, and for nAg, the literature reflects a production volume
97 below 1,000 tons per year (Giese et al., 2018). Use of TiO₂ for inhibition of microbial proliferation
98 in food is one of the most important ways to prolong the shelf life of packaged products (Abutalib &
99 Rajeh., 2020). In addition, a panel from EFSA concluded that E171 can no longer be considered as
100 safe when used as a food additive (EFSA Journal 2021).

101 Gottschalk et al., (2009) calculated environmental concentrations using a probabilistic life-cycle
102 analysis of ENM containing products. The authors modelled nTiO₂, nZnO, nAg, carbon nanotubes
103 (CNT), and fullerenes for the U.S., Europe, and Switzerland. The concentrations in the environment

104 were calculated through probabilistic density functions and compared to ecotoxicology data. In the
105 simulations, the values ranged from 0.003 ng L⁻¹ (fullerenes) to 21 ng L⁻¹ (nTiO₂) for surface waters
106 and from 4 ng L⁻¹ for fullerenes, to 4 µg L⁻¹ for nTiO₂ for sewage treatment effluents. In Europe and
107 the U.S., ENMs increased annually in sludge-treated soil, and ranged from 1 ng kg⁻¹ for fullerenes to
108 89 µg kg⁻¹ for nTiO₂ (Gottschalk et al. 2009).

109 Importantly, quantum dots (QDs), as well as many carbon- and metal-based ENMs have been shown
110 to produce negative effects on animals and plants as a function of dose, including accumulation,
111 alteration of physiological and biochemical traits, and reduced growth or yield (Oh et al., 2016;
112 Zuverza-Mena et al., 2017). Quantum dots have been shown to enter plant roots and to damage the
113 cell wall and dysregulate metabolism (Marmioli et al. 2020). While mercaptoacetic acid (MAA)-
114 coated CdSe/ZnS QDs induced minimal toxicity on maize seedlings, pristine cadmium/tellurium
115 (Cd/Te) QDs induced chromatin stress, mitochondrial damage and inhibition in green gram sprouts
116 (*Phaseolus radiatus* L.) growth (Zuverza-Mena et al., 2017). There are several routes by which ENM

117 can enter the soil-plant system. This includes the agricultural application of sewage sludges which
118 often contain nSiO₂, nTiO₂, nZnO, and nAg; as well as the application of nano-enabled
119 agrochemicals. Although global food production has generally increased over time, the distribution
120 has been far from equitable, with more than 820 million people having insufficient food and many
121 more consuming low-quality diets leading directly to micronutrient deficiencies (Willett et al. 2019;
122 Zhong et al., 2020). In the past 20 years, and particularly in the past decade, engineered nanomaterials
123 (ENMs) have seen dramatically increasing use and had an equally significant impact on ecosystems
124 services and human society. As such, studies focused on the implications associated with their use
125 are critical. In fact, it is known that ENMs exert important, but not completely understood, effects on
126 biota; a particular topic of concern include the effect on crops, food production, and trophic transfer
127 (Gardea-Torresdey et al., 2014; Ma et al., 2018; White et al., 2022).

128 The interplay between plant growth, dissolution, evaporation, and aggregation are key aspects of the
129 dynamic behavior of ENMs in the environment. Directional aggregation can result in the formation

Formatted: Highlight

130 of larger particles with a more complex morphology. However, given the complexity of natural
131 environments, most nanomaterials can be found in hetero-aggregated composites of different
132 inorganic and organic materials (Judy et al., 2012; Ma et al 2018). These aggregates can be very
133 different from original simple pristine morphologies and may even form highly branched structures
134 similar to fractals, all of which subsequently dramatically affect their reactivity and transport (Ma et
135 al., 2018; Huangfu et al., 2019).

136 Sectors with a large nanomaterial application such as medicine and food production may experience
137 greater risks of ENMs exposure due to their uses, with thousands of tons of ENMs that are eventually
138 discarded into the three main environmental matrices: soil, water, and air (Keller et al., 2013;
139 Zuverza-Mena et al., 2017). ENMs with the greatest historical use include nanoscale ceria (nCeO₂),
140 silica (nSiO₂), titania (nTiO₂), as well as nanoscale copper oxide (nCuO), zinc oxide (nZnO) and
141 nanosilver (nAg), and as such, release in the environment has been investigated (Keller & Lazareva,
142 2014; Mitrano et al., 2015). Considering the long-time span of use of select materials (1950 to 2050),
143 efforts to estimate ENMs release through commercial and associated activity into the environment
144 have been undertaken. For example, for nSiO₂, a global production between 100,000 and three million
145 tons per year has been estimated, while for nCeO₂, levels likely reach the upper limit of 10,000 tons
146 per year, and for nAg, the literature reflects a production volume below 1,000 tons per year (Giese et
147 al., 2018). The use of nTiO₂ for the inhibition of microbial proliferation in food is one of the most
148 important ways to prolong the shelf life of packaged products (Abutalib & Rajeh., 2020). However,
149 a panel from EFSA concluded that E171 (TiO₂) can no longer be considered as safe when used as a
150 food additive (EFSA Journal 2021).

151 Gottschalk et al., (2009) calculated environmental concentrations using a probabilistic LCA (life
152 cycle analysis) of ENM containing products. The authors modelled nTiO₂, nZnO, nAg, carbon
153 nanotubes (CNT), and fullerenes for the U.S., Europe, and Switzerland. The concentrations in the
154 environment were calculated through probabilistic density functions and compared to ecotoxicology
155 data. In the simulations, the values ranged from 0.003 ng L⁻¹ (fullerenes) to 21 ng L⁻¹ (nTiO₂) for

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Superscript

Formatted: Superscript

Formatted: Subscript

156 surface waters and from 4 ng L⁻¹ for fullerenes to 4 µg L⁻¹ for nTiO₂ for sewage treatment effluents.
157 In Europe and the U.S., ENMs increased annually in sludge treated soil, and ranged from 1 ng kg⁻¹
158 for fullerenes to 89 µg kg⁻¹ for nTiO₂ (Gottschalk et al. 2009; Keller et al., 2013; Keller & Lazareva,
159 2014; Rincon, 2019).
160 Importantly, quantum dots (QDs), as well as many carbon- and metal-based ENMs, have been shown
161 to produce negative effects on animals and plants as a function of dose, including accumulation,
162 alteration of physiological and biochemical parameters, and reduced growth or yield (Oh et al., 2016;
163 Zuverza-Mena et al., 2017). Quantum dots have shown to enter plant roots and to damage the cell
164 wall, dysregulating metabolism (Marmioli et al., 2020). While mercaptoacetic acid (MAA)-coated
165 CdSe/ZnS QDs induced minimal toxicity on maize seedlings, pristine cadmium/tellurium (Cd/Te)
166 QDs induced chromatin stress, mitochondrial damage and inhibition on green gram sprouts
167 (*Phaseolus radiatus* L.) growth (Song et al., 2013). There are several routes by which ENM can enter
168 the soil-plant system. These include agricultural application of sewage sludges which often contain
169 nSiO₂, nTiO₂, nZnO, and nAg; as well as the application of nano-enabled agrochemicals, resulting in
170 the direct entry of nSiO₂, nTiO₂, nZnO, nFeOx, nCuO, CeO₂ and nAg into agricultural soils (Lv et
171 al., 2019; Verma et al., 2022).

Formatted: Superscript

Formatted: Superscript

Formatted: Subscript

Formatted: Superscript

Formatted: Superscript

Formatted: Subscript

Formatted: Font: Italic

Formatted: Subscript

Formatted: Subscript

173 4.1.2. *Conceiving and studying the ENM biotransformation*

174 Environmental and soil physico-chemical characteristics may significantly impact on ENMs
175 aggregation, and dissolution, which may modify ENM bioavailability, uptake, translocation, and
176 accumulation into terrestrial plants. In fact, light and temperature may induce potential changes on
177 the ENM structure, such as during foliar spray (Hong et al., 2021). Once within plant tissues, ENM
178 biotransformation may alter particle stability and behaviour in terms of interactions with
179 biomolecules, triggering differential plant defense mechanisms (Ma et al., 2018; Rawat et al., 2018).
180 ENMs are subject to a range of processes that may lead to their partial dissolution or result in
181 structural modifications (Milosevic et al., 2020; Marmioli et al., 2020). A schematic representation

Formatted: Indent: Left: 0", Hanging: 0.3", Outline numbered + Level: 2 + Numbering Style: 1, 2, 3, ... + Start at: 2 + Alignment: Left + Aligned at: 0.3" + Indent at: 0.55"

182 ~~is reported in Figure 1. Once accumulated by plants, ENMs are subject to a range of processes that~~
183 ~~may induce partial dissolution or result in structural modifications (Milosevic et al., 2020; Marmioli~~
184 ~~et al., 2020). Nanoparticle biotransformation is a highly complex and poorly understood series of~~
185 ~~events and has been shown to occur during weathering in the soil, trophic transfer, and translocation~~
186 ~~within plant tissues. These reactions are highly dynamic and alter the original pristine structure of the~~
187 ~~nanoparticles in a number of ways, potentially causing the release of ions, but also the consequent~~
188 ~~restructuring (or destructuring) of the nanoparticle (Servin et al., 2017a). Biotransformation of~~
189 ~~nanomaterials may rest on the interaction with biological molecules that stabilize their external~~
190 ~~reactivity, such as peptides including those involved in detoxification, (e.g., glutathione), fatty acids,~~
191 ~~secondary metabolites, and even components of cell membranes (Marmioli et al., 2020). Particle~~
192 ~~properties such as size, stability, charge, and dissolution may strongly influence other~~
193 ~~biotransformation mechanisms, potentially promoting enzymatic modification and functionalization~~
194 ~~with proteins (e.g., corona protein) present in the cytoplasm and organelles (Ma et al., 2018;~~
195 ~~Marmioli et al., 2020). ENMs may maintain crystal structure when internalized by cells or may be~~
196 ~~disassembled and converted into less complex structures (by biological modification or chelation),~~
197 ~~thus reducing toxicity, and the risk of their accumulation and translocation (Wang et al., 2022). These~~
198 ~~post-uptake structural modifications involve specific parameters such as bond distance with other~~
199 ~~atoms or nature of the ligand atoms. In consideration of this, one objective in biotransformation~~
200 ~~studies is to investigate the physico-chemical forms (e.g., nanocrystal structure) within exposed~~
201 ~~tissues and to characterize the structural differences within the new biotransformed molecules,~~
202 ~~including identification of the biomolecules interacting with the ENMs (Castillo-Michel et al., 2017;~~
203 ~~Marmioli et al., 2020). Nanoparticle biotransformation is highly complex and poorly understood~~
204 ~~series of reactions and has been shown to occur during soil weathering, trophic transfer, translocation~~
205 ~~within plant tissues; these reactions are highly dynamic and alter the original pristine structure of the~~
206 ~~nanoparticles in a myriad of ways, potentially causing the release of ions but also the consequent~~
207 ~~restructuring (or destructuring) of the nanoparticle physico-chemical form (Servin et al., 2017a).~~

Formatted: Font: Italic

Formatted: Font: Italic

208 ~~Biotransformation of nanomaterials may lead to the attachment of biological molecules that stabilize~~
209 ~~their external reactivity, such as proteins, fatty acids, secondary metabolites, and even cell membranes~~
210 ~~(Marmiroli et al., 2020). Particle properties such as size, stability, charge, and dissolution influence~~
211 ~~the biotransformation mechanisms, potentially promoting enzymatic modification and~~
212 ~~functionalization with proteins (e.g., corona protein) present in the cytoplasm and organelles~~
213 ~~(Marmiroli et al., 2020). Importantly, ENMs may maintain crystal structure when internalized by~~
214 ~~cells or may be disassembled and transformed into less complex structures (by biological~~
215 ~~modification or chelation), thus minimizing toxicity and influencing the risk of their accumulation~~
216 ~~and translocation. These post uptake structural modifications involve specific parameters such as~~
217 ~~bond distance with other atoms or nature of the ligand atoms. In consideration of this, one objective~~
218 ~~in biotransformation studies is to investigate the physico-chemical forms (e.g., nanocrystal structure)~~
219 ~~within exposed tissues and to characterize the structural differences evident in the new~~
220 ~~biotransformed molecules, including identification of the key biomolecules interacting with the ENM~~
221 ~~(Castillo-Michel et al., 2017; Marmiroli et al., 2020). It has furthermore to consider how genetic~~
222 ~~diversity across different plant species and within the same plant species (in different cultivars) may~~
223 ~~influence the ENM uptake and translocation (Deng et al., 2020).~~

224 Some of the more interesting discoveries on the biotransformation and localization of metal based-
225 ENMs into plants have been achieved with to the synchrotron-based techniques of imaging, elemental
226 speciation, and atomic neighbors' identification. This transformation will be also influenced by the
227 environment, these reactions of the same particle will be different on the leaf surface, in the
228 mesophyll, in the vascular tissue, in different organelles, in the roots and in the rhizosphere (Sarret et
229 al. 2013; Castillo-Michel et al., 2017).

230 Micro-X-Ray Fluorescence (μ -XRF) and micro-X-Ray Adsorption Spectroscopy (μ -XAS) K-, L- or
231 L_{III} -edge EXAFS and XANES spectra have been used to study the biotransformation of coated
232 nanomaterials present in plants and soil (Lopez-Moreno et al., 2010; Judy et al., 2012). μ -XRF is
233 used for qualitative elemental analysis of heterogeneous biological samples. The interaction of the

234 sample with high-energy X-ray radiation, which leads to X-ray absorption and emission of the
235 fingerprint X-ray spectra for each element, is the key feature of this powerful analytical method. The
236 absorption/excitation effect and relaxation process lead to atoms emitting fluorescence photons
237 characterized by elemental specific energy (Chebakova et al., 2021). Extended X-ray absorption fine
238 structure (EXAFS) is a technique that utilizes oscillations of the X-ray absorption coefficient on the
239 high-energy side of an absorption edge. Such oscillations can reach up to 1000 eV above the edge
240 and may have a magnitude of 10% or more. In addition, it is necessary to consider that atoms are not
241 stationary. Thermal vibrations will obscure the EXAFS oscillations, and in the harmonic
242 approximation, this can be accounted for by considering a Debye-Waller-type term. This led to a
243 great improvement in the theoretical understanding of EXAFS and it is now established that a single
244 scattering short-range order theory is adequate under most circumstances (Gurman, 1995). In
245 addition, electrons that have undergone inelastic losses will not interfere in the process. This is
246 considered by an exponential damping term. It is the limited range of the photoelectrons in the energy
247 region of interest 50-1000 eV that allows for a short-range order description of EXAFS also in
248 crystalline materials (Gurman, 1995). The region which includes the pre-edge, edge-jump and post-
249 edge to approximately 30-50 eV is referred to as the X-ray Absorption Near Edge Structure
250 (XANES), which describes the structural component of the X-ray absorption near-edge as an
251 extension of the EXAFS, largely due to the long mean free path of the photoelectron and the
252 dominance of high order multiple scattering contributions (Gräfe et al., 2014).

253 Synchrotron-based techniques use photons, which do not have mass; therefore the factor 1.22 is
254 substituted by the non-relativistic mass of the electron which is $m=5.485 \cdot 10^{-4}$ Da. This makes the
255 resolution even smaller and increases penetration depth into the sample. On the other hand, EDX
256 depends on the acceleration voltage of the particle or of the photon. Every element has its own orbital
257 energies, and the acceleration voltage allows excitation one or more of these, independently if it
258 comes from a TEM or from a synchrotron (Goldstein et al., 2003).

259 From the perspective of application, μ -XRF can provide information on the presence and localization
260 of specific elements within tissues, while XANES and EXAFS spectroscopy can provide information
261 related to the valence state and coordination environment of the element of interest, as well as the
262 molecular species present in the sample. The use of μ -XRF and μ -XANES for the analysis of
263 nanoparticles in plants have been thoroughly reviewed by Castillo-Michel et al. (2017).
264 Importantly, these powerful methodologies open the possibility to mechanistically address many
265 important environmental issues, such as the chemical activities of environmental pollutants, to trace
266 environmental elemental cycles, element speciation in complex matrices, and to characterize the
267 natural/anthropogenic complex matrixes that are not amendable for standard analytical and structural
268 analyses (Puri et al., 2019). In many synchrotrons around the world, there is increasing use of
269 dedicated beamlines to study environmental and plant tissues exposed to contaminants such as ENMs
270 (Proux et al., 2017; d'Acapito et al., 2019).

271

272 ~~This~~ present review aims to describe the current understanding of metal based-ENMs
273 biotransformation mechanisms in ~~plants, and plants and~~ focuses on correlating available physiological
274 and molecular data with the information obtained by synchrotron-based techniques. This evaluation
275 not only highlights biotransformation as one of the major driving forces mediating the biological
276 effects of ENMs on plants, but also offers some perspective on intentional and safer-by-design
277 strategies that can ensure more sustainable application of these materials. Moreover, the study on
278 plants is instrumental to the application of the REACH normative within European Union for
279 toxicological and ecotoxicological studies (Replacement, Reduction and Refinement). Plants are
280 higher eukaryotes, characterized by large nuclear genomes and organellar genomic information
281 (within chloroplasts and mitochondria) that provide an effective model for many complex species
282 (Chang et al., 2016).

283

284

285 *2. Physiological and molecular effects as indirect evidence of ENM biotransformation in plants*

286 The physiological behaviour and related molecular pathways of response are important to
287 characterizing and understanding ENM biotransformation. An important part of this involves
288 comparing the effects of a nanomaterial with that of the bulk and ionic counterparts, as well as by
289 investigating different exposure times and by exposing different plant organs and tissues (Schwab et
290 al., 2016; Marmiroli et al., 2021). Studies have added significant molecular data on the effects of
291 ENMs exposure in plants (Schwab et al., 2016; Ma et al., 2018). Different results are often observed
292 for the same element as a function of its form or size, i.e. nanostructured, bulk, or ionic species
293 (Pagano et al., 2016; Wang et al., 2022).~~A number of important studies have added significant~~
294 ~~molecular understanding to the effects of ENMs exposure in plants (Schwab et al., 2016; Ma et al.,~~
295 ~~2018). Different results are often observed for the same element as a function of form or size; i.e.~~
296 ~~nanostructured, bulk, or ionic species (Pagano et al., 2016).~~ Detailed study of differential
297 transcriptional regulation, protein abundance or metabolomic profiling (Huang et al., 2018;
298 Majumdar et al., 2019; Gallo et al., 2021) are critical to demonstrating the nano-specificity of plant
299 response. The study of organellar genome stability and the related stoichiometric variations during
300 ENM treatment has also provided important mechanistic insight into plant to ENM exposure (Pagano
301 et al., 2022). Advanced synchrotron-based techniques may either help to systematically understand
302 the nano-bio interactions, with regard to physical and chemical reactions at the biomolecular surface:
303 biomolecules may interact with ENMs, generating biomolecular corona, which change the ENM
304 surface properties, and interfere with its functionality/reactivity (Hameed et al., 2022).
305 Regarding the physiological effects of ENMs on plants of agronomic interest, these studies have
306 provided a better understanding of the specific properties of the ENMs that may enable sustainable
307 use in the agrifood sector. Beyond the potential adverse effects upon bioaccumulation from soil or
308 other exposure routes, there is an increasing interest in exploiting the potential positive effects of
309 ENMs on plants, aiming to improve crop yields and quality. A range of mechanisms, including direct
310 use as nanofertilizers (Verma et al., 2022), nanocarriers (Karny et al., 2018), smart delivery systems

Formatted: Font: (Default) Times New Roman, Bold, Italic

Formatted: Indent: Left: 0", Hanging: 0.2", Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 0.25" + Indent at: 0.5"

§11 ~~(Xu et al., 2022) or when in association to plant growth-promoting bacteria, are considered (Prado de~~
§12 ~~Moraes et al., 2021). Beyond the potential adverse effects upon accumulation from the soil or other~~
§13 ~~exposure routes, there is increasing interest in exploring the potential positive effects of nanomaterials~~
§14 ~~on plants, aiming to improve crop yields and productivity by a range of mechanisms, including direct~~
§15 ~~use as nanofertilizers (Verma et al., 2022), nanocarriers (Karny et al., 2018), or when in association~~
§16 ~~to plant growth promoting bacteria (Prado de Moraes et al., 2021).~~ In addition, ENMs may act
§17 indirectly by protecting plants from biotic (*e.g.*, nanopesticides) or abiotic stressors (*e.g.*, wastewater
§18 and soil treatment) (Liu et al., 2015; Kah et al., 2018; Kumari et al., 2019). Due to the many variables
§19 involved, it is essential to obtain robust safety data regardless of the end use: ENM type, the modes
§20 and time of exposure, concentrations tested, and the plants used are all important considerations. Any
§21 recurring effects that occur under these different conditions are of particular interest and are explored
§22 below (see Figure 24 and Tables 1). Table 2 summarizes the major outcomes related to metal based-
§23 ENM biotransformation in plants, including the principal mechanisms involved, and the major
§24 physiological and molecular insights observed from exposure.

§26 2.1. *Lanthanides based ENMs*

§27 Cerium Oxide (nCeO₂) has shown significant potential for agricultural applications, largely due to its
§28 properties as an ROS scavenger (Ma et al., 2015; Servin et al., 2017b). While CeO₂ as a bulk crystal
§29 mainly consists of Ce(IV), the reduction to nCeO₂ significantly enhances the relative amount of
§30 Ce(III), resulting in a higher catalytic effects comparable to the capabilities of a biological antioxidant
§31 (Eriksson et al., 2018). Servin et al. (2017b) used μ -XRF and μ -XANES to analyze the interactions
§32 between nCeO₂ and different biochars in soil, observing that much of the Ce remained in nCeO₂ form
§33 within the plant tissues. The dissolution rate of the nanoform can increase in acidic environments to
§34 generate Ce(III), as reported by Hernandez-Viezcas et al. (2013) who analyzed in *Glycine max* L. the
§35 effects of nCeO₂ (1000 mg L⁻¹, 48d exposure). Results have been confirmed by Rui et al. (2015), who
§36 used XANES on exposed cucumber (*Cucumis sativus* L.) tissues (2000 mg L⁻¹, 21d exposure) to

Formatted: Outline numbered + Level: 2 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 0" + Indent at: 0.25"

Formatted: Font: (Default) Times New Roman, Italic

337 observe nCeO₂ association with phosphate. These properties highly impact not only reactivity but
338 also nCeO₂ translocation. In zucchini (*Cucurbita pepo* L.), treated with 500 mg L⁻¹ of nCeO₂, the
339 nanoform is mainly present in the roots and stems, with limited translocation to the leaves (Pagano et
340 al., 2016). However, co-contamination with other ENMs (*e.g.*, CdS QDs) under same experimental
341 conditions resulted in increased translocation to the shoots from 1000 to 3000 mg kg⁻¹ (Pagano et al.,
342 2017). Similar results have been reported in several plant species: for example, Rossi et al (2017)
343 nCeO₂ under co-exposure with ionic Cd in soybean (*Glycine max* L.) showed an altered (1-2 fold
344 increased) translocation to the shoots. Interestingly, bulk CeO₂ translocation resulted similar to the
345 nano-form, whereas ionic Ce was translocated in greater amounts to the shoots (Pagano et al., 2016).
346 This analysis was supplemented with molecular data; the transcriptional profiles were evaluated in
347 *C. pepo* and *S. lycopersicum* as a function of nCeO₂, nLa₂O₃ and nCuO exposure and were compared
348 with bulk and ionic forms using a set of 38 genes based on the *A. thaliana* orthologs as potential
349 biomarkers of exposure/effects (Marmioli et al., 2014). The responses observed were generally
350 different in term of up- or down-regulation as a function of Ce form (Pagano et al., 2016). Of
351 particular interest are impacts on the chloroplast are the *PetL* and *PSBN* genes, which encode for a
352 structural component of the cytochrome b₆f complex and low molecular weight protein located on
353 thylakoid membrane as a component of the photosystem II (PSII), respectively (Figure 24, Table 1).
354 These two chloroplastic genes were differentially expressed across nano-, bulk, and ionic forms. A
355 similar trend was also evident for nLa₂O₃ and nCuO exposure scenarios. Interestingly, by analyzing
356 the effects on chloroplast and mitochondrial genomes in *A. thaliana* in terms of copy number, the
357 effects of nCeO₂ and CeCl₃ exposure were rather limited as compared to the untreated control, which
358 ~~is in agreement~~ agrees with the limited translocation to the shoots (Pagano et al., 2022). With regard
359 to proteomic analysis, Majumdar et al. (2015) conducted a quantitative proteomic analysis of kidney
360 beans (*Phaseous vulgaris* L.) seeds after nCeO₂ exposure and reported that the major seed proteins
361 associated with nutrient storage (phaseolin) and carbohydrate metabolism (lectins) were significantly

362 reduced by nCeO₂ (62.5-500 mg kg⁻¹, 50d exposure) in a dose dependent manner. Interesting, the
363 plants did not exhibit overt toxicity.

364 In fact, at the physiological level cerium-based nanoparticles generally do not produce phytotoxicity
365 (Ma et al., 2015; Rui et al., 2014; Lizzi et al., 2020; Rodrigues et al., 2021), though some have
366 highlighted a positive impact on biomass and on physiological indicators such as chlorophyll and
367 photosynthesis at selected doses (Rossi et al., 2017; Gui et al., 2017). Another important aspect of
368 nCeO₂ seems to involve enhance tolerance to saline stress, leading to improved phenotypic and
369 enzymatic performances and ROS elimination in seeds priming (An et al., 2020; Liu et al., 2021;
370 Hassanpouraghdam et al., 2022; Chen et al., 2022).

371 Compared to nCeO₂, nanoscale lanthanum oxide (nLa₂O₃) exhibits lower stability, increased ion
372 dissolution, greater translocation from roots and shoots, all of which seems to lead to higher
373 phytotoxicity. ~~The limited stability of nLa₂O₃ has been confirmed by μ -XRF analysis performed in~~
374 ~~*Cucumis sativus* L. (Ma et al., 2015).~~ The limited stability of nLa₂O₃, as compared to nCeO₂, has
375 been confirmed by μ -XRF analysis in *Cucumis sativus* L. through element speciation, dissolution
376 studies in aqueous solution and *in planta*. After 14d treatment, the nCeO₂ structure in the roots
377 remains mostly preserved (more than 80%) while pristine nLa₂O₃ structure was observed at levels
378 below 10% (Ma et al., 2015).

379 Interestingly, co-contamination with nCeO₂ strongly reduces the uptake of nLa₂O₃ (Pagano et al.,
380 2017). The different behaviour of the two ENMs was evident in the transcriptomic profile: only 7 out
381 of 38 genes were commonly modulated between nCeO₂ and nLa₂O₃; these genes were involved in
382 primary metabolic functions, protein synthesis and stress response (Pagano et al., 2016). Several
383 publications using different model plants were compared, and the reported effects due to nLa₂O₃
384 exposure in soil include reduction in root and leaf biomass (Ma et al., 2015), decreased transpiration
385 (Yue et al., 2019), decreased photosynthesis (Xiao et al., 2021) and reduced pigment concentration
386 (Neves et al., 2019). The decrease in photosynthetic activity is also reflected by altered root
387 morphology, including root cracking (Xiao et al., 2021) and the presence of apoplastic barriers (Yue

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

388 et al., 2019). Interestingly, the adverse effect of nLa₂O₃ on plant biomass was alleviated under binary
389 exposure combinations with ENMs such as nCuO and nZnO (Pagano et al., 2017). As ideal case
390 study, information on RedOx state and potential translocation of lanthanide-based nanoforms within
391 plant tissues become fundamental in the mechanistic understanding on the physiological and
392 molecular effects, with regard to application in the agrifood sector.

393

394 2.2. *Titanium oxide ENM*

395 Titanium dioxide (nTiO₂) has been largely studied as a potential environmental and agricultural
396 contaminant (Servin et al., 2012; 2013). nTiO₂ has demonstrated a high stability, both in anatase and
397 rutile crystal form (Servin et al., 2012). Translocation of nTiO₂ (0-750 mg kg⁻¹, 150d exposure) from
398 soil to roots and to shoots in *C. sativus* is generally limited, though the two crystal structures were
399 evident in the leaf trichomes and fruit by μ-XANES spectra (Servin et al., 2013). Ruotolo et al. (2018)
400 analyzed and reviewed the molecular responses of *A. thaliana* and other model species to nTiO₂ and
401 reported that exposure triggers an abiotic stress response at the transcriptomic level, involving ROS
402 detoxification systems, triterpenoid and phenylpropanoid metabolism, and hormone signaling
403 pathways involving in the response to salicylic acid, jasmonic acid, ethylene, and brassinosteroids.
404 At the post-transcriptional level, several miRNAs were strongly modulated, including miR395 and
405 miR399 as key regulators of plant adaptive responses to nutrient starvation (Pagano et al., 2021).
406 Thus, the ability of nTiO₂ to modulate ROS signaling is particularly effective under abiotic stress
407 conditions. Here, the presence of this ENMs enhances plant physiological parameters by stimulating
408 the activation of several defense mechanisms. Several studies (in plants such as *C. sativus*, *S.*
409 *lycopersicum*, *V. faba*) have shown that in both saline soils and under drought conditions, the addition
410 of nTiO₂ increases root length, plant biomass, and other parameters such as H₂O₂ level, antioxidant
411 activity, sugar content, and chlorophyll amount (Servin et al., 2012; Nasir Kahn, 2016; Abdel Latef
412 et al., 2018; Mustafa et al., 2021). However, higher concentrations can result in phytotoxicity, likely
413 due to aggregation and subsequent excessive ROS production (Mattiello et al., 2015; Gohari et al.,

Formatted: Outline numbered + Level: 2 + Numbering
Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned
at: 0" + Indent at: 0.25"

Formatted: Font: (Default) Times New Roman, Italic

414 2020). However ROS are “double blade” sword because they can also trigger production of defensive
415 molecules as shown recently by Castro et al., (2021). Interestingly, when either considering the
416 utilization of pristine and coated nTiO₂ (hydrophilic or hydrophobic) in carrot (*Daucus carota* L.),
417 responses observed depended mainly on the nTiO₂ surface coating, concentration and in soil
418 weathering (Wang et al., 2021a; 2021b). Taproot and leaf fresh biomass and plant height were all
419 increased with exposure, as well as nutrient uptake (Fe in leaves; Mg in taproots; Ca, Zn, K in roots).
420 Conversely, sugar and starch contents were negatively affected, compromising the nutritional quality
421 (Wang et al., 2021b).

Formatted: Subscript

Formatted: Font: Italic

Formatted: Subscript

424 2.3. *Gold and silver nanoparticles*

425 Similar to nTiO₂, gold nanoparticles (nAu) are highly stable in plants: nAu remained mostly as Au⁰
426 within the plant tissues (*Nicotiana tabacum* L. cv. Xanthi nc.-), even if accumulated and translocated
427 (Sabo-Attwood et al., 2011). Specifically, XANES analyses demonstrated that nAu maintained its
428 nanoparticle structure without any biotransformation or ionic release. There are no actual uses for
429 gold nanoparticles and plants, it just used as a tool to study NP-plant interactions. nAu levels in
430 biosolids would ever be high enough to be considered phytotoxic. It is known that nAu toxicity
431 depends on concentration, particle size and shape: nAu with a smaller particle size (3.5nm,
432 concentration of 48 mg L⁻¹) were evenly biodistributed across the plant in comparison with the
433 18.5nm nAu (in a concentration of 76 mg L⁻¹), even leading to the formation of necrotic leaf lesions
434 and plant death after 30 days (Sabo-Attwood et al., 2011). Other studies have shown that nAu
435 exposure improved radical scavenging and antioxidant enzymatic activities and modulated miRNA
436 expression implicated plant abiotic stress response (miR398, miR408). In particular, the regulation
437 of superoxide dismutase (SOD) led to an increased ROS scavenging activity, root elongation,
438 seedling growth, and seed yield (Arora et al. 2012; Kumar et al., 2013; Siddiqi & Husen, 2017).

Formatted: Outline numbered + Level: 2 + Numbering
Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned
at: 0" + Indent at: 0.25"

Formatted: Font: (Default) Times New Roman, Italic

439 Given the widespread commercial utilization and environmental relevance (*e.g.*, wastewater
440 treatment; fertilization) of silver nanoformulations (nAg), the effect on plant species has been a topic
441 of robust study. Stegemeier et al. (2015) analyzed the nAg and nAg₂S speciation in *Medicago sativa*
442 L., demonstrating that nAg accumulates in the root elongation area but that nAg₂S remains adhered
443 to the root surface; Ag ions accumulate more uniformly throughout the root tissues. Notably, the Ag
444 accumulation in the root apoplast was determined by XRF. The presence of nAg in root cell walls
445 demonstrated the uptake of partially dissolved nAg and translocation along the apoplast. Larue et al.
446 (2014) localized and determined nAg speciation in *L. sativa* after foliar spray treatment through μ -
447 XRF and μ -XAS techniques; the authors reported that nAg was able to cross the foliar cuticle,
448 penetrating in the leaf tissue through the stomata. Moreover, nAg biotransformed through oxidation
449 and complexation with thiol-containing molecules such as glutathione (GSH). These findings
450 correlated well with the transcriptomics analyses of *A. thaliana* exposed to different types of nAg:
451 plant response included defensin-like proteins, plant thionin, β -glucosidases, cytochrome P450
452 proteins, and glutathione-S-transferase (GST) members (Kaveh et al., 2013). Although some studies
453 point out that the morphological and physiological effects of nAg exposure were strictly dependent
454 on particle size and concentration and that sublethal concentrations may have also beneficial effects
455 (Wang et al., 2013; Syu et al., 2014), most of reports demonstrated reduced root elongation and shoot
456 biomass, together with decreased levels of chlorophyll, pigments, micronutrients, and increased level
457 of ROS and activity of enzymes involved in the oxidative stress response (Yin et al., 2011; Zuverza-
458 Mena et al., 2016; Yang et al., 2018; Lahuta et al., 2022).

459

460 2.4. *Iron-based ENMs*

461 Iron-based nanomaterials, including iron oxides (nFeOx) and zero valent iron (nZVI), have been
462 investigated in plant systems and the reports highlight two major routes of entry: i) a reductive and
463 proton-promoted process able to modify the structure of the ENM or ii) through the secretion of plant
464 transporters (*e.g.*, phytosiderophores) with a high affinity for Fe (III) (Morrissey & Guerinot, 2009).

Formatted: Outline numbered + Level: 2 + Numbering
Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned
at: 0" + Indent at: 0.25"

465 Dwivedi et al. (2018) investigated nZVI exposure in *C. sativus* and reported that transformed nZVI
466 was stored in the root cell membrane and vacuoles of the leaf parenchyma. XAS identified ferric
467 citrate and iron (oxyhydr)oxides as the main transformation products in roots and shoots, albeit in
468 different proportions. The major pathways of nZVI biotransformation involve interaction with low
469 molecular weight organic acid ligands and on the dissolution/precipitation of the mineral products.
470 Transcriptional analyses performed on H⁺-ATPase genes (*CsHA1*, *CsHA2*) showed an upregulation
471 of these genes upon nZVI exposure (and relative root acidification), indicating that the plant-
472 promoted transformation of nZVI can be driven by protons released by the roots.

473 A separate study investigated the effects of nFe₂O₃ and nFe₃O₄ on *A. thaliana*, highlighting
474 differences in the response between nanoparticle forms and metal salts through a nanoscale-specific
475 response pathway involving energy production and oxidative stress. The differential response was
476 ascribed to the ENM and the metal salt dissolution rates and the toxicity of the metal ion, which is
477 more compatible with biotransformation processes in the plant tissues. Importantly, specific effects
478 on plastid and mitochondrial genomes were evident, with nFeOx causing a 1- to 3-fold increase in
479 ptDNA and mtDNA copy numbers depending on the stability of the nanoform utilized (Pagano et al.,
480 2022).

481 Given their widespread application in soil and water remediation, a primary concern with iron
482 nanoparticles is a potential toxicity from excessive accumulation in the environment. However,
483 several studies have shown that plant exposure to this type of nanoparticle does not result in
484 phytotoxicity. For example, Dwivedi et al. (2018) evaluated the potential environmental impact of
485 nZVI on *C. sativus* in soil and in hydroponic culture, and reported no instances of reduced plant
486 biomass even at the highest doses (from 250 to 1000 mg L⁻¹) and for *O. sativa*, the low doses (50-500
487 mg L⁻¹) of nZVI and nFe₃O₄ improved plant growth (Li et al., 2021). The use of this nanomaterial as
488 a soil conditioner for remediation of metal-contaminated soils is confirmed by the demonstration of
489 improved plant growth in Cd-contaminated soils (Rizwan et al., 2019; Manzoor et al., 2021);

490 mechanistically, this involves limiting cadmium translocation and the promotion of antioxidant
491 activity.

492 In summary, the extent and the degree of biotransformation of nZVI, which consists in the
493 biochemical alteration of chemical compounds within a living tissue, are reflected in the physico-
494 chemical properties, macromolecular interaction, and biologically mediated pathways observed.

495

496 2.5. Zinc-based ENMs

497 Zinc-based nanomaterials have been applied to plants to increase food safety, promote food
498 production and enhance sustainability by reducing oxidative stress symptoms induced by abiotic
499 stressors (Faizan et al., 2021). nZnO is characterized by a low stability, and a high dissolution rate
500 (Lv et al., 2021). Hernandez-Viezcas et al. (2013) exposed *Glycine max* L. to nZnO (500 mg kg⁻¹,
501 48d exposure): μ -XRF analysis showed no detectable ZnO NPs within the tissues, while μ -XANES
502 data showed O-bound Zn in a form resembling Zn citrate. Lv et al. (2015) studied the effects of nZnO
503 in *Z. mays* L. and used μ -XANES to demonstrate that the majority of accumulated Zn was derived
504 from Zn²⁺ released from the nanoparticles and was accumulated mainly as Zn phosphate in epidermis,
505 cortex, and root tip cells. The results were correlated to transcriptomic analyses in which gene
506 ontology (GO) performed in nZnO-exposed *A. thaliana* revealed significant commonalities with the
507 response to Zn²⁺ ions, particularly with proteins involved in metal binding, transport, metal
508 homeostasis and detoxification. This suggests that Zn ion release by nZnO is a key in mediating the
509 overall effect on plant species (Landa et al., 2015). These findings have been extended to other
510 species, such as *C. pepo* L.; here nZnO treatment was shown to modulate genes that encode for
511 transporters of heavy metals, cellular response to abiotic stress, decreased chlorophyll production,
512 and induction of secondary metabolite biosynthesis (Pagano et al., 2017).

513 In recent years other forms of Zn-based nanomaterials have been tested for a potential plant
514 remediation purpose, such as ZnS QDs (Imperiale et al., 2022). An analysis of the effect of ZnS QDs
515 and ionic Zn exposure on mitochondrial and plastid genome copy number demonstrates that both

Formatted: Indent: Left: -0.01", Hanging: 0.3", Outline
numbered + Level: 2 + Numbering Style: 1, 2, 3, ... +
Start at: 1 + Alignment: Left + Aligned at: 0" + Indent
at: 0.25"

516 increase by 1 to 3-fold), but that ZnS QDs dissolution alone does not explain the phenomenon; this
517 suggests that ZnS QDs biotransformation may occur within the plant tissues and organs to a form
518 more similar to ionic than nanoscale Zn (Pagano et al., 2022). Zinc-based nanomaterials have also
519 shown interesting properties as nanofertilizers, including mitigating abiotic and biotic stress (*e.g.*, salt
520 stress, infections), regulating micronutrient uptake, improving water use efficiency, and promoting
521 detoxification of heavy metals (Akhtar et al., 2021; Zafar et al., 2022). Under drought conditions, the
522 nZnO (5 mg kg⁻¹) significantly increased grain yield in sorghum (*Sorghum vulgare* Moench) and fruit
523 yield in eggplant (*Solanum melongena* L.), respectively by 22-183% and 12-23% (Dimkpa et al.,
524 2019; Semida et al., 2021).

525

526 2.6. -Copper oxide

527 Copper oxide nanomaterials (nCuO) are among the most utilized ENMs with plants, including use as
528 a nanopesticide or nanofertilizer (Elmer et al., 2018; Lowry et al., 2019; Xu et al., 2022). ~~Copper~~
529 ~~oxide nanomaterials (nCuO) are one of the most utilized ENMs with plants, including use as a~~
530 ~~nanofertilizer (Elmer et al., 2018; Lowry et al., 2019).~~ nCuO dissolution within the plant tissues has
531 been demonstrated (in *C. pepo*), and this was shown to depend not only on uptake, and translocation,
532 but also on the interaction with important biomolecules (Tamez et al., 2019; Marmioli et al., 2021).
533 EXAFS (Marmioli et al., 2021) demonstrated that the local Cu environment in the higher shells
534 shows small differences between roots and flowers. A second Cu-O shell path was present in both
535 flowers and roots; a Cu-Cu bond was also observed in ~~roots, but~~ roots but was not observed in flowers.
536 A full transcriptomics analysis by RNAseq was performed to highlight the differential responses
537 between nano-, bulk and ionic forms in roots, leaves and pollen (Marmioli et al., 2021). The results
538 highlighted the nano-specificity of the responses; the modulated genes (significantly up- or down-
539 regulated genes) observed were more significant in the roots and decreased with translocation to
540 leaves and pollen. However, the portion of the response common to the three Cu forms tested was
541 shown to increase following the translocation from roots to shoots (Marmioli et al., 2021). A

Formatted: Indent: Left: -0.01", Hanging: 0.3", Outline numbered + Level: 2 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 0" + Indent at: 0.25"

542 characterization of the main steps and implications involved in this phenomenon, as well as some
543 relevant biomarkers observed in different plant species, is presented in Figure 2+ (details reported in
544 Table 1).

545 Additional data was presented by Servin et al. (2017a), who studied nCuO weathering in *Lactuca*
546 *sativa* L.: lettuce was exposed to unweathered and 70d-weathered nCuO, and corresponding bulk and
547 ionic form (0–400 mg kg⁻¹) for 70 d in soil. To assess nCuO trophic transfer, leaves were fed to
548 crickets (*Acheta domestica* L.) as primary consumer, followed by insect feeding to lizards (*Anolis*
549 *carolinensis* L.) as secondary consumer, in both cases for 15d. The authors used μ -XANES to show
550 that Cu(II) was reduced to Cu(I) within the plant roots, and used a transcriptional analysis of to show
551 that several biomarkers, including *CCH* and *COPT5*, which encodes for a copper chaperon and a
552 copper ion transporter, respectively, were significantly decreased by weathering.

553 In spite of being widely used, results regarding the physiological effects upon nCuO exposure are
554 rather discordant. For example, Deng et al. (2022a) reported that, unlike the bulk counterpart, nCuO
555 (0-600 mg kg⁻¹ of soil) does not produce toxicity in rice (*O. sativa*), but rather improves the supply
556 of essential elements, including increasing content of sugar and starch, as well as overall yield.

557 The grain of weedy and cultivated rice were differentially impacted by nCuO, bulk or ionic forms,
558 showing also a cultivar-specific and concentration-dependent response. Cu translocation directly
559 influenced plant yield, sugar production, starch content, protein content, and expression of auxin
560 associated genes in grain (Deng et al., 2022b). Analyzing the effect of citric acid (CA) coated copper
561 oxide (CA-nCuO) and its application (foliar spray or soil exposure) on the growth and physiology of
562 soybean (*Glycine max* L.), nCuO appeared to be more accessible for plant uptake, as compared to
563 CA-nCuO, decreasing the protein content, and inhibiting plant growth. CA reduced CuO NPs
564 toxicity, demonstrating that surface modification may change the toxic properties of NPs. (Deng et
565 al., 2022c).

566 Treatment of *Lactuca sativa* L. with nCuO significantly increased biomass as compared to CuO
567 microparticles (Wang et al., 2019). In addition, plants can benefit from nCuO treatment through

Formatted: Font: Italic

Formatted: Font: Italic

568 enhanced defensive pathways, and through direct antimicrobial and antifungal activities (Elmer et al.,
569 2018). Similarly, *L. sativa* treatment with nCuO significantly increased biomass relative to CuO
570 microparticles (Wang et al., 2019). In addition, nCuO can benefit plants through enhanced defensive
571 pathways, as well as through direct antimicrobial and antifungal activities (Elmer et al., 2018). For
572 example, exposure of nCuO to *Solanum lycopersicum* increased root and stem length, leaf number,
573 and chlorophyll content, and also inhibited the mycelial growth of *Fusarium oxysporum* sp.
574 *Lycopersici* (Lopez-Lima et al., 2021). Conversely, some authors report toxic and inhibitory effects
575 on the growth in plants such as lettuce (*Lactuca sativa* L., 0-1000 mg L⁻¹, 5-15d exposure by foliar
576 spray), turnip (*Brassica rapa* L., 50-500 mg L⁻¹, 14d exposure), and wheat (*Triticum aestivum* L., 50
577 mg kg⁻¹ in sand, 1-14d exposure) upon nCuO treatment. The toxic effects are largely ascribed to the
578 redox reactivity and ROS generation of the nanoparticle form (Dimkpa et al., 2012; Chung et al.,
579 2019; Xiong et al., 2020). Others have reported no significant impact at the physiological level
580 (Servin et al., 2017a; Tamez et al., 2019; Marmioli et al., 2021; Roubeau Dumont et al., 2022), which
581 highlights the importance of the experimental variables and design, including dose, particle
582 properties, exposure conditions and endpoints.

583

584 2.7. Quantum dots

585 Cadmium-based nanomaterials, and cadmium sulfide quantum dots (CdS QDs) in particular, have
586 been used as a model material to elucidate physiological mechanisms and molecular pathways
587 involved in the response plant response to exposure (Marmioli et al., 2014; Imperiale et al., 2022).
588 A Systems biology approach gave a complete picture of the targets in both model (*A. thaliana*) and
589 crop (*C. pepo*) species (Marmioli et al., 2014; Marmioli et al., 2015; Pagano et al., 2017; Gallo et
590 al., 2021; Marmioli et al., 2020; Pagano et al., 2022). In *A. thaliana*, CdS QDs tolerant mutants were
591 used to establish *in vitro* inhibition concentrations for growth (80 mg L⁻¹) in an attempt to elucidate
592 the mechanisms involved in the plant response; the results largely implicated metabolic functions and
593 chloroplast energy production as sensitive targets (Marmioli et al., 2014). The results demonstrate

Formatted: Indent: Left: 0", Hanging: 0.3", Outline numbered + Level: 2 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 0" + Indent at: 0.25"

594 that CdS QDs and ionic Cd were exploiting different pathways in the plant, highlighting that the
595 tolerance to CdS QDs did not ~~overlapped~~overlap with the tolerance to CdSO₄. Conversely, Cd
596 sensitive mutants of Arabidopsis (Howden & Cobbett, 1992) that were exposed to CdS QDs did not
597 exhibit differences in growth as compared to the wild type line (Marmioli et al., 2014). A
598 transcriptomic analysis and proteomic comparison between wild type and tolerant mutants
599 highlighted that only a few genes were commonly modulated upon ionic Cd and CdS QDs treatment
600 (Marmioli et al., 2015, Gallo et al., 2021). Marmioli et al., (2020) used EXAFS to investigate the
601 cadmium environment in planta and showed that the spectra were compatible with a mixed O/S
602 coordination; while Cd–S distances did not show relevant variations, Cd–O distances varied in
603 samples grown with QDs compared with those grown with CdSO₄. The number of Cd–S bonds in
604 plants grown with QDs was higher than Cd–O bonds. This EXAFS analysis demonstrated that CdS
605 QDs were biotransformed after uptake: the QD original structure was modified but not completely
606 absent within the plant cell, and Cd atoms were not released as Cd ions. Interestingly, CdS QDs
607 showed a relatively high stability; once accumulated by the plant, the QD may go through different
608 stages in the response pathways: i) exposure: explained by the different genetic mechanisms behind
609 the physiological/molecular response between the wild type and tolerant phenotypes; ii)
610 reactivity/biotransformation: explained by a transition phase in which the structure of CdS QDs is
611 modified to decrease particle reactivity, and this can be detected by XANES and EXAFS analyses;
612 iii) effects/detoxification: transcriptomic, proteomic and metabolomic response related to the
613 physico-chemical forms after QDs biotransformation. Additionally, the effects on organelle genomes
614 (ptDNA and mtDNA) demonstrate how QDs biotransformation may modify the genomes
615 stoichiometry or sub-stoichiometry, likely through potential morpho-functional adaptive response
616 triggered by modifications in the bioenergetic redox balance, or a reduction of photosynthesis or
617 cellular respiration rates after QD exposure (Pagano et al., 2022).

618 Similarly to what was observed in *A. thaliana*, CdS QDs induced analogous effects other plant species
619 of agricultural interest: Pagano et al. (2017) analyzed the effects of the CdS QDs in a context of

620 ENMs binary co-contamination, highlighting a similar response as in *A. thaliana*; specific and
621 common biomarkers were involved between CdS QDs and other the ENMs tested (nCeO₂, nLa₂O₃,
622 nCuO, nZnO). Majumdar et al. (2019) investigated the effect of differently functionalized CdS QDs
623 in *G. max*; the authors used proteomic and metabolomic endpoints to demonstrate how the
624 transmembrane proteins involved uptake and related genes including *NRAMP6* and *HMA8* were
625 differently regulated in CdS QDs and ion treated plants. In addition, ATP-dependent ion transporters
626 in the membranes presented feedback mechanisms in the soybean roots to restrict the uptake of CdS
627 QDs and simultaneously to alter the mineral acquisition. Moreover, CdS QDs altered major metabolic
628 functions, including glutathione metabolism, the tricarboxylic acid cycle, glycolysis, fatty acid
629 oxidation and phenylpropanoid and amino acids biosynthesis. Physiologically, CdS QDs, induced
630 oxidative stress, decreased biomass, reduced chlorophyll and carotenoids content, and damaged
631 primary roots (Majumdar et al., 2019; Pagano et al., 2022).

632

633

634 3. *Biotransformation as a perspective to comprehend ENM response in plant*

635 ENMs have been rather extensively tested in recent years, with data indicating that several physico-
636 chemical parameters are fundamental to explaining their behaviour during exposure, including
637 composition, stability, surface charge, and functionalization. These ENM properties become
638 biologically relevant and mediate subsequent biotransformation processes, including: i) the
639 possibility to be translocated within organs, tissues, and cells; ii) the ability to interact with the
640 biologically active environment within the plant (*e.g.*, phospholipids, nucleic acids, proteins,
641 secondary metabolites, reactive oxygen species); iii) the dissolution rate and the consequent ion
642 release. Importantly, to fully comprehend the plant response to ENMs exposure, one must consider
643 the biologically modified ENMs forms that are indicative of the highly complex interactions between
644 plants and ENMs interaction. Integration of the information from physiological and molecular
645 analyses with physical evidence (*e.g.*, types and number of atoms surrounding the ENM, radial

Formatted: Indent: Left: 0", Hanging: 0.2", Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 2 + Alignment: Left + Aligned at: 0" + Indent at: 0.25"

Formatted: Font: Italic

646 distance between atoms of the interactors and atoms constituting the ENM; ENM crystal structure)
647 obtained through high energy X-ray spectroscopy platforms such as synchrotron-based techniques
648 will enable a more realistic, mechanistic, and systems-level picture of ~~of~~-plant response to ENM
649 exposure.

650 This review describes some of the primary biological constraints that determine ENM
651 biotransformation in plants (Figure 24, Table 2). For ENMs characterized by high stability, such as
652 nCeO₂, nTiO₂ or nAu, limited dissolution and translocation has been observed, even considering
653 differences determined by structure and atomic properties related to the redox state (*e.g.*, the redox
654 state of Ce). Conversely, ENMs with higher dissolution such as Fe- or Zn-based ENMs, nCuO, nAg
655 or nLa₂O₃, exhibit greater translocation rates, likely involving a dynamic process of particle
656 interaction with the plant biomolecules that increase ENM solubility and bioavailability, as
657 exemplified with nCuO (Marmiroli et al., 2021).

658 The importance of in planta ENM biotransformation is corroborated indirectly at molecular level by
659 “omic” analyses that can describe the effects on the plant at genetic and epigenetic level (including
660 genome stability) by measuring transcriptional modulation, protein abundance and metabolite
661 synthesis, as well as on physiological (phenotypical) level by observing the plant redox state, ROS
662 production, photosynthetic activity, and cellular respiration rate in response to stress (Marmiroli et
663 al., 2020; Gallo et al., 2021). The direct measurement of changes upon ENM biotransformation within
664 the plant tissues by synchrotron-based techniques (μ -XRF, μ -XANES, and XAS) provide critical
665 information in terms of distribution, atomic redox state, and atomic local structure, and add critical
666 knowledge necessary to understand the ENM-plant interactions. This information is highly relevant
667 with regard to potential applicability: ENMs can interact with sensitive ecosystem components within
668 trophic food chains, affect microbial populations in soil, enter into the plant and where they can be
669 translocated to different tissues and organs, including the edible tissues or organs (Holden et al., 2013;
670 Liu et al., 2015). Biotransformation can occur at each step within these processes, modifying and/or
671 amplifying ENM effects at organism level. These interactions from the level of ecosystem, organism,

672 tissue, cell, and organelles become key factors when applying “ENM biotransformation” as a concept
673 for a safer design, when considering applications for agriculture and food production, and for
674 minimizing the adverse biological impact (Burello & Worth, 2015; Pagano et al., 2018; Lowry et al.,
675 2019; Kah et al., 2019; Zulfiqar et al., 2019; Ma et al., 2020; Hameed et al., 2022; Xu et al., 2022)~~This~~
676 ~~information is highly relevant with regard to applications: ENMs can clearly interact with sensitive~~
677 ~~ecosystem components and within the trophic food chains, alter microbial populations in soil, enter~~
678 ~~in plants and be translocated to different tissues and organs, including edible tissues (Holden et al.,~~
679 ~~2013; Liu et al., 2015). Biotransformation can occur at each step of these processes, modifying and/or~~
680 ~~amplifying the effects ENM effects at the level organisms. These interactions at the level of~~
681 ~~ecosystem, organism, tissue, cell, and organelles become key factors in applying “ENM~~
682 ~~biotransformation” as a concept for safer design when considering applications for agriculture and~~
683 ~~food production, for minimizing the adverse biological effects (Burello & Worth, 2015; Pagano et~~
684 ~~al., 2018; Lowry et al., 2019; Kah et al., 2019; Zulfiqar et al., 2019; Ma et al., 2020; Hameed et al.,~~
685 ~~2022).~~

688 *Author contributions*

689 Conceptualization: LP, MM; original draft preparation: LP, MM, RR; review and editing: JCW, NM.

690 All authors revised and agreed on the final version of the manuscript.

692 *Acknowledgements*

693 This work was supported by European Union's HORIZON 2020 research and innovation Programme
694 [grant number 818431 SIMBA], European Union's PRIMA Programme [grant number 1811
695 SUSTAINOLIVE], and FIL (Fondi Locali per la Ricerca). The funding sources had no involvement
696 in study design, collection, analysis and interpretation of data, writing, decision to submit the paper.

698 ***Declaration of competing interest***

699 The authors declare no competing financial interests.

700

701

702 **References**

703 Abutalib M.M., Rajeh A. 2020. Enhanced structural, electrical, mechanical properties and
704 antibacterial activity of Cs/PEO doped mixed nanoparticles (Ag/TiO₂) for food packaging
705 applications. *Polymer Testing*. 107013.

706

707 Abdel Latef A.A.H., Srivastava A.K., El-sadek M.S.A., Kordrostami M., Tran L.-S.P. 2018. Titanium
708 dioxide nanoparticles improve growth and enhance tolerance of broad bean plants under saline soil
709 conditions. *Land Degrad. Dev.* 29, 1065–1073.

710

711 Akhtar N., Ilyas N., Meraj T.A., Pour-Aboughadareh A., Sayyed R.Z., Mashwani Z.U.R., Pocza P.
712 2022. Improvement of Plant Responses by Nanobiofertilizer: A Step towards Sustainable Agriculture.
713 *Nanomaterials*. 12, 965.

714

715 An J., Hu P., Li F., Wu H., Shen Y., White J.C., Tian X., Li Z., Giraldo J.P. 2020. Molecular
716 mechanisms of plant salinity stress tolerance improvement by seed priming with cerium oxide
717 nanoparticles. *Environ. Sci: Nano*. 7, 2214.

718

719 Arora S., Sharma P., Kumar S., Nayan R., Khanna P.K., Zaidi M.G.H. 2012. Gold-nanoparticle
720 induced enhancement in growth and seed yield of *Brassica juncea*. *Plant Growth Regul.* 66, 303–
721 310.

722

723 Burello E., Worth A.P. 2015. A rule for designing safer nanomaterials: do not interfere with the
724 cellular redox equilibrium. *Nanotoxicology*. 9, 116-117.

725

726 Castillo-Michel H.A., Larue C., Pradas del Real A.E., Cotte M., Sarret G. 2017. Practical review on
727 the use of synchrotron based micro- and nano- X-ray fluorescence mapping and X-ray absorption
728 spectroscopy to investigate the interactions between plants and engineered nanomaterials. *Plant*
729 *Physiol Biochem*. 110, 13-32.

730

731 Castro B., Citterico M., Kimura S., Stevens D.M., Wrzaczek M., Coaker G. 2021. Stress-induced
732 reactive oxygen species compartmentalization, perception and signalling. *Nat. Plants* 7, 403–412.

733

734 Chebakova K.A., Dzidziguri E.L., Sidorova E.N., Vasiliev A.A., Ozherlkov D.Y., Pelevin I.A.,
735 Gromov A.A., Nalivaiko A.Y. 2021. X-ray Fluorescence Spectroscopy Features of Micro- and
736 Nanoscale Copper and Nickel Particle Compositions. *Nanomaterials*. 14, 11(9), 2388.

737

738 Chen L., Peng Y., Zhu L., Huang Y., Bie Z., Wu H. 2022. CeO₂ nanoparticles improved cucumber
739 salt tolerance is associated with its induced early stimulation on antioxidant system. *Chemosphere*.
740 299, 134474.

741

742 Chang C., Bowman J.L., Meyerowitz E.M. 2016. Field Guide to Plant Model Systems. *Cell*. 167,
743 325-339.

744

745 Chung I.M., Rekha K., Venkidasamy B., Thiruvengadam M. 2019. Effect of Copper Oxide
746 Nanoparticles on the Physiology, Bioactive Molecules, and Transcriptional Changes in *Brassica rapa*
747 ssp. *rapa* Seedlings. *Water. Air. Soil Pollut.* 230, 48.

748

749 d'Acapito F., Lepore G.O., Puri A., Laloni A., La Manna F., Dettona E., De Luisa A., Martin A. 2019.
750 The LISA beamline at ESRF. J. Synchrotron Rad. 26, 551-558.

751
752 Deng C., Wang Y., Cota-Ruiz K., Reyes A., Sun Y., Peralta-Videa J.R., Hernandez-Viezcas J.A.,
753 Turley R.S., Niu G., Li C., Gardea-Torresdey J.L. 2020. Bok choy (*Brassica rapa*) grown in copper
754 oxide nanoparticles-amended soils exhibits toxicity in a phenotype-dependent manner: Translocation,
755 biodistribution and nutritional disturbance. J Haz Mater. 398, 122978.

756
757 Deng C., Wang Y., Navarro G., Sun Y., Cota-Ruiz K., Hernandez-Viezcas J.A., Niu G., Li C., White
758 J.C., Gardea-Torresdey J. 2022a. Copper oxide (CuO) nanoparticles affect yield, nutritional quality,
759 and auxin associated gene expression in weedy and cultivated rice (*Oryza sativa* L.) grains. Sci. Total
760 Environ. 810, 152260.

761
762 Deng C., Wang Y., Navarro G., Sun Y., Cota-Ruiz K., Hernandez-Viezcas J.A., Niu G., Li C., White
763 J.C., Gardea-Torresdey J. 2022b. Copper oxide (CuO) nanoparticles affect yield, nutritional quality,
764 and auxin associated gene expression in weedy and cultivated rice (*Oryza sativa* L.) grains, Scie Tot
765 Environ. 810, 152260.

766
767 Deng C., Wang Y., Cantu J.M., Valdes C., Navarro G., Cota-Ruiz K., Hernandez-Viezcas J.A., Li
768 C., Elmer W.H., Dimkpa C.O., White J.C., Gardea-Torresdey, J.L. 2022c. Soil and foliar exposure
769 of soybean (*Glycine max*) to Cu: Nanoparticle coating-dependent plant responses, NanoImpact,
770 26.100406.

771
772 Dimkpa C.O., McLean J.E., Latta D.E., Manangón E., Britt D.W., Johnson W.P., Boyanov M.I.,
773 Anderson A.J. 2012. CuO and ZnO nanoparticles: Phytotoxicity, metal speciation, and induction of
774 oxidative stress in sand-grown wheat. J. Nanoparticle Res. 14, 1125.

- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: Italian (Italy)
- Formatted: English (United States)
- Formatted: Font: Italic, English (United States)
- Formatted: English (United States)
- Formatted: English (United States)
- Formatted: English (United States)

Formatted: Font: Italic

775

776 Dimkpa C.O., Singh U., Bindraban P.S., Elmer W.H., Gardea-Torresdey J.L., White J.C. 2019. Zinc
777 oxide nanoparticles alleviate drought-induced alterations in sorghum performance, nutrient
778 acquisition, and grain fortification. *Sci. Total Environ.* 688, 926–934.

779

780 Dwivedi A.D., Yoon H., Singh J.P., Chae K.H., Rho S-C., Hwang D.S., Chang Y-S. 2018. Uptake,
781 Distribution, and Transformation of Zerovalent Iron Nanoparticles in the Edible Plant *Cucumis*
782 *sativus*. *Environ Sci Technol.* 52 (17), 10057-10066.

783

784 EFSA. 2021. Safety assessment of titanium dioxide (E171) as a food additive. *EFSA Journal.* 19(5),
785 6585.

786

787 Elmer W., De la Torre-Roche R., Pagano L., Majumdar S., Zuverza-Mena N., Dimkpa C., Gardea-
788 Torresdey, J.L., White J.C. 2018. Effect of Metalloid and Metal Oxide Nanoparticles on Fusarium
789 Wilt of Watermelon. *Plant Dis.* 102, 1394–1401.

790

791 Eriksson P., Tal A.A., Skallberg A., Brommesson C., Hu Z., Boyd R.D., Olovsson W., Fairley N.,
792 Abrikosov I.A., Zhang X., Uvdal K. 2018. Cerium Oxide Nanoparticles with Antioxidant Capabilities
793 And Gadolinium Integration For MRI Contrast Enhancement. *Sci. Rep.* 8, 6999.

794

795 Faizan M., Yu F., Chen C., Faraz A., Hayat S. Zinc Oxide Nanoparticles Help to Enhance Plant
796 Growth and Alleviate Abiotic Stress: A Review. *Curr Protein Pept Sci.* 2021, 22(5), 362-375.

797

798 Gallo V., Zappettini A., Villani M., Marmioli N., Marmioli M. 2021. Comparative Analysis of
799 Proteins Regulated During Cadmium Sulfide Quantum Dots Response in *Arabidopsis thaliana* Wild
800 Type and Tolerant Mutants. *Nanomaterials.* 11, 615.

801
802 [Gardea-Torresdey J.L., Rico C.M., White J.C. 2014. Trophic transfer, transformation, and impact of](#)
803 [engineered nanomaterials in terrestrial environments. Environ. Sci. Technol. 48, 2526–2540.](#)
804
805 Giese B., Klaessig F., Park B., Kaegi R., Steinfeldt M., Wigger H., von Gleich A., Gottschalk F.
806 2018. Risks, Release and Concentrations of Engineered Nanomaterial in the Environment. Sci Rep
807 8, 1565.
808
809 Gohari G., Mohammadi A., Ak A., Panahirad S. 2020. Titanium dioxide nanoparticles (TiO₂ NPs)
810 promote growth and ameliorate salinity stress effects on essential oil profile and biochemical
811 attributes of *Dracocephalum moldavica*. Sci. Rep. 10, 912.
812
813 [Goldstein J.I., Newbury D.E., Echlin P., Joy D.C., Lyman C.E., Lifshin E., Sawyer L., Michael J.R.](#)
814 [Editors. Scanning Electron Microscopy and X-Ray Microanalysis, Third Edition. 2003 Springer](#)
815 [Science+Business Media LLC, 233 Spring Street, New York, NY 10013 USA.](#)
816
817 Gottschalk F., Sonderer T., Scholz R.W., Nowack B. 2009. Modeled Environmental Concentrations
818 of Engineered Nanomaterials (TiO₂, ZnO, Ag, CNT, Fullerenes) for Different Regions. Environ. Sci.
819 Technol. 43, 24, 9216–9222.
820
821 Gräfe M., Donner E., Collins R.N., Lombi E. 2014. Speciation of metal(loid)s in environmental
822 samples by X-ray absorption spectroscopy: A critical review. Analytica Chimica Acta. 822, 1-22.
823
824 Gui X., Rui M., Song Y., Yuhui M., Rui Y., Zhang P., He X., Li Y., Zhang Z., Liu L. 2017.
825 Phytotoxicity of CeO₂ nanoparticles on radish plant (*Raphanus sativus*). Environ. Sci. Pollut. Res.
826 24, 13775–13781.

827

828 Gurman S.J. 1995. Interpretation of EXAFS Data. *J. Synchr. Rad.* 2, (1), 56-63.

829

830 Hameed S., Baimanov D., Li X., Liua -K., Wang L. 2022. Synchrotron radiation-based analysis of
831 interactions at the nano–bio interface. *Environ. Sci.: Nano*. Doi: 10.1039/D2EN00408A.

832

833 Hassanpouraghdam M.B., Mehrabani L.V., Bonabian Z., Aazami M.A., Rasouli F., Feldo M.,
834 Strzemiński M., Dresler S. 2022. Foliar Application of Cerium Oxide-Salicylic Acid Nanoparticles
835 (CeO₂:SA Nanoparticles) Influences the Growth and Physiological Responses of *Portulaca oleracea*
836 L. under Salinity. *Int. J. Mol. Sci.* 23, 1–19.

837

838 Hernandez-Viezcas J.A., Castillo-Michel H., Andrews J.C., Cotte M., Rico C., Peralta-Videa J.R.,
839 Ge Y., Priester J.H., Holden P.A., Gardea-Torresdey J.L. 2013. In situ synchrotron X-ray
840 fluorescence mapping and speciation of CeO₂ and ZnO nanoparticles in soil cultivated soybean
841 (*Glycine max*). *ACS Nano*. 26, 7(2),1415-1423.

842

843 Holden P.A., Nisbet R.M., Lenihan H.S., Miller R.J., Cherr G.N., Schimel J.P., Gardea-Torresdey
844 J.L. 2013. Ecological Nanotoxicology: Integrating Nanomaterial Hazard Considerations Across the
845 Subcellular, Population, Community, and Ecosystems Levels. *Acc. Chem. Res.* 46, 813–822.

846

847 [Hong J., Wang C., Wagner D.C., Gardea-Torresdey J.L., He F., Rico C.M. 2021, Foliar application](#)
848 [of nanoparticles: mechanisms of absorption, transfer, and multiple impacts. *Environ. Sci.: Nano*, 8,](#)
849 [1196-1210.](#)

850

851 Howden R., Cobbett C.S. 1992. Cadmium-sensitive mutants of *Arabidopsis thaliana*. *Plant Physiol.*
852 99, 100–107.

Formatted: English (United States)

853

854 Huang Y., Li W., Minakova A.S., Anumol T., Keller A.A. 2018. Quantitative analysis of changes in
855 amino acids levels for cucumber (*Cucumis sativus*) exposed to nano copper. NanoImpact. 12, 9–17.

856

857 Huangfu X., Xu Y., Liu C., He Q., Ma J., Ma C., Huang R. 2019. A review on the interactions between
858 engineered nanoparticles with extracellular and intracellular polymeric substances from wastewater
859 treatment aggregates. Chemosphere. 219, 766-783.

860

861 Imperiale D., Lencioni G., Marmiroli M., Zappettini A., White J.C., Marmiroli N. 2022. Interaction
862 of hyperaccumulating plants with Zn and Cd nanoparticles. Sci Tot Environ. 817, 152741.

863

864 Judy J.D., Unrine J.M., Rao W., Wirick S., Bertsch P.M. 2012. Bioavailability of gold nanomaterials
865 to plants: importance of particle size and surface coating. Environ Sci Technol. 7, 8467-8474.

866

867 Kah M., Kookana R.S., Gogos A., Bucheli T.D. 2018. A critical evaluation of nanopesticides and
868 nanofertilizers against their conventional analogues. Nat. Nanotechnol. 13, 677–684.

869

870 Kah M., Tufenkji N., White J.C. 2019. Nano-enabled strategies to enhance crop nutrition and
871 protection. Nat. Nanotechnol. 14, 532–540.

872

873 Karny A., Zinger A., Kagal A., Shainsky-Roitman J., Schroeder A. 2018. Therapeutic nanoparticles
874 penetrate leaves and deliver nutrients to agricultural crops. Sci Rep. 8(1), 7589.

875

876 Kaveh R., Li Y.S., Ranjbar S., Tehrani R., Brueck C.L., Van Aken B. 2013. Changes in *Arabidopsis*
877 *thaliana* gene expression in response to silver nanoparticles and silver ions. Environ. Sci. Technol.
878 47 (18), 10637–10644.

Formatted: English (United States)

879

880 [Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered](#)
881 [nanomaterials. J. Nanopart. Res. 15, 1692–1709.](#)

882

883 [Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional](#)
884 [to Local. Environ. Sci. Technol. Lett. 2014, 1, 1, 65–70.](#)

885

886 Kumar V., Guleria P., Kumar V., Yadav S.K. 2013. Gold nanoparticle exposure induces growth and
887 yield enhancement in *Arabidopsis thaliana*. Sci. Total Environ. 461–462, 462–468.

888

889 Kumari P., Alam M., Siddiqi W.A. 2019. Usage of nanoparticles as adsorbents for wastewater
890 treatment: An emerging trend. Sustain. Mater. Technol. 22, e00128.

891

892 Lahuta L.B., Szablińska-Piernik J., Głowacka K., Stańlanowska K., Railean-Plugaru V., Horbowicz
893 M., Pomastowski P., Buszewski B. 2022. The Effect of Bio-Synthesized Silver Nanoparticles on
894 Germination, Early Seedling Development, and Metabolome of Wheat (*Triticum aestivum* L.).
895 Molecules. 27, 2303.

896

897 Landa P., Prerostova S., Petrova S., Knirsch V., Vankova R., Vanek T. 2015. The Transcriptomic
898 Response of *Arabidopsis thaliana* to Zinc Oxide: A Comparison of the Impact of Nanoparticle, Bulk,
899 and Ionic Zinc. Environ. Sci. Technol. 49 (24), 14537–14545.

900

901 Larue C., Castillo-Michel H., Sobanska S., Cécillon L., Bureau S., Barthès V., Ouerdane L., Carrière
902 M., Sarret G. 2014. Foliar exposure of the crop *Lactuca sativa* to silver nanoparticles: Evidence for
903 internalization and changes in Ag speciation. J Haz Mat. 264, 98-106.

904

905 Li M., Zhang P., Adeel M., Guo Z., Chetwynd A.J., Ma C., Bai T., Hao Y., Rui Y. 2021. Physiological
906 impacts of zero valent iron, Fe₃O₄ and Fe₂O₃ nanoparticles in rice plants and their potential as Fe
907 fertilizers. *Environ. Pollut.* 269, 116134.

908

909 Liu W., Tian S., Zhao X., Xie W., Gong Y., Zhao D. 2015. Application of Stabilized Nanoparticles
910 for In Situ Remediation of Metal-Contaminated Soil and Groundwater: a Critical Review. *Curr.*
911 *Pollut. Reports.* 1, 280–291.

912

913 Liu Y., Persson D.P., Li J., Liang Y., Li T. 2021. Exposure of cerium oxide nanoparticles to the
914 hyperaccumulator *Sedum alfredii* decreases the uptake of cadmium via the apoplastic pathway. *J.*
915 *Hazard. Mater.* 417, 125955.

916

917 Lizzi D., Mattiello A., Piani B., Fellet G., Adamiano A., Marchiol L. 2020. Germination and early
918 development of three spontaneous plant species exposed to nanoceria (nCeO₂) with different
919 concentrations and particle sizes. *Nanomaterials.* 10, 1–16.

920

921 Lopez-Lima D., Mtz-Enriquez A.I., Carrión G., Basurto-Cereceda S., Pariona N. 2021. The
922 bifunctional role of copper nanoparticles in tomato: Effective treatment for Fusarium wilt and plant
923 growth promoter. *Sci. Hortic.* 2, 277.

924

925 López-Moreno M.L., de la Rosa G., Hernández-Viezcas J.A., Castillo-Michel H., Botez C.E., Peralta-
926 Videá J.R., Gardea-Torresdey J.L. 2010. Evidence of the Differential Biotransformation and
927 Genotoxicity of ZnO and CeO₂ Nanoparticles on Soybean (*Glycine max*) Plants. *Environ. Sci.*
928 *Technol.* 44, 19, 7315–7320.

929

930 Lowry G.V., Avellan A., Gilbertson L.M. 2019. Opportunities and challenges for nanotechnology in
931 the agri-tech revolution. *Nat. Nanotechnol.* 14, 517–522.
932

933 Lv J., Zhang S., Luo L., Zhang J., Yang K., Christie P. 2015. Accumulation, speciation and uptake
934 pathway of ZnO nanoparticles in maize. *Environ. Sci.: Nano.* 2, 68.
935

936 Lv J., Christie P., Zhang S. 2019. Uptake, translocation, and transformation of metal-based
937 nanoparticles in plants: recent advances and methodological challenges. *Environ. Sci.: Nano.* 6, 41-
938 59.
939

940 Lv Z., Sun H., Du W., Li R., Mao H., Kopittke P.M. 2021. Interaction of different-sized ZnO
941 nanoparticles with maize (*Zea mays*): Accumulation, biotransformation and phytotoxicity. *Sci Tot*
942 *Environ.* 796, 148927.
943

944 Ma C., White J.C., Zhao J., Zhao Q., Xing B. 2018. Uptake of Engineered Nanoparticles by Food
945 Crops: Characterization, Mechanisms, and Implications. *Annu. Rev. Food Sci. Technol.* 9, 129–153.
946

947 Ma C., Borgatta J., Hudson B.G., Tamijani A.A., De La Torre-Roche R., Zuverza-Mena N., Shen Y.,
948 Elmer W., Xing B., Mason S. E., Hamers R. J., White J.C. 2020. Advanced material modulation of
949 nutritional and phytohormone status alleviates damage from soybean sudden death syndrome. *Nat.*
950 *Nanotechnol.* 15, 1033–1042.
951

952 Ma Y., Zhang P., Zhang Z., He H., Li Y., Zhang J., Zheng L., Chu S., Yang K., Zhao Y., Chai Z.
953 2015. Origin of the different phytotoxicity and biotransformation of cerium and lanthanum oxide
954 nanoparticles in cucumber. *Nanotoxicology.* 9(2), 262–270.
955

956 Majumdar S., Almeida I.C., Arigi E.A., Choi H., VerBerkmoes N.C., Trujillo-Reyes J., Flores-
957 Margez J.P., White J.C., Peralta-Videa J.R., Gardea-Torresdey J.L. 2015. Environmental Effects of
958 Nanoceria on Seed Production of Common Bean (*Phaseolus vulgaris*): A Proteomic Analysis.
959 Environ. Sci. Technol. 49, 22, 13283–13293.

960

961 Majumdar S., Pagano L., Wohlschlegel J.A., Villani M., Zappettini A., White J.C., Keller A.A. 2019.
962 Proteomic, Gene And Metabolite Characterization Reveal The Uptake And Toxicity Mechanisms Of
963 Cadmium Sulfide Quantum Dots In Soybean Plants. Environ. Sci.: Nano. 6, 3010–3026.

964

965 Manzoor N., Ahmed T., Noman M., Shahid M., Mudassir M., Ali L., Alnusaire T.S., Li B., Schulin
966 R., Wang G. 2021. Science of the Total Environment Iron oxide nanoparticles ameliorated the
967 cadmium and salinity stresses in wheat plants, facilitating photosynthetic pigments and restricting
968 cadmium uptake. Sci. Total Environ. 769, 145221.

969

970 Marmiroli M., Pagano L., Savo Sardaro M.L., Villani M., Marmiroli N. 2014. Genome-wide
971 approach in *Arabidopsis thaliana* to assess the toxicity of cadmium sulfide quantum dots. Environ.
972 Sci. Technol. Environ. Sci. Technol. 48, 5902–5909.

973

974 Marmiroli M., Imperiale D., Pagano L., Villani M., Zappettini A., Marmiroli N. 2015. The Proteomic
975 Response of *Arabidopsis thaliana* to Quantum Dots, and Its Correlation with the Transcriptomic
976 Response. Front. Plant Sci. 6, 1104.

977

978 Marmiroli M., Lepore G.O., Pagano L., d'Acapito F., Gianoncelli A., Villani M., Lazzarini L., White
979 J.C., Marmiroli N. 2020. The fate of CdS Quantum Dots in plants as revealed by Extended X-ray
980 Absorption Fine Structure (EXAFS) analysis. Environ. Sci. Nano 7, 1150–1162.

981

982 Marmiroli M., Pagano L., Rossi R., De La Torre-Roche R., Lepore G.O., Ruotolo R., Gariani G.,
983 Bonanni V., Pollastri S., Puri A., Gianoncelli A., Aquilanti G., d'Acapito F., White J.C., Marmiroli
984 N. 2021. Copper Oxide nanomaterial fate in plant tissue: Nanoscale impacts on reproductive tissues.
985 Environ Sci Technol. 55, 15, 10769–10783.

986
987 Mattiello A., Filippi A., Poscic F., Musetti R., Salvatici M.C., Giordano C., Vischi M., Bertolini A.,
988 Marchiol L. 2015. Evidence of Phytotoxicity and Genotoxicity in *Hordeum vulgare* L. Exposed to
989 CeO₂ and TiO₂ Nanoparticles. Front. Plant Sci. 6, 1043.

990
991 Milosevic A., Romeo D., Wick P. 2020. Understanding Nanomaterial Biotransformation: An Unmet
992 Challenge to Achieving Predictive Nanotoxicology. Small. 1907650.

993
994 [Mitrano D.M., Motellier S., Clavaguera S., Nowack B., 2015. Review of nanomaterial aging and](#)
995 [transformations through the life cycle of nano-enhanced products, Environment International, 77,](#)
996 [132-147.](#)

997
998 Morrissey J., Guerinot M.L. 2009. Iron uptake and transport in plants: the good, the bad, and the
999 ionome. Chem. Rev. 109 (10), 4553–67.

1000
1001 Mustafa H., Ilyas N., Akhtar N., Iqbal N., Zainab T. 2021. Biosynthesis and characterization of
1002 titanium dioxide nanoparticles and its effects along with calcium phosphate on physicochemical
1003 attributes of wheat under drought stress. Ecotoxicol. Environ. Saf. 223, 112519.

1004
1005 Nasir Khan M. 2016. Nano-titanium Dioxide (Nano-TiO₂) Mitigates NaCl Stress by Enhancing
1006 Antioxidative Enzymes and Accumulation of Compatible Solutes in Tomato (*Lycopersicon*
1007 *esculentum* Mill.). J. Plant Sciences. 11, 1-11.

Formatted: English (United States)

1008

1009 Neves V.M., Heidrich G.M., Rodrigues E.S., Enders M.S.P., Muller E.I., Nicoloso F.T., Carvalho
1010 H.W.P. De, Dressler V.L. 2019. La₂O₃ Nanoparticles: Study of Uptake and Distribution in *Pfaffia*
1011 *glomerata* (Spreng.) Pedersen by LA-ICP-MS and μ -XRF. Environ. Sci. Technol. 53, 10827–10834.

1012

1013 Oh E., Liu R., Nel A, Boeneman Gemill K., Bilal M., Cohen Y., Medintz I.L. 2016. Meta-analysis of
1014 cellular toxicity for cadmium-containing quantum dots. Nature Nanotech. 11, 479–486.

1015

1016 Pagano L., Servin A.D., De La Torre-Roche R., Mukherjee A., Majumdar S., Hawthorne J.,
1017 Marmiroli M., Maestri E., Marra R.E., Isch S.M., Dhankher O.P., White J. C., Marmiroli N. 2016.
1018 Molecular Response of Crop Plants to Engineered Nanomaterials. Environ. Sci. Technol. 50 (13),
1019 7198–7207.

1020

1021 Pagano L., Pasquali F., Majumdar S., De La Torre-Roche R., Zuverza-Mena N., Villani M.,
1022 Zappettini A., Marra R.E., Isch S.M., Marmiroli M., Maestri E., Dhankher O.P., White J.C.,
1023 Marmiroli N. 2017. Exposure of *Cucurbita pepo* to binary combinations of engineered nanomaterials:
1024 Physiological and molecular response. Environ. Sci.: Nano. 4, 1579–1590.

1025

1026 Pagano L., Maestri E., White J.C., Marmiroli N., Marmiroli M. 2018. Quantum dots exposure in
1027 plants: Minimizing the adverse response. COESH. 6, 71–76.

1028

1029 Pagano L., Rossi R., Paesano L., Marmiroli N., Marmiroli M. 2021. miRNA regulation and stress
1030 adaptation in plants. Env. Exp. Bot. 104369.

1031

1032 Pagano L., Marmioli M., Villani M., Magnani J., Rossi R., Zappettini A., White J.C., Marmioli N.
1033 2022. Engineered nanomaterial exposure affects organelle genetic material replication in *Arabidopsis*
1034 *thaliana*. ACS Nano. 16, 2249–2260.

1035

1036 Prado de Moraes A.C., Ribeiro L. da S., de Camargo E.R., Lacava P.T. 2021. The potential of
1037 nanomaterials associated with plant growth-promoting bacteria in agriculture. 3 Biotech. 11, 1–17.

1038

1039 Proux O., Lahera E., Del Net W., Kieffer I., Rovezzi M., Testemale D., Irar M., Thomas S., Aguilar-
1040 Tapia A., Bazarkina E.F., Prat A., Tella M., Auffan M., Rose J., Hazemann J.L. 2017. High-Energy
1041 Resolution Fluorescence Detected X-Ray Absorption Spectroscopy: A Powerful New Structural Tool
1042 in Environmental Biogeochemistry Sciences. J Environ Qual. 46, 1146-1157.

1043

1044 Puri A., Lepore G.O., d’Acapito F. 2019. The New Beamline LISA at ESRF: Performances and
1045 Perspectives for Earth and Environmental Sciences. Condensed Matter. 4, 12.

1046

1047 [Rawat S., Pullagurala V.L.R., Adisa I.O., Wang Y., Peralta-Videa J.R., Gardea-Torresdey J.L. 2018.](#)
1048 [Factors affecting fate and transport of engineered nanomaterials in terrestrial environments, COESH,](#)
1049 [6, 47-53.](#)

1050

1051 [Rincon A.M. 2019. Chapter 6 - Presence of nanomaterials on consumer products: food, cosmetics,](#)
1052 [and drugs. pp. 165-181. Marmioli N., White J.C., Song J., Eds: In Micro and Nano Technologies,](#)
1053 [Exposure to Engineered Nanomaterials in the Environment, Elsevier, Amsterdam, The Netherlands.](#)

1054

1055 Rizwan M., Ali S., Ali B., Adrees M., Arshad M., Hussain A., Zia ur Rehman M., Waris A.A. 2019.
1056 Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and
1057 cadmium concentration in wheat. Chemosphere. 214, 269–277.

1058

1059 Rodrigues E.S., Montanha G.S., de Almeida E., Fantucci H., Santos R.M., de Carvalho H.W.P. 2021.
1060 Effect of nano cerium oxide on soybean (*Glycine max* L. Merrill) crop exposed to environmentally
1061 relevant concentrations. *Chemosphere*. 273, 128492.

1062

1063 Rossi L., Zhang W., Schwab A.P., Ma X. 2017. Uptake, Accumulation, and in Planta Distribution of
1064 Coexisting Cerium Oxide Nanoparticles and Cadmium in *Glycine max* (L.) Merr. *Environ. Sci.*
1065 *Technol.* 51, 12815–12824.

1066

1067 Roubeau Dumont E., Elger A., Azéma C., Castillo H., Surble S., Larue C. 2022. Cutting-edge
1068 spectroscopy techniques highlight toxicity mechanisms of copper oxide nanoparticles in the aquatic
1069 plant *Myriophyllum spicatum*. *Sci. Total Environ.* 803, 150001.

1070

1071 Rui Y., Zhang P., Zhang T., Ma Y., He X., Gui X., Li Y., Zhang J., Zheng L., Chu S., Guo Z., Chai
1072 Z., Zhao Y., Zhang Z. 2015. Transformation of ceria nanoparticles in cucumber plants is influenced
1073 by phosphate, *Environmental Pollution*. 198, 8-14.

1074

1075 Ruotolo R., Maestri E., Pagano L., Marmioli M., White J.C., Marmioli N. 2018. Plant response to
1076 metal-containing engineered nanomaterials: an omics-based perspective. *Environ Sci Technol.* 52, 5,
1077 2451-2467.

1078

1079 Sabo-Attwood T., Unrine J.M., Stone J.W., Murphy C.J., Ghoshroy S., Blom D., Bertsch P.M.,
1080 Newman L.A. 2011. Uptake, distribution and toxicity of gold nanoparticles in tobacco (*Nicotiana*
1081 *xanthi*) seedlings. *Nanotoxicology*. 6(4), 353-360.

1082

1083 Sarret G., Pilon Smits E.A.H., Castillo Michel H., Isaure M.P., Zhao F.J., Tappero R. 2013. Chapter
1084 One - Use of Synchrotron-Based Techniques to Elucidate Metal Uptake and Metabolism in Plants,
1085 Adv. Agron. 119, 1–82.
1086
1087 Schwab F., Zhai G., Kern M., Turner A., Schnoor J.L., Wiesner M.R. 2016. Barriers, pathways and
1088 processes for uptake, translocation and accumulation of nanomaterials in plants – Critical review.
1089 Nanotoxicology. 10, 257–278.
1090
1091 Semida W.M., Abdelkhalik A., Mohamed G.F., Abd El-Mageed T.A., Abd El-Mageed S.A., Rady
1092 M.M., Ali E.F. 2021. Foliar application of zinc oxide nanoparticles promotes drought stress tolerance
1093 in eggplant (*Solanum melongena* L.). Plants. 10, 1–18.
1094
1095 Servin A.D., Castillo-Michel H., Hernandez-Viezcas J.A., Corral Diaz B., Peralta-Videa J.R.,
1096 Gardea-Torresdey J.L. 2012. Synchrotron Micro-XRF and Micro-XANES Confirmation of the
1097 Uptake and Translocation of TiO₂ Nanoparticles in Cucumber (*Cucumis sativus*) Plants. Environ.
1098 Sci. Technol. 46, 14, 7637–7643.
1099
1100 Servin A.D., Morales M.I., Castillo-Michel H., Hernandez-Viezcas J.A., Munoz B., Zhao L., Nunez
1101 J.E., Peralta-Videa J.R., Gardea-Torresdey J.L. 2013. Synchrotron Verification of TiO₂
1102 Accumulation in Cucumber Fruit: A Possible Pathway of TiO₂ Nanoparticle Transfer from Soil into
1103 the Food Chain. Environ. Sci. Technol. 47, 20, 11592–11598.
1104
1105 Servin A.D., Pagano L., Castillo-Michel H., De la Torre-Roche R., Hawthorne J., Hernandez-Viezcas
1106 J.A., Loredó-Portales R., Majumdar S., Gardea-Torresdey J.L., Dhankher O.P., White J.C. 2017a.
1107 Weathering in soil increases nanoparticle CuO bioaccumulation within a terrestrial food chain.
1108 Nanotoxicology. 11, 98–111.

1109

1110 Servin A.D., De la Torre-Roche R., Castillo-Michel H., Pagano L., Hawthorne J., Musante C.,
1111 Pignatello J., Uchimiya M., White J.C. 2017b. Exposure of agricultural crops to nanoparticle CeO₂
1112 in biochar-amended soil. *Plant Physiology and Biochemistry*. 110, 147-157.

1113

1114 Siddiqi K.S., Husen A. 2017. Engineered Gold Nanoparticles and Plant Adaptation Potential.
1115 *Nanoscale Res Lett*. 11, 400-409.

1116

1117 Song U., Jun H., Waldman B., Roh J., Kim Y., Yi J., Lee E.J. 2013. Functional analyses of
1118 nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon*
1119 *esculentum*). *Ecotoxicol. Environ. Saf.*, 93, 60-67.

1120

1121 Stegemeier J.P., Schwab F., Colman B.P., Webb S.M., Newville M., Lanzirotti A., Winkler C.,
1122 Wiesner M.R., Lowry G.V. 2015. Speciation Matters: Bioavailability of Silver and Silver Sulfide
1123 Nanoparticles to Alfalfa (*Medicago sativa*). *Environ Sci Technol*. 49 (14), 8451-8460.

1124

1125 Syu Y., Hung J.H., Chen J.C., Chuang H. 2014. Impacts of size and shape of silver nanoparticles on
1126 Arabidopsis plant growth and gene expression. *Plant Physiol. Biochem.* 83, 57-64.

1127

1128 Tamez C., Hernandez-Molina M., Hernandez-Viezcas J.A., Gardea-Torresdey J.L. 2019. Uptake,
1129 transport, and effects of nano-copper exposure in zucchini (*Cucurbita pepo*). *Sci. Total Environ*. 665,
1130 100-106.

1131

1132 Verma K.K., Song X.-P., Joshi A., Tian D.-D., Rajput V.D., Singh M., Arora J., Minkina T., Li Y.-
1133 R. 2022. Recent Trends in Nano-Fertilizers for Sustainable Agriculture under Climate Change for
1134 Global Food Security. *Nanomaterials*. 12, 173.

Formatted: Font: Italic

Formatted: English (United States)

1135

1136 Wang J., Koo Y., Alexander A., Yang Y., Westerhof S., Zhang Q., Schnoor J.L., Colvin V.L., Braam
1137 J., Alvarez P.J.J. 2013. Phytostimulation of poplars and Arabidopsis exposed to silver nanoparticles
1138 and Ag⁺ at sublethal concentrations. *Environ. Sci. Technol.* 47, 5442–5449.

1139

1140 Wang Y., Lin Y., Xu Y., Yin Y., Guo H., Du W. 2019. Divergence in response of lettuce (var. ramosa
1141 Hort.) to copper oxide nanoparticles/microparticles as potential agricultural fertilizer. *Environ. Pollut.*
1142 *Bioavail.* 31, 80–84.

1143

1144 [Wang Y., Deng C., Cota-Ruiz K., Tan W., Reyes A., Peralta-Videa J.R., Hernandez-Viezcas J.A., Li
1145 C., Gardea-Torresdey J.L. 2021a. Effects of different surface-coated nTiO₂ on full-grown carrot
1146 plants: Impacts on root splitting, essential elements, and Ti uptake. *J Haz Mater.* 402, 123768.](#)

1147

1148 [Wang Y., Deng C., Cota-Ruiz K., Peralta-Videa J.R., Hernandez-Viezcas J.A., Gardea-Torresdey
1149 J.L. 2021b. Soil-aged nano titanium dioxide effects on full-grown carrot: Dose and surface-coating
1150 dependent improvements on growth and nutrient quality. *Scie Tot Environ.* 774, 145699.](#)

1151

1152 [Wang Y., Deng C., Elmer W.H., Dimkpa C.O., Sharma S., Navarro G., Wang Z., LaReau J., Steven
1153 B.T., Wang Z., Zhao L., Li C., Parkash Dhankher O., Gardea-Torresdey J.L., Xing B., White J.C.
1154 2022. Therapeutic Delivery of Nanoscale Sulfur to Suppress Disease in Tomatoes: In Vitro Imaging
1155 and Orthogonal Mechanistic Investigation. *ACS Nano.* 16, 7, 11204–11217.](#)

1156

1157 [White J.C., Zuverza-Mena N., Elmer W.H. 2022. From nanotoxicology to nano-enabled agriculture:
1158 Following the science at the Connecticut Agricultural Experiment Station \(CAES\). *Plant Nano
1159 Biology.* 1, 100007. Doi: 10.1016/j.plana.2022.100007.](#)

1160

Formatted: English (United States)

1161 Willett W., Rockström J., Loken B., Springmann M., Lang T., Vermeulen S., Garnett T., Tilman D.,
1162 DeClerck F., Wood A., Jonell M., Clark M., Gordon L.J., Fanzo J., Hawkes C., Zurayk R., Rivera
1163 J.A., De Vries W., Sibanda L.M., Afshin A., Chaudhary A., Herrero M., Agustina R., Branca F.,
1164 Lartey A., Fan S., Crona B., Fox E., Bignet V., Troell M., Lindahl T., Singh S., Cornell S.E., Reddy
1165 K.S., Narain S., Nishtar S., Murray C.J.L. 2019. Food in the Anthropocene: the EAT–Lancet
1166 Commission on healthy diets from sustainable food systems. *Lancet*. 393, 447–492.
1167
1168 Xiao Z., Yue L., Wang C. Chen F., Ding Y., Liu Y., Cao X., Chen Z., Rasmann S., Wang Z. 2021.
1169 Downregulation of the photosynthetic machinery and carbon storage signaling pathways mediate
1170 La₂O₃ nanoparticle toxicity on radish taproot formation. *J. Hazard. Mater.* 411, 124971.
1171
1172 Xiong Z., Zhang T., Xian Y., Kang Z., Zhang S., Dumat C., Shahid M., Li S. 2021. Foliar uptake,
1173 biotransformation, and impact of CuO nanoparticles in *Lactuca sativa* var. *ramosa* Hort. *Environ.*
1174 *Geochem. Health.* 43(1), 423-439.
1175
1176 [Xu T., Wang Y., Aytac Z., Zuverza-Mena N., Zhao Z., Hu X., Ng K.W., White J.C., Demokritou P.](#)
1177 [2022. Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli](#)
1178 [Responsive Core–Shell Nanostructures. *ACS Nano* 16, 4, 6034–6048.](#)
1179
1180 Yang J., Jiang F., Ma C., Rui Y., Rui M., Adeel M., Cao W., Xing B. 2018. Alteration of Crop Yield
1181 and Quality of Wheat upon Exposure to Silver Nanoparticles in a Life Cycle Study. *J. Agric. Food*
1182 *Chem.* 66, 2589–2597.
1183
1184 Yin L., Cheng Y., Espinasse B., Colman B.P., Au M., Wiesner M., Rose J., Liu J., Bernhardt E.S.
1185 2011. More than the Ions: The Effects of Silver Nanoparticles on *Lolium multiflorum*. *Environ. Sci.*
1186 *Technol.* 45, 2360–2367.

Formatted: Italian (Italy)

1187

1188 Yue L., Chen F., Yu K., Xiao Z., Yu X., Wang Z., Xing B. 2019. Early development of apoplastic
1189 barriers and molecular mechanisms in juvenile maize roots in response to La_2O_3 nanoparticles. *Sci.*
1190 *Total Environ.* 653, 675–683.

1191

1192 Zafar S, Perveen S, Kamran Khan M, Shaheen MR, Hussain R, Sarwar N, Rashid S., Nafees M.,
1193 Farid G., Alamri S., Shah A.A., Javed T., Irfan M., Siddiqui M.H. 2022. Effect of zinc nanoparticles
1194 seed priming and foliar application on the growth and physio-biochemical indices of spinach
1195 (*Spinacia oleracea* L.) under salt stress. *PLoS ONE*, 17(2), e0263194.

1196

1197 Zhong Z., Zhu L., Young S. 2020. Approximation Framework of Embodied Energy of Safety:
1198 Insights and Analysis. *Energies*. 13, 4230.

1199

1200 Zulfiqar F., Navarro M., Ashraf M., Akram N.A., Munné-Bosch S. 2019. Nanofertilizer Use For
1201 Sustainable Agriculture: Advantages And Limitations. *Plant Science*. 289, 110270.

1202

1203 Zuverza-Mena N., Armendariz R., Peralta-Videa J.R., Gardea-Torresdey J.L. 2016. Effects of silver
1204 nanoparticles on radish sprouts: Root growth reduction and modifications in the nutritional value.
1205 *Front. Plant Sci.* 7, 1–11.

1206

1207 Zuverza-Mena N., Martínez-Fernandez D., Du W., Hernandez-Viezcás J.A., Bonilla-Bird N., Lopez-
1208 Moreno M.L., Komarek M., Peralta-Videa J.R., Gardea-Torresdey J.L. 2017. Exposure of engineered
1209 nanomaterials to plants: insights into the physiological and biochemical responses-A review. *Plant*
1210 *Physiol. Biochem.* 110, 236-264.

1211

1212

1213

1214

1215 **Figure captions and Tables**

1216

1217

1218 Figure 1. Schematic representation of principal effects emerging after biotransformation of ENMs in
1219 soil, on foliar surface and within the plant tissues. ENMs in soil, due to particle and soil physico-
1220 chemical interactions, may remain unmodified, or may undergo to potential dissolution (with ions
1221 release), or undergo homo-/hetero-aggregation, which may highly influence particle stability and
1222 potential bioavailability (and consequently their uptake). On the foliar surface, temperature and light
1223 may also affect particle stability before uptake into the leaf tissues. Once within the plant, ENMs
1224 pristine, or modified, can interact with several biomolecule types (peptides, sugars, lipids, nucleic
1225 acids, secondary metabolites) leading to phenomena such as coating, enzymatic degradation,
1226 chelation or functionalization, which may influence the biotransformed particle at level of
1227 translocation, storage or reactivity. These parameters may also influence the interaction within the
1228 plant cell, triggering differential responses (e.g., toxicity, oxidative stress, ROS production), which
1229 may be indirectly measured by physiological and molecular assays, but directly observed through
1230 physical strategies, including synchrotron-based methods.

1231

1232 Figure 24. Principal mechanisms effects of ENM biotransformation in plant and relevant biomarkers
1233 observed in different plant species from model organisms (*A. thaliana*) to crops (*C. pepo*; *S.*
1234 *lycopersicum*; *G. max*) and different tissues (roots, leaves and flowers/pollen). Relevant ENM
1235 parameters such as size, stability, dissolution may influence the translocation from roots to shoots.
1236 Potential biotransformation mechanisms that may occur within plant tissues are also reported:
1237 enzymatic degradation, protein functionalization, functionalization at the level of cytoplasm and
1238 organelles (organic acids, thiol-containing compounds, aminoacids, sugars, secondary metabolites).

1239 In this scenario, chloroplast become not only a in important actor in the energy production but also
 1240 one of the key targets and main regulators involved in the ENM exposure and response. Details on
 1241 the biomarkers generated are reported in Table 1.

1242
 1243
 1244
 1245
 1246
 1247
 1248
 1249
 1250
 1251

1252 Table 1. Genes as potential biomarkers of exposure/effect observed in roots, leaves and pollen, in
 1253 different plant species (reported in Figure 2). Genes reported cannot be considered only as modulated
 1254 in the different plant organs by the different type of ENMs, but also they showed a nano-specificity
 1255 during the ENM response. It is also important to observe how, depending on the ENM type,
 1256 biotransformation, and as indirect consequence, the transcriptomic response can be convergent
 1257 between different forms (nano, bulk, ion) of the same element (see Figure 2).~~Table 1. Gene list of~~
 1258 ~~potential biomarkers observed in roots, leaves and pollen, in different plant species (reported in Figure~~
 1259 ~~1).~~

flower & pollen					
plant	gene	function	pathway	ENM	reference
<i>Cucurbita pepo L.</i>	Cp4.1LG00g07430	Beta-galactosidase	primary metabolism		
	Cp4.1LG13g06380	Pectinesterase	primary metabolism		
	Cp4.1LG12g04750	Phosphatidylinositol 3-/4-kinase family protein	primary metabolism	nCuO	Marmioli <i>et al.</i> , 2021
	Cp4.1LG03g02560	Delta-1-pyrroline-5-carboxylate synthetase	primary metabolism		
	Cp4.1LG02g07240	Leucine-rich repeat family protein	signaling, stress response		

Cp4.1LG02g12750	Protein EFR3 like	signaling
-----------------	-------------------	-----------

leaf					
plant	gene	function	pathway	ENM	reference
<i>Cucurbita pepo</i> L.	Cp4.1LG01(*)	PSBN, photosystem II reaction center protein N	chloroplast electron transport	nCeO2 nLa2O3	Pagano <i>et al.</i> , 2016 Pagano <i>et al.</i> , 2017
	Cp4.1LG00(*)	PetL, component of Cytochrome b6f	chloroplast electron transport	nCuO nZnO CdS QD	
<i>Solanum lycopersicum</i> L.	Solyc09g074540	PetL, component of Cytochrome b6f	chloroplast electron transport	nCeO2 nLa2O3 nCuO	Pagano <i>et al.</i> , 2016
<i>Glicine max</i> L.	Glyma12g36130	PetL, component of Cytochrome b6f	chloroplast electron transport	CdS QD	Majumdar <i>et al.</i> , 2019
<i>Arabidopsi thaliana</i> L.	AtCg00590	PetL, component of Cytochrome b6f	chloroplast electron transport	CdS QD	Marmioli <i>et al.</i> , 2014
	AtCg00270	PSBN, photosystem II reaction center protein N	chloroplast electron transport		

root					
plant	gene	function	pathway	ENM	reference
<i>Cucurbita pepo</i> L.	Cp4.1LG16g08630	BIP3, Heat shock protein 70 family protein	protein folding, stress response	nCeO2 nLa2O3 nCuO nZnO CdS QD	Pagano <i>et al.</i> , 2016 Pagano <i>et al.</i> , 2017
	Cp4.1LG00g00090	GPT2, glucose-6-phosphate/phosphate translocator	primary metabolism		
	Cp4.1LG05g08050	RPS12, ribosomal protein S12A	protein synthesis		
	Cp4.1LG05g11200	PLP2, phospholipase	biotic/abiotic stress response		
<i>Solanum lycopersicum</i> L.	Solyc08g006150	ChaC-like family protein	glutathione degradation	nCeO2 nLa2O3 nCuO	Pagano <i>et al.</i> , 2016
	Solyc03g081240	PRR5, pseudo-response regulator 5	biotic/abiotic stress response		
	Solyc10g005080	LHY1, Homeodomain-like superfamily protein	stress response		
<i>Glicine max</i> L.	Glyma19g45030	LHY1, Homeodomain-like superfamily protein	stress response	CdS QD	Majumdar <i>et al.</i> , 2019
	Glyma15g06800	PR1, pathogenesis-related gene 1	biotic/abiotic stress response		

1260
1261
1262
1263
1264
1265
1266
1267

Table 2. Principal evidence of the ENM biotransformation in plant observed by physiological, molecular and synchrotron-based analyses.

ENM	plant	physiological response	molecular response	techniques	biotransformation	reference
-----	-------	------------------------	--------------------	------------	-------------------	-----------

nCeO2	<i>Cucumis sativus L.</i>	nCeO2 exposure had no significant effects on the biomass production under both the +P and -P conditions. However, the uptake of Ce in the plants is different under the two conditions	-	TEM XANES NEXAFS	high stability	<i>Rui et al., 2015</i>
	<i>Lactuca sativa L.</i> <i>Cucurbita pepo L.</i> <i>Zea mays L.</i> <i>Glycine max L.</i>	biomass in the agricultural soil amended with biochar 600°C was largely unaffected	-	SEM μ-XRF μ-XANES	modified redox state, from Ce(IV) to Ce(III) low translocation from roots to shoots	<i>Servin et al., 2017</i>
nCeO2 nZnO	<i>Glycine max L.</i>	-	-	μ-XRF μ-XANES		<i>Hernandez-Viezcas et al., 2013</i>
nCeO2 nLa2O3	<i>Cucumis sativus L.</i>	nCeO2 had no phytotoxicity to cucumber at all tested concentrations, while nLa2O3 showed significant inhibition on root elongation, shoot elongation, root biomass, and shoot biomass, as well as induced more reactive oxygen species and cell death in roots	-	μ-XRF XAS	higher dissolution compared to nCeO2 moderate translocation from roots to shoots	<i>Ma et al., 2014</i>
nTiO2	<i>Cucumis sativus L.</i>	at all concentrations, nTiO2 significantly increased root length (average >300%)	-	μ-XRF μ-XANES	high stability	<i>Servin et al., 2012</i>
	<i>Cucumis sativus L.</i>	In nTiO2-treated plants, the chlorophyll content in leaves increased as the external concentration of NPs increased. nTiO2 treatments increased CAT activity in leaves.	-	μ-XRF μ-XANES FTIR	low translocation from roots to shoots	<i>Servin et al., 2013</i>
nAu	<i>Nicotiana tabacum L.</i>	leaf necrosis was observed after 14 days of exposure to 3.5 nm nAu	-	μ-XRF	high stability no changes in Au valence	<i>Sabo-Attwood et al., 2012</i>
nAg	<i>Lolium multiflorum L.</i>	nAg and ionic silver significantly reduced growth, resulting in shorter shoots and roots and lower biomass. The growth inhibition from nAg was stronger than that from AgNO3. Higher concentrations of AgNPs caused broken epidermis and rootcap. Cell structures were unaltered in AgNO3 treated roots.	-	μ-XRF XANES	low stability high translocation from roots to shoots	<i>Yin et al., 2011</i>
	<i>Lactuca sativa L.</i> <i>Cucurbita pepo L.</i> <i>Zea mays L.</i> <i>Glycine max L.</i>	fresh foliar biomass was unchanged. Chlorophyll a, chlorophyll b, carotenoid and pheophytin contents were not affect	-	SEM μ-XRF XANES		<i>Larue et al., 2013</i>
	<i>Medicago sativa L.</i>	-	-	TEM XRF		<i>Stegemeier et al., 2015</i>
nZVI	<i>Cucumis sativus L.</i>	nZVI treatments did not affect the biomass of plants in hydroponic or soil systems. Only nZVI treated plant shoots grown under hydroponic conditions exhibited increased biomass (15%). Chlorosis observed in the leaves of the control plants but not in the plants treated with nZVI	ATPase isoforms increased their expression in the roots of plant exposed to nZVI.	EXAFS	low stability limited translocation from roots to shoots modified particle structure	<i>Dwivedi et al., 2018</i>

Table 2 continue in the next page...

ENM	plant	physiological response	molecular response	techniques	biotransformation	reference
nZnO	<i>Zea Mays L.</i>	-	-	μ-XRF XANES	low stability	<i>Ly et al., 2015</i>

1268
1269
1270

	<i>Zea Mays L.</i>	By the 7th day, the treatment of 9 nm nZnO and ZnSO ₄ significantly reduced the dry weight of roots by 44% and 58% respectively, compared to the unexposed control plants. In general, ZnSO ₄ treatment had the greatest effect on root biomass, followed by 9 nm nZnO and finally 40 nm nZnO	-	μ-XRF	high translocation from roots to shoots	<i>Ly et al., 2021</i>
nCuO	<i>Nicotiana tabacum L.</i>	When exposed to equivalent weight of Cu, nCu ₂ O exhibited higher toxicity than nCuO, implying that the transformation may elevate the toxicity upon nCuO exposure	-	TEM XANES		<i>Dai et al., 2019</i>
	<i>Lactuca sativa L.</i>	Cu exposure had limited impacts on lettuce biomass. For the unweathered exposures, only the root biomass of NP-exposed plants was less than in bulk treatment; no other tissue-specific differences were evident. In the W exposure, the total biomass ranged from 8.2 g (W NP) to 9.5 g (unexposed control); nCuO and ion-treated plant biomass was significantly less than the unexposed controls. With regard to individual tissues in the W exposure, there were no differences of significance in the root biomass.	The expression level of nine genes involved in Cu transport shows that the mechanisms of nCuO and bulk CuO response-accumulation are different from ionic Cu	μ-XRF XANES	low stability high translocation from roots to shoots consistent with an increased ion release modified redox state, from Cu(II) to Cu (I)	<i>Servin et al., 2017</i>
	<i>Cucurbita pepo L.</i>	no impact on zucchini biomass, photosynthetic activity or cellular respiration.	RNA-seq analyses on vegetative and reproductive tissues highlighted common and nanoscale-specific components of the response. Mitochondrial and chloroplast functions were uniquely modulated in response to ENM exposure as compared with bulk and salt forms	μ-XRF XANES EXAFS		<i>Marmioli et al., 2021</i>
CdS QD	<i>Arabidopsis thaliana L.</i>	treatment with CdS QDs caused a slight stress that increased the biomass in the mutants, but not in the wt, while CdSO ₄ caused modest phytotoxicity to both the wt and mutants	-	EXAFS	high stability limited ion release high translocation modification in bonds distance	<i>Marmioli et al., 2020</i>

1 **Nanomaterials biotransformation: In planta mechanisms of action**

2

3 Luca Pagano,¹ Riccardo Rossi,^{1,2} Jason C. White,³ Nelson Marmiroli,^{1,4} Marta Marmiroli^{1,5*}

4

5 ¹, Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma,
6 43124 Parma, Italy.

7 ², Centro Interdipartimentale per l'Energia e l'Ambiente (CIDEA), University of Parma, 43124 Parma,
8 Italy.

9 ³, The Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504, United States.

10 ⁴, Consorzio Interuniversitario Nazionale per le Scienze Ambientali (CINSA), University of Parma,
11 43124 Parma, Italy.

12 ⁵, Interdepartmental Centre for Food Safety, Technologies and Innovation for Agri-food
13 (SITEIA.PARMA), 43124 Parma, Italy.

14 *, corresponding author: marta.marmiroli@unipr.it.

15

16 **Abstract**

17 Research on engineered nanomaterials (ENMs) exposure has continued to expand rapidly, with a
18 focus on uncovering the underlying mechanisms. The EU largely limits the number and the type of
19 organisms that can be used for experimental testing through the 3R normative. There are different
20 routes through which ENMs can enter the soil-plant system: this includes the agricultural application
21 of sewage sludges, and the distribution of nano-enabled agrochemicals. However, a thorough
22 understanding of the physiological and molecular implications of ENMs dispersion and chronic low-
23 dose exposure remains elusive, thus requiring new evidence and a more mechanistic overview of
24 pathways and major effectors involved in plants. Plants can offer a reliable alternative to conventional
25 model systems to elucidate the concept of ENM biotransformation within tissues and organs, as a
26 crucial step in understanding the mechanisms of ENM-organism interaction. To facilitate the

27 understanding of the physico-chemical forms involved in plant response, synchrotron-based
28 techniques have added new potential perspectives in studying the interactions between ENMs and
29 biota. These techniques are providing new insights on the interactions between ENMs and
30 biomolecules. The present review discusses the principal outcomes for ENMs after intake by plants,
31 including possible routes of biotransformation which make their final fate less uncertain, and
32 therefore require further investigation.

33

34 **Keywords:** biotransformation, plant, nanomaterials, synchrotron-based analyses, molecular response

35

36 **Highlights**

- 37 • Biotransformation is a fundamental phenomenon for understanding ENM-organism response
- 38 mechanisms
- 39 • Synchrotron-based methodological analyses are critical for investigating ENM
- 40 biotransformation
- 41 • Biotransformation of ENMs may have positive or negative effects when considering the agri-
- 42 food application

43

44

45 **1. Engineered nanomaterial (ENM) biotransformation**

46 *1.1. ENMs: from exposure to biotransformation*

47 Although global food production has generally increased over time, the distribution has been far from
48 equitable, with more than 820 million people having insufficient food and many more consuming
49 low-quality diets leading directly to micronutrient deficiencies (Willett et al 2019; Zhong et al., 2020).
50 In the past 20 years, and particularly in the past decade, engineered nanomaterials (ENMs) have seen
51 dramatically increasing use and had an equally significant impact on ecosystems services and human
52 society. As such, studies focused on the implications associated with their use are critical. In fact, it

53 is known that ENMs exert important, but not completely understood, effects on biota; a particular
54 topic of concern include the effect on crops, food production, and trophic transfer (Gardea-Torresdey
55 et al., 2014; Ma et al., 2018; White et al., 2022).

56 The interplay between plant growth, dissolution, evaporation, and aggregation are key aspects of the
57 dynamic behavior of ENMs in the environment. Directional aggregation can result in the formation
58 of larger particles with a more complex morphology. However, given the complexity of natural
59 environments, most nanomaterials can be found in hetero-aggregated composites of different
60 inorganic and organic materials (Judy et al., 2012; Ma et al 2018). These aggregates can be very
61 different from original simple pristine morphologies and may even form highly branched structures
62 similar to fractals, all of which subsequently dramatically affect their reactivity and transport (Ma et
63 al., 2018; Huangfu et al., 2019).

64 Sectors with a large nanomaterial application such as medicine and food production may experience
65 greater risks of ENMs exposure due to their uses, with thousands of tons of ENMs that are eventually
66 discarded into the three main environmental matrices: soil, water, and air (Keller et al., 2013;
67 Zuverza-Mena et al., 2017). ENMs with the greatest historical use include nanoscale ceria ($n\text{CeO}_2$),
68 silica ($n\text{SiO}_2$), titania ($n\text{TiO}_2$), as well as nanoscale copper oxide ($n\text{CuO}$), zinc oxide ($n\text{ZnO}$) and
69 nanosilver ($n\text{Ag}$), and as such, release in the environment has been investigated (Keller & Lazareva,
70 2014; Mitrano et al., 2015). Considering the long-time span of use of select materials (1950 to 2050),
71 efforts to estimate ENMs release through commercial and associated activity into the environment
72 have been undertaken. For example, for $n\text{SiO}_2$, a global production between 100,000 and three million
73 tons per year has been estimated, while for $n\text{CeO}_2$, levels likely reach the upper limit of 10,000 tons
74 per year, and for $n\text{Ag}$, the literature reflects a production volume below 1,000 tons per year (Giese et
75 al., 2018). The use of $n\text{TiO}_2$ for the inhibition of microbial proliferation in food is one of the most
76 important ways to prolong the shelf life of packaged products (Abutalib & Rajeh., 2020). However,
77 a panel from EFSA concluded that E171 (TiO_2) can no longer be considered as safe when used as a
78 food additive (EFSA Journal 2021).

79 Gottschalk et al., (2009) calculated environmental concentrations using a probabilistic LCA (life
80 cycle analysis) of ENM containing products. The authors modelled nTiO₂, nZnO, nAg, carbon
81 nanotubes (CNT), and fullerenes for the U.S., Europe, and Switzerland. The concentrations in the
82 environment were calculated through probabilistic density functions and compared to ecotoxicology
83 data. In the simulations, the values ranged from 0.003 ng L⁻¹ (fullerenes) to 21 ng L⁻¹ (nTiO₂) for
84 surface waters and from 4 ng L⁻¹ for fullerenes to 4 µg L⁻¹ for nTiO₂ for sewage treatment effluents.
85 In Europe and the U.S., ENMs increased annually in sludge treated soil, and ranged from 1 ng kg⁻¹
86 for fullerenes to 89 µg kg⁻¹ for nTiO₂ (Gottschalk et al. 2009; Keller et al., 2013; Keller & Lazareva,
87 2014; Rincon, 2019).

88 Importantly, quantum dots (QDs), as well as many carbon- and metal-based ENMs, have been shown
89 to produce negative effects on animals and plants as a function of dose, including accumulation,
90 alteration of physiological and biochemical parameters, and reduced growth or yield (Oh et al., 2016;
91 Zuverza-Mena et al., 2017). Quantum dots have shown to enter plant roots and to damage the cell
92 wall, dysregulating metabolism (Marmioli et al., 2020). While mercaptoacetic acid (MAA)-coated
93 CdSe/ZnS QDs induced minimal toxicity on maize seedlings, pristine cadmium/tellurium (Cd/Te)
94 QDs induced chromatin stress, mitochondrial damage and inhibition on green gram sprouts
95 (*Phaseolus radiatus* L.) growth (Song et al., 2013). There are several routes by which ENM can enter
96 the soil-plant system. These include agricultural application of sewage sludges which often contain
97 nSiO₂, nTiO₂, nZnO, and nAg; as well as the application of nano-enabled agrochemicals, resulting in
98 the direct entry of nSiO₂, nTiO₂, nZnO, nFeOx, nCuO, CeO₂ and nAg into agricultural soils (Lv et
99 al., 2019; Verma et al., 2022).

100

101 *1.2. Conceiving and studying the ENM biotransformation*

102 Environmental and soil physico-chemical characteristics may significantly impact on ENMs
103 aggregation, and dissolution, which may modify ENM bioavailability, uptake, translocation, and
104 accumulation into terrestrial plants. In fact, light and temperature may induce potential changes on

105 the ENM structure, such as during foliar spray (Hong et al., 2021). Once within plant tissues, ENM
106 biotransformation may alter particle stability and behaviour in terms of interactions with
107 biomolecules, triggering differential plant defense mechanisms (Ma et al., 2018; Rawat et al., 2018).
108 ENMs are subject to a range of processes that may lead to their partial dissolution or result in
109 structural modifications (Milosevic et al., 2020; Marmioli et al., 2020). A schematic representation
110 is reported in Figure 1. Nanoparticle biotransformation is a highly complex and poorly understood
111 series of events and has been shown to occur during weathering in the soil, trophic transfer, and
112 translocation within plant tissues. These reactions are highly dynamic and alter the original pristine
113 structure of the nanoparticles in a number of ways, potentially causing the release of ions, but also
114 the consequent restructuring (or destructuring) of the nanoparticle (Servin et al., 2017a).
115 Biotransformation of nanomaterials may rest on the interaction with biological molecules that
116 stabilize their external reactivity, such as peptides including those involved in detoxification, (*e.g.*,
117 glutathione), fatty acids, secondary metabolites, and even components of cell membranes (Marmioli
118 et al., 2020). Particle properties such as size, stability, charge, and dissolution may strongly influence
119 other biotransformation mechanisms, potentially promoting enzymatic modification and
120 functionalization with proteins (*e.g.*, corona protein) present in the cytoplasm and organelles (Ma et
121 al., 2018; Marmioli et al., 2020). ENMs may maintain crystal structure when internalized by cells or
122 may be disassembled and converted into less complex structures (by biological modification or
123 chelation), thus reducing toxicity, and the risk of their accumulation and translocation (Wang et al.,
124 2022). These post-uptake structural modifications involve specific parameters such as bond distance
125 with other atoms or nature of the ligand atoms. In consideration of this, one objective in
126 biotransformation studies is to investigate the physico-chemical forms (*e.g.*, nanocrystal structure)
127 within exposed tissues and to characterize the structural differences within the new biotransformed
128 molecules, including identification of the biomolecules interacting with the ENMs (Castillo-Michel
129 et al., 2017; Marmioli et al., 2020). It has furthermore to consider how genetic diversity across
130 different plant species and within the same plant species (in different cultivars) may influence the

131 ENM uptake and translocation (Deng et al., 2020). Some of the more interesting discoveries on the
132 biotransformation and localization of metal based-ENMs into plants have been achieved with to the
133 synchrotron-based techniques of imaging, elemental speciation, and atomic neighbors' identification.
134 This transformation will be also influenced by the environment, these reactions of the same particle
135 will be different on the leaf surface, in the mesophyll, in the vascular tissue, in different organelles,
136 in the roots and in the rhizosphere (Sarret et al. 2013; Castillo-Michel et al., 2017).

137 Micro-X-Ray Fluorescence (μ -XRF) and micro-X-Ray Adsorption Spectroscopy (μ -XAS) K-, L- or
138 L_{III}-edge EXAFS and XANES spectra have be used to study the biotransformation of coated
139 nanomaterials present in plants and soil (Lopez-Moreno et al., 2010; Judy et al., 2012). μ -XRF is
140 used for qualitative elemental analysis of heterogeneous biological samples. The interaction of the
141 sample with high-energy X-ray radiation, which leads to X-ray absorption and emission of the
142 fingerprint X-ray spectra for each element, is the key feature of this powerful analytical method. The
143 absorption/excitation effect and relaxation process lead to atoms emitting fluorescence photons
144 characterized by elemental specific energy (Chebakova et al., 2021). Extended X-ray absorption fine
145 structure (EXAFS) is a technique that utilizes oscillations of the X-ray absorption coefficient on the
146 high-energy side of an absorption edge. Such oscillations can reach up to 1000 eV above the edge
147 and may have a magnitude of 10% or more. In addition, it is necessary to consider that atoms are not
148 stationary. Thermal vibrations will obscure the EXAFS oscillations, and in the harmonic
149 approximation, this can be accounted for by considering a Debye-Waller-type term. This led to a
150 great improvement in the theoretical understanding of EXAFS and it is now established that a single
151 scattering short-range order theory is adequate under most circumstances (Gurman, 1995). In
152 addition, electrons that have undergone inelastic losses will not interference in the process. This is
153 considered by an exponential damping term. It is the limited range of the photoelectrons in the energy
154 region of interest 50-1000 eV that allows for a short-range order description of EXAFS also in
155 crystalline materials (Gurman, 1995). The region which includes the pre-edge, edge-jump and post-
156 edge to approximately 30-50 eV is referred to as the X-ray Absorption Near Edge Structure

157 (XANES), which describes the structural component of the X-ray absorption near-edge as an
158 extension of the EXAFS, largely due to the long mean free path of the photoelectron and the
159 dominance of high order multiple scattering contributions (Gräfe et al., 2014).

160 Synchrotron-based techniques use photons, which do not have mass; therefore the factor 1.22 is
161 substituted by the non-relativistic mass of the electron which is $m=5.485 \cdot 10^{-4}$ Da. This makes the
162 resolution even smaller and increases penetration depth into the sample. On the other hand, EDX
163 depends on the acceleration voltage of the particle or of the photon. Every element has its own orbital
164 energies, and the acceleration voltage allows excitation one or more of these, independently if it
165 comes from a TEM or from a synchrotron (Goldstein et al., 2003).

166 From the perspective of application, μ -XRF can provide information on the presence and localization
167 of specific elements within tissues, while XANES and EXAFS spectroscopy can provide information
168 related to the valence state and coordination environment of the element of interest, as well as the
169 molecular species present in the sample. The use of μ -XRF and μ -XANES for the analysis of
170 nanoparticles in plants have been thoroughly reviewed by Castillo-Michel et al. (2017).

171 Importantly, these powerful methodologies open the possibility to mechanistically address many
172 important environmental issues, such as the chemical activities of environmental pollutants, to trace
173 environmental elemental cycles, element speciation in complex matrices, and to characterize the
174 natural/anthropogenic complex matrixes that are not amendable for standard analytical and structural
175 analyses (Puri et al., 2019). In many synchrotrons around the world, there is increasing use of
176 dedicated beamlines to study environmental and plant tissues exposed to contaminants such as ENMs
177 (Proux et al., 2017; d'Acapito et al., 2019).

178

179 The present review aims to describe the current understanding of metal based-ENMs
180 biotransformation mechanisms in plants and focuses on correlating available physiological and
181 molecular data with the information obtained by synchrotron-based techniques. This evaluation not
182 only highlights biotransformation as one of the major driving forces mediating the biological effects

183 of ENMs on plants, but also offers some perspective on intentional and safer-by-design strategies that
184 can ensure more sustainable application of these materials. Moreover, the study on plants is
185 instrumental to the application of the REACH normative within European Union for toxicological
186 and ecotoxicological studies (Replacement, Reduction and Refinement). Plants are higher eukaryotes,
187 characterized by large nuclear genomes and organellar genomic information (within chloroplasts and
188 mitochondria) that provide an effective model for many complex species (Chang et al., 2016).

189

190

191 ***2. Physiological and molecular effects as indirect evidence of ENM biotransformation in plants***

192 The physiological behaviour and related molecular pathways of response are important to
193 characterizing and understanding ENM biotransformation. An important part of this involves
194 comparing the effects of a nanomaterial with that of the bulk and ionic counterparts, as well as by
195 investigating different exposure times and by exposing different plant organs and tissues (Schwab et
196 al., 2016; Marmioli et al., 2021). Studies have added significant molecular data on the effects of
197 ENMs exposure in plants (Schwab et al., 2016; Ma et al., 2018). Different results are often observed
198 for the same element as a function of its form or size, *i.e.* nanostructured, bulk, or ionic species
199 (Pagano et al., 2016; Wang et al., 2022). Detailed study of differential transcriptional regulation,
200 protein abundance or metabolomic profiling (Huang et al., 2018; Majumdar et al., 2019; Gallo et al.,
201 2021) are critical to demonstrating the nano-specificity of plant response. The study of organellar
202 genome stability and the related stoichiometric variations during ENM treatment has also provided
203 important mechanistic insight into plant to ENM exposure (Pagano et al., 2022). Advanced
204 synchrotron-based techniques may either help to systematically understand the nano-bio interactions,
205 with regard to physical and chemical reactions at the biomolecular surface: biomolecules may interact
206 with ENMs, generating biomolecular corona, which change the ENM surface properties, and interfere
207 with its functionality/reactivity (Hameed et al., 2022).

208 Regarding the physiological effects of ENMs on plants of agronomic interest, these studies have
209 provided a better understanding of the specific properties of the ENMs that may enable sustainable
210 use in the agrifood sector. Beyond the potential adverse effects upon bioaccumulation from soil or
211 other exposure routes, there is an increasing interest in exploiting the potential positive effects of
212 ENMs on plants, aiming to improve crop yields and quality. A range of mechanisms, including direct
213 use as nanofertilizers (Verma et al., 2022), nanocarriers (Karny et al., 2018), smart delivery systems
214 (Xu et al., 2022) or when in association to plant growth-promoting bacteria, are considered (Prado de
215 Moraes et al., 2021). In addition, ENMs may act indirectly by protecting plants from biotic (*e.g.*,
216 nanopesticides) or abiotic stressors (*e.g.*, wastewater and soil treatment) (Liu et al., 2015; Kah et al.,
217 2018; Kumari et al., 2019). Due to the many variables involved, it is essential to obtain robust safety
218 data regardless of the end use: ENM type, the modes and time of exposure, concentrations tested, and
219 the plants used are all important considerations. Any recurring effects that occur under these different
220 conditions are of particular interest and are explored below (see Figure 2 and Table 1). Table 2
221 summarizes the major outcomes related to metal based-ENM biotransformation in plants, including
222 the principal mechanisms involved, and the major physiological and molecular insights observed
223 from exposure.

224

225 2.1. Lanthanides based ENMs

226 Cerium Oxide ($n\text{CeO}_2$) has shown significant potential for agricultural applications, largely due to its
227 properties as an ROS scavenger (Ma et al., 2015; Servin et al., 2017b). While CeO_2 as a bulk crystal
228 mainly consists of Ce(IV), the reduction to $n\text{CeO}_2$ significantly enhances the relative amount of
229 Ce(III), resulting in a higher catalytic effects comparable to the capabilities of a biological antioxidant
230 (Eriksson et al., 2018). Servin et al. (2017b) used $\mu\text{-XRF}$ and $\mu\text{-XANES}$ to analyze the interactions
231 between $n\text{CeO}_2$ and different biochars in soil, observing that much of the Ce remained in $n\text{CeO}_2$ form
232 within the plant tissues. The dissolution rate of the nanoform can increase in acidic environments to
233 generate Ce(III), as reported by Hernandez-Viezcas et al. (2013) who analyzed in *Glycine max* L. the

234 effects of nCeO₂ (1000 mg L⁻¹, 48d exposure). Results have been confirmed by Rui et al. (2015), who
235 used XANES on exposed cucumber (*Cucumis sativus* L.) tissues (2000 mg L⁻¹, 21d exposure) to
236 observe nCeO₂ association with phosphate. These properties highly impact not only reactivity but
237 also nCeO₂ translocation. In zucchini (*Cucurbita pepo* L.), treated with 500 mg L⁻¹ of nCeO₂, the
238 nanoform is mainly present in the roots and stems, with limited translocation to the leaves (Pagano et
239 al., 2016). However, co-contamination with other ENMs (e.g., CdS QDs) under same experimental
240 conditions resulted in increased translocation to the shoots from 1000 to 3000 mg kg⁻¹ (Pagano et al.,
241 2017). Similar results have been reported in several plant species: for example, Rossi et al (2017)
242 nCeO₂ under co-exposure with ionic Cd in soybean (*Glycine max* L.) showed an altered (1-2 fold
243 increased) translocation to the shoots. Interestingly, bulk CeO₂ translocation resulted similar to the
244 nano-form, whereas ionic Ce was translocated in greater amounts to the shoots (Pagano et al., 2016).
245 This analysis was supplemented with molecular data; the transcriptional profiles were evaluated in
246 *C. pepo* and *S. lycopersicum* as a function of nCeO₂, nLa₂O₃ and nCuO exposure and were compared
247 with bulk and ionic forms using a set of 38 genes based on the *A. thaliana* orthologs as potential
248 biomarkers of exposure/effects (Marmioli et al., 2014). The responses observed were generally
249 different in term of up- or down-regulation as a function of Ce form (Pagano et al., 2016). Of
250 particular interest are impacts on the chloroplast are the *PetL* and *PSBN* genes, which encode for a
251 structural component of the cytochrome b₆f complex and low molecular weight protein located on
252 thylakoid membrane as a component of the photosystem II (PSII), respectively (Figure 2, Table 1).
253 These two chloroplastic genes were differentially expressed across nano-, bulk, and ionic forms. A
254 similar trend was also evident for nLa₂O₃ and nCuO exposure scenarios. Interestingly, by analyzing
255 the effects on chloroplast and mitochondrial genomes in *A. thaliana* in terms of copy number, the
256 effects of nCeO₂ and CeCl₃ exposure were rather limited as compared to the untreated control, which
257 agrees with the limited translocation to the shoots (Pagano et al., 2022). With regard to proteomic
258 analysis, Majumdar et al. (2015) conducted a quantitative proteomic analysis of kidney beans
259 (*Phaseous vulgaris* L.) seeds after nCeO₂ exposure and reported that the major seed proteins

260 associated with nutrient storage (phaseolin) and carbohydrate metabolism (lectins) were significantly
261 reduced by nCeO₂ (62.5-500 mg kg⁻¹, 50d exposure) in a dose dependent manner. Interesting, the
262 plants did not exhibit overt toxicity.

263 In fact, at the physiological level cerium-based nanoparticles generally do not produce phytotoxicity
264 (Ma et al., 2015; Rui et al., 2014; Lizzi et al., 2020; Rodrigues et al., 2021), though some have
265 highlighted a positive impact on biomass and on physiological indicators such as chlorophyll and
266 photosynthesis at selected doses (Rossi et al., 2017; Gui et al., 2017). Another important aspect of
267 nCeO₂ seems to involve enhance tolerance to saline stress, leading to improved phenotypic and
268 enzymatic performances and ROS elimination in seeds priming (An et al., 2020; Liu et al., 2021;
269 Hassanpouraghdam et al., 2022; Chen et al., 2022).

270 Compared to nCeO₂, nanoscale lanthanum oxide (nLa₂O₃) exhibits lower stability, increased ion
271 dissolution, greater translocation from roots and shoots, all of which seems to lead to higher
272 phytotoxicity. The limited stability of nLa₂O₃, as compared to nCeO₂, has been confirmed by μ -XRF
273 analysis in *Cucumis sativus* L. through element speciation, dissolution studies in aqueous solution
274 and *in planta*. After 14d treatment, the nCeO₂ structure in the roots remains mostly preserved (more
275 than 80%) while pristine nLa₂O₃ structure was observed at levels below 10% (Ma et al., 2015).

276 Interestingly, co-contamination with nCeO₂ strongly reduces the uptake of nLa₂O₃ (Pagano et al.,
277 2017). The different behaviour of the two ENMs was evident in the transcriptomic profile: only 7 out
278 of 38 genes were commonly modulated between nCeO₂ and nLa₂O₃; these genes were involved in
279 primary metabolic functions, protein synthesis and stress response (Pagano et al., 2016). Several
280 publications using different model plants were compared, and the reported effects due to nLa₂O₃
281 exposure in soil include reduction in root and leaf biomass (Ma et al., 2015), decreased transpiration
282 (Yue et al., 2019), decreased photosynthesis (Xiao et al., 2021) and reduced pigment concentration
283 (Neves et al., 2019). The decrease in photosynthetic activity is also reflected by altered root
284 morphology, including root cracking (Xiao et al., 2021) and the presence of apoplastic barriers (Yue
285 et al., 2019). Interestingly, the adverse effect of nLa₂O₃ on plant biomass was alleviated under binary

286 exposure combinations with ENMs such as nCuO and nZnO (Pagano et al., 2017). As ideal case
287 study, information on RedOx state and potential translocation of lanthanide-based nanoforms within
288 plant tissues become fundamental in the mechanistic understanding on the physiological and
289 molecular effects, with regard to application in the agrifood sector.

290

291 2.2. Titanium oxide ENM

292 Titanium dioxide (nTiO₂) has been largely studied as a potential environmental and agricultural
293 contaminant (Servin et al., 2012; 2013). nTiO₂ has demonstrated a high stability, both in anatase and
294 rutile crystal form (Servin et al., 2012). Translocation of nTiO₂ (0-750 mg kg⁻¹, 150d exposure) from
295 soil to roots and to shoots in *C. sativus* is generally limited, though the two crystal structures were
296 evident in the leaf trichomes and fruit by μ -XANES spectra (Servin et al., 2013). Ruotolo et al. (2018)
297 analyzed and reviewed the molecular responses of *A. thaliana* and other model species to nTiO₂ and
298 reported that exposure triggers an abiotic stress response at the transcriptomic level, involving ROS
299 detoxification systems, triterpenoid and phenylpropanoid metabolism, and hormone signaling
300 pathways involving in the response to salicylic acid, jasmonic acid, ethylene, and brassinosteroids.
301 At the post-transcriptional level, several miRNAs were strongly modulated, including miR395 and
302 miR399 as key regulators of plant adaptive responses to nutrient starvation (Pagano et al., 2021).
303 Thus, the ability of nTiO₂ to modulate ROS signaling is particularly effective under abiotic stress
304 conditions. Here, the presence of this ENMs enhances plant physiological parameters by stimulating
305 the activation of several defense mechanisms. Several studies (in plants such as *C. sativus*, *S.*
306 *lycopersicum*, *V. faba*) have shown that in both saline soils and under drought conditions, the addition
307 of nTiO₂ increases root length, plant biomass, and other parameters such as H₂O₂ level, antioxidant
308 activity, sugar content, and chlorophyll amount (Servin et al., 2012; Nasir Kahn, 2016; Abdel Latef
309 et al., 2018; Mustafa et al., 2021). However, higher concentrations can result in phytotoxicity, likely
310 due to aggregation and subsequent excessive ROS production (Mattiello et al., 2015; Gohari et al.,
311 2020). However ROS are “double blade” sword because they can also trigger production of defensive

312 molecules as shown recently by Castro et al., (2021). Interestingly, when either considering the
313 utilization of pristine and coated nTiO₂ (hydrophilic or hydrophobic) in carrot (*Daucus carota* L.),
314 responses observed depended mainly on the nTiO₂ surface coating, concentration and in soil
315 weathering (Wang et al., 2021a; 2021b). Taproot and leaf fresh biomass and plant height were all
316 increased with exposure, as well as nutrient uptake (Fe in leaves; Mg in taproots; Ca, Zn, K in roots).
317 Conversely, sugar and starch contents were negatively affected, compromising the nutritional quality
318 (Wang et al., 2021b).

319

320 2.3. Gold and silver nanoparticles

321 Similar to nTiO₂, gold nanoparticles (nAu) are highly stable in plants: nAu remained mostly as Au⁰
322 within the plant tissues (*Nicotiana tabacum* L. cv. Xanthi nc.), even if accumulated and translocated
323 (Sabo-Attwood et al., 2011). Specifically, XANES analyses demonstrated that nAu maintained its
324 nanoparticle structure without any biotransformation or ionic release. There are no actual uses for
325 gold nanoparticles and plants, it just used as a tool to study NP-plant interactions. nAu levels in
326 biosolids would ever be high enough to be considered phytotoxic. It is known that nAu toxicity
327 depends on concentration, particle size and shape: nAu with a smaller particle size (3.5nm,
328 concentration of 48 mg L⁻¹) were evenly biodistributed across the plant in comparison with the
329 18.5nm nAu (in a concentration of 76 mg L⁻¹), even leading to the formation of necrotic leaf lesions
330 and plant death after 30 days (Sabo-Attwood et al., 2011). Other studies have shown that nAu
331 exposure improved radical scavenging and antioxidant enzymatic activities and modulated miRNA
332 expression implicated plant abiotic stress response (miR398, miR408). In particular, the regulation
333 of superoxide dismutase (SOD) led to an increased ROS scavenging activity, root elongation,
334 seedling growth, and seed yield (Arora et al. 2012; Kumar et al., 2013; Siddiqi & Husen, 2017).
335 Given the widespread commercial utilization and environmental relevance (e.g., wastewater
336 treatment; fertilization) of silver nanoformulations (nAg), the effect on plant species has been a topic
337 of robust study. Stegemeier et al. (2015) analyzed the nAg and nAg₂S speciation in *Medicago sativa*

338 L., demonstrating that nAg accumulates in the root elongation area but that nAg₂S remains adhered
339 to the root surface; Ag ions accumulate more uniformly throughout the root tissues. Notably, the Ag
340 accumulation in the root apoplast was determined by XRF. The presence of nAg in root cell walls
341 demonstrated the uptake of partially dissolved nAg and translocation along the apoplast. Larue et al.
342 (2014) localized and determined nAg speciation in *L. sativa* after foliar spray treatment through μ -
343 XRF and μ -XAS techniques; the authors reported that nAg was able to cross the foliar cuticle,
344 penetrating in the leaf tissue through the stomata. Moreover, nAg biotransformed through oxidation
345 and complexation with thiol-containing molecules such as glutathione (GSH). These findings
346 correlated well with the transcriptomics analyses of *A. thaliana* exposed to different types of nAg:
347 plant response included defensin-like proteins, plant thionin, β -glucosidases, cytochrome P450
348 proteins, and glutathione-S-transferase (GST) members (Kaveh et al., 2013). Although some studies
349 point out that the morphological and physiological effects of nAg exposure were strictly dependent
350 on particle size and concentration and that sublethal concentrations may have also beneficial effects
351 (Wang et al., 2013; Syu et al., 2014), most of reports demonstrated reduced root elongation and shoot
352 biomass, together with decreased levels of chlorophyll, pigments, micronutrients, and increased level
353 of ROS and activity of enzymes involved in the oxidative stress response (Yin et al., 2011; Zuverza-
354 Mena et al., 2016; Yang et al., 2018; Lahuta et al., 2022).

355

356 2.4. Iron-based ENMs

357 Iron-based nanomaterials, including iron oxides (nFeOx) and zero valent iron (nZVI), have been
358 investigated in plant systems and the reports highlight two major routes of entry: i) a reductive and
359 proton-promoted process able to modify the structure of the ENM or ii) through the secretion of plant
360 transporters (*e.g.*, phytosiderophores) with a high affinity for Fe (III) (Morrissey & Guerinot, 2009).
361 Dwivedi et al. (2018) investigated nZVI exposure in *C. sativus* and reported that transformed nZVI
362 was stored in the root cell membrane and vacuoles of the leaf parenchyma. XAS identified ferric
363 citrate and iron (oxyhydr)oxides as the main transformation products in roots and shoots, albeit in

364 different proportions. The major pathways of nZVI biotransformation involve interaction with low
365 molecular weight organic acid ligands and on the dissolution/precipitation of the mineral products.
366 Transcriptional analyses performed on H⁺-ATPase genes (*CsHA1*, *CsHA2*) showed an upregulation
367 of these genes upon nZVI exposure (and relative root acidification), indicating that the plant-
368 promoted transformation of nZVI can be driven by protons released by the roots.

369 A separate study investigated the effects of nFe₂O₃ and nFe₃O₄ on *A. thaliana*, highlighting
370 differences in the response between nanoparticle forms and metal salts through a nanoscale-specific
371 response pathway involving energy production and oxidative stress. The differential response was
372 ascribed to the ENM and the metal salt dissolution rates and the toxicity of the metal ion, which is
373 more compatible with biotransformation processes in the plant tissues. Importantly, specific effects
374 on plastid and mitochondrial genomes were evident, with nFeOx causing a 1- to 3-fold increase in
375 ptDNA and mtDNA copy numbers depending on the stability of the nanoform utilized (Pagano et al.,
376 2022).

377 Given their widespread application in soil and water remediation, a primary concern with iron
378 nanoparticles is a potential toxicity from excessive accumulation in the environment. However,
379 several studies have shown that plant exposure to this type of nanoparticle does not result in
380 phytotoxicity. For example, Dwivedi et al. (2018) evaluated the potential environmental impact of
381 nZVI on *C. sativus* in soil and in hydroponic culture, and reported no instances of reduced plant
382 biomass even at the highest doses (from 250 to 1000 mg L⁻¹) and for *O. sativa*, the low doses (50-500
383 mg L⁻¹) of nZVI and nFe₃O₄ improved plant growth (Li et al., 2021). The use of this nanomaterial as
384 a soil conditioner for remediation of metal-contaminated soils is confirmed by the demonstration of
385 improved plant growth in Cd-contaminated soils (Rizwan et al., 2019; Manzoor et al., 2021);
386 mechanistically, this involves limiting cadmium translocation and the promotion of antioxidant
387 activity.

388 In summary, the extent and the degree of biotransformation of nZVI, which consists in the
389 biochemical alteration of chemical compounds within a living tissue, are reflected in the physico-
390 chemical properties, macromolecular interaction, and biologically mediated pathways observed.

391

392 2.5. Zinc-based ENMs

393 Zinc-based nanomaterials have been applied to plants to increase food safety, promote food
394 production and enhance sustainability by reducing oxidative stress symptoms induced by abiotic
395 stressors (Faizan et al., 2021). nZnO is characterized by a low stability, and a high dissolution rate
396 (Lv et al., 2021). Hernandez-Viezcas et al. (2013) exposed *Glycine max* L. to nZnO (500 mg kg⁻¹,
397 48d exposure): μ -XRF analysis showed no detectable ZnO NPs within the tissues, while μ -XANES
398 data showed O-bound Zn in a form resembling Zn citrate. Lv et al. (2015) studied the effects of nZnO
399 in *Z. mays* L. and used μ -XANES to demonstrate that the majority of accumulated Zn was derived
400 from Zn²⁺ released from the nanoparticles and was accumulated mainly as Zn phosphate in epidermis,
401 cortex, and root tip cells. The results were correlated to transcriptomic analyses in which gene
402 ontology (GO) performed in nZnO-exposed *A. thaliana* revealed significant commonalities with the
403 response to Zn²⁺ ions, particularly with proteins involved in metal binding, transport, metal
404 homeostasis and detoxification. This suggests that Zn ion release by nZnO is a key in mediating the
405 overall effect on plant species (Landa et al., 2015). These findings have been extended to other
406 species, such as *C. pepo* L.; here nZnO treatment was shown to modulate genes that encode for
407 transporters of heavy metals, cellular response to abiotic stress, decreased chlorophyll production,
408 and induction of secondary metabolite biosynthesis (Pagano et al., 2017).

409 In recent years other forms of Zn-based nanomaterials have been tested for a potential plant
410 remediation purpose, such as ZnS QDs (Imperiale et al., 2022). An analysis of the effect of ZnS QDs
411 and ionic Zn exposure on mitochondrial and plastid genome copy number demonstrates that both
412 increase by 1 to 3-fold), but that ZnS QDs dissolution alone does not explain the phenomenon; this
413 suggests that ZnS QDs biotransformation may occur within the plant tissues and organs to a form

414 more similar to ionic than nanoscale Zn (Pagano et al., 2022). Zinc-based nanomaterials have also
415 shown interesting properties as nanofertilizers, including mitigating abiotic and biotic stress (*e.g.*, salt
416 stress, infections), regulating micronutrient uptake, improving water use efficiency, and promoting
417 detoxification of heavy metals (Akhtar et al., 2021; Zafar et al., 2022). Under drought conditions, the
418 nZnO (5 mg kg⁻¹) significantly increased grain yield in sorghum (*Sorghum vulgare* Moench) and fruit
419 yield in eggplant (*Solanum melongena* L.), respectively by 22-183% and 12-23% (Dimkpa et al.,
420 2019; Semida et al., 2021).

421

422 2.6. Copper oxide

423 Copper oxide nanomaterials (nCuO) are among the most utilized ENMs with plants, including use as
424 a nanopesticide or nanofertilizer (Elmer et al., 2018; Lowry et al., 2019; Xu et al., 2022). nCuO
425 dissolution within the plant tissues has been demonstrated (in *C. pepo*), and this was shown to depend
426 not only on uptake, and translocation, but also on the interaction with important biomolecules (Tamez
427 et al., 2019; Marmiroli et al., 2021).

428 EXAFS (Marmiroli et al., 2021) demonstrated that the local Cu environment in the higher shells
429 shows small differences between roots and flowers. A second Cu-O shell path was present in both
430 flowers and roots; a Cu-Cu bond was also observed in roots but was not observed in flowers. A full
431 transcriptomics analysis by RNAseq was performed to highlight the differential responses between
432 nano-, bulk and ionic forms in roots, leaves and pollen (Marmiroli et al., 2021). The results
433 highlighted the nano-specificity of the responses; the modulated genes (significantly up- or down-
434 regulated genes) observed were more significant in the roots and decreased with translocation to
435 leaves and pollen. However, the portion of the response common to the three Cu forms tested was
436 shown to increase following the translocation from roots to shoots (Marmiroli et al., 2021). A
437 characterization of the main steps and implications involved in this phenomenon, as well as some
438 relevant biomarkers observed in different plant species, is presented in Figure 2 (details reported in
439 Table 1).

440 Additional data was presented by Servin et al. (2017a), who studied nCuO weathering in *Lactuca*
441 *sativa* L.: lettuce was exposed to unweathered and 70d-weathered nCuO, and corresponding bulk and
442 ionic form (0–400 mg kg⁻¹) for 70 d in soil. To assess nCuO trophic transfer, leaves were fed to
443 crickets (*Acheta domestica* L.) as primary consumer, followed by insect feeding to lizards (*Anolis*
444 *carolinensis* L.) as secondary consumer, in both cases for 15d. The authors used μ -XANES to show
445 that Cu(II) was reduced to Cu(I) within the plant roots, and used a transcriptional analysis of to show
446 that several biomarkers, including *CCH* and *COPT5*, which encodes for a copper chaperon and a
447 copper ion transporter, respectively, were significantly decreased by weathering.

448 In spite of being widely used, results regarding the physiological effects upon nCuO exposure are
449 rather discordant. For example, Deng et al. (2022a) reported that, unlike the bulk counterpart, nCuO
450 (0-600 mg kg⁻¹ of soil) does not produce toxicity in rice (*O. sativa*), but rather improves the supply
451 of essential elements, including increasing content of sugar and starch, as well as overall yield.

452 The grain of weedy and cultivated rice were differentially impacted by nCuO, bulk or ionic forms,
453 showing also a cultivar-specific and concentration-dependent response. Cu translocation directly
454 influenced plant yield, sugar production, starch content, protein content, and expression of auxin
455 associated genes in grain (Deng et al., 2022b). Analyzing the effect of citric acid (CA) coated copper
456 oxide (CA-nCuO) and its application (foliar spray or soil exposure) on the growth and physiology of
457 soybean (*Glycine max* L.), nCuO appeared to be more accessible for plant uptake, as compared to
458 CA-nCuO, decreasing the protein content, and inhibiting plant growth. CA reduced CuO NPs
459 toxicity, demonstrating that surface modification may change the toxic properties of NPs (Deng et
460 al., 2022c).

461 Treatment of *Lactuca sativa* L. with nCuO significantly increased biomass as compared to CuO
462 microparticles (Wang et al., 2019). In addition, plants can benefit from nCuO treatment through
463 enhanced defensive pathways, and through direct antimicrobial and antifungal activities (Elmer et al.,
464 2018). For example, exposure of nCuO to *Solanum lycopersicum* increased root and stem length, leaf
465 number, and chlorophyll content, and also inhibited the mycelial growth of *Fusarium oxysporum* sp.

466 Lycopersici (Lopez-Lima et al., 2021). Conversely, some authors report toxic and inhibitory effects
467 on the growth in plants such as lettuce (*Lactuca sativa* L., 0-1000 mg L⁻¹, 5-15d exposure by foliar
468 spray), turnip (*Brassica rapa* L., 50-500 mg L⁻¹, 14d exposure), and wheat (*Triticum aestivum* L., 50
469 mg kg⁻¹ in sand, 1-14d exposure) upon nCuO treatment. The toxic effects are largely ascribed to the
470 redox reactivity and ROS generation of the nanoparticle form (Dimkpa et al., 2012; Chung et al.,
471 2019; Xiong et al., 2020). Others have reported no significant impact at the physiological level
472 (Servin et al., 2017a; Tamez et al., 2019; Marmioli et al., 2021; Roubeau Dumont et al., 2022), which
473 highlights the importance of the experimental variables and design, including dose, particle
474 properties, exposure conditions and endpoints.

475

476 2.7. Quantum dots

477 Cadmium-based nanomaterials, and cadmium sulfide quantum dots (CdS QDs) in particular, have
478 been used as a model material to elucidate physiological mechanisms and molecular pathways
479 involved in the response plant response to exposure (Marmioli et al., 2014; Imperiale et al., 2022).
480 A Systems biology approach gave a complete picture of the targets in both model (*A. thaliana*) and
481 crop (*C. pepo*) species (Marmioli et al., 2014; Marmioli et al., 2015; Pagano et al., 2017; Gallo et
482 al., 2021; Marmioli et al., 2020; Pagano et al., 2022). In *A. thaliana*, CdS QDs tolerant mutants were
483 used to establish *in vitro* inhibition concentrations for growth (80 mg L⁻¹) in an attempt to elucidate
484 the mechanisms involved in the plant response; the results largely implicated metabolic functions and
485 chloroplast energy production as sensitive targets (Marmioli et al., 2014). The results demonstrate
486 that CdS QDs and ionic Cd were exploiting different pathways in the plant, highlighting that the
487 tolerance to CdS QDs did not overlap with the tolerance to CdSO₄. Conversely, Cd sensitive mutants
488 of Arabidopsis (Howden & Cobbett, 1992) that were exposed to CdS QDs did not exhibit differences
489 in growth as compared to the wild type line (Marmioli et al., 2014). A transcriptomic analysis and
490 proteomic comparison between wild type and tolerant mutants highlighted that only a few genes were
491 commonly modulated upon ionic Cd and CdS QDs treatment (Marmioli et al., 2015; Gallo et al.,

492 2021). Marmiroli et al., (2020) used EXAFS to investigate the cadmium environment in planta and
493 showed that the spectra were compatible with a mixed O/S coordination; while Cd–S distances did
494 not show relevant variations, Cd–O distances varied in samples grown with QDs compared with those
495 grown with CdSO₄. The number of Cd–S bonds in plants grown with QDs was higher than Cd–O
496 bonds. This EXAFS analysis demonstrated that CdS QDs were biotransformed after uptake: the QD
497 original structure was modified but not completely absent within the plant cell, and Cd atoms were
498 not released as Cd ions. Interestingly, CdS QDs showed a relatively high stability; once accumulated
499 by the plant, the QD may go through different stages in the response pathways: i) exposure: explained
500 by the different genetic mechanisms behind the physiological/molecular response between the wild
501 type and tolerant phenotypes; ii) reactivity/biotransformation: explained by a transition phase in
502 which the structure of CdS QDs is modified to decrease particle reactivity, and this can be detected
503 by XANES and EXAFS analyses; iii) effects/detoxification: transcriptomic, proteomic and
504 metabolomic response related to the physico-chemical forms after QDs biotransformation.
505 Additionally, the effects on organelle genomes (ptDNA and mtDNA) demonstrate how QDs
506 biotransformation may modify the genomes stoichiometry or sub-stoichiometry, likely through
507 potential morpho-functional adaptive response triggered by modifications in the bioenergetic redox
508 balance, or a reduction of photosynthesis or cellular respiration rates after QD exposure (Pagano et
509 al., 2022).

510 Similarly to what was observed in *A. thaliana*, CdS QDs induced analogous effects other plant species
511 of agricultural interest: Pagano et al. (2017) analyzed the effects of the CdS QDs in a context of
512 ENMs binary co-contamination, highlighting a similar response as in *A. thaliana*; specific and
513 common biomarkers were involved between CdS QDs and other the ENMs tested (nCeO₂, nLa₂O₃,
514 nCuO, nZnO). Majumdar et al. (2019) investigated the effect of differently functionalized CdS QDs
515 in *G. max*; the authors used proteomic and metabolomic endpoints to demonstrate how the
516 transmembrane proteins involved uptake and related genes including *NRAMP6* and *HMA8* were
517 differently regulated in CdS QDs and ion treated plants. In addition, ATP-dependent ion transporters

518 in the membranes presented feedback mechanisms in the soybean roots to restrict the uptake of CdS
519 QDs and simultaneously to alter the mineral acquisition. Moreover, CdS QDs altered major metabolic
520 functions, including glutathione metabolism, the tricarboxylic acid cycle, glycolysis, fatty acid
521 oxidation and phenylpropanoid and amino acids biosynthesis. Physiologically, CdS QDs, induced
522 oxidative stress, decreased biomass, reduced chlorophyll and carotenoids content, and damaged
523 primary roots (Majumdar et al., 2019; Pagano et al., 2022).

524

525

526 ***3. Biotransformation as a perspective to comprehend ENM response in plant***

527 ENMs have been rather extensively tested in recent years, with data indicating that several physico-
528 chemical parameters are fundamental to explaining their behaviour during exposure, including
529 composition, stability, surface charge, and functionalization. These ENM properties become
530 biologically relevant and mediate subsequent biotransformation processes, including: i) the
531 possibility to be translocated within organs, tissues, and cells; ii) the ability to interact with the
532 biologically active environment within the plant (*e.g.*, phospholipids, nucleic acids, proteins,
533 secondary metabolites, reactive oxygen species); iii) the dissolution rate and the consequent ion
534 release. Importantly, to fully comprehend the plant response to ENMs exposure, one must consider
535 the biologically modified ENMs forms that are indicative of the highly complex interactions between
536 plants and ENMs interaction. Integration of the information from physiological and molecular
537 analyses with physical evidence (*e.g.*, types and number of atoms surrounding the ENM, radial
538 distance between atoms of the interactors and atoms constituting the ENM; ENM crystal structure)
539 obtained through high energy X-ray spectroscopy platforms such as synchrotron-based techniques
540 will enable a more realistic, mechanistic, and systems-level picture of plant response to ENM
541 exposure.

542 This review describes some of the primary biological constraints that determine ENM
543 biotransformation in plants (Figure 2, Table 2). For ENMs characterized by high stability, such as

544 nCeO₂, nTiO₂ or nAu, limited dissolution and translocation has been observed, even considering
545 differences determined by structure and atomic properties related to the redox state (*e.g.*, the redox
546 state of Ce). Conversely, ENMs with higher dissolution such as Fe- or Zn-based ENMs, nCuO, nAg
547 or nLa₂O₃, exhibit greater translocation rates, likely involving a dynamic process of particle
548 interaction with the plant biomolecules that increase ENM solubility and bioavailability, as
549 exemplified with nCuO (Marmiroli et al., 2021).

550 The importance of in planta ENM biotransformation is corroborated indirectly at molecular level by
551 “omic” analyses that can describe the effects on the plant at genetic and epigenetic level (including
552 genome stability) by measuring transcriptional modulation, protein abundance and metabolite
553 synthesis, as well as on physiological (phenotypical) level by observing the plant redox state, ROS
554 production, photosynthetic activity, and cellular respiration rate in response to stress (Marmiroli et
555 al., 2020; Gallo et al., 2021). The direct measurement of changes upon ENM biotransformation within
556 the plant tissues by synchrotron-based techniques (μ -XRF, μ -XANES, and XAS) provide critical
557 information in terms of distribution, atomic redox state, and atomic local structure, and add critical
558 knowledge necessary to understand the ENM-plant interactions. This information is highly relevant
559 with regard to potential applicability: ENMs can interact with sensitive ecosystem components within
560 trophic food chains, affect microbial populations in soil, enter into the plant and where they can be
561 translocated to different tissues and organs, including the edible tissues or organs (Holden et al., 2013;
562 Liu et al., 2015). Biotransformation can occur at each step within these processes, modifying and/or
563 amplifying ENM effects at organism level. These interactions from the level of ecosystem, organism,
564 tissue, cell, and organelles become key factors when applying “ENM biotransformation” as a concept
565 for a safer design, when considering applications for agriculture and food production, and for
566 minimizing the adverse biological impact (Burello & Worth, 2015; Pagano et al., 2018; Lowry et al.,
567 2019; Kah et al., 2019; Zulfiqar et al., 2019; Ma et al., 2020; Hameed et al., 2022; Xu et al., 2022).

568

569 *Author contributions*

570 Conceptualization: LP, MM; original draft preparation: LP, MM, RR; review and editing: JCW, NM.
571 All authors revised and agreed on the final version of the manuscript.

572

573 *Acknowledgements*

574 This work was supported by European Union's HORIZON 2020 research and innovation Programme
575 [grant number 818431 SIMBA], European Union's PRIMA Programme [grant number 1811
576 SUSTAINOLIVE], and FIL (Fondi Locali per la Ricerca). The funding sources had no involvement
577 in study design, collection, analysis and interpretation of data, writing, decision to submit the paper.

578

579 *Declaration of competing interest*

580 The authors declare no competing financial interests.

581

582

583 **References**

584 Abutalib M.M., Rajeh A. 2020. Enhanced structural, electrical, mechanical properties and
585 antibacterial activity of Cs/PEO doped mixed nanoparticles (Ag/TiO₂) for food packaging
586 applications. Polymer Testing. 107013.

587

588 Abdel Latef A.A.H., Srivastava A.K., El-sadek M.S.A., Kordrostami M., Tran L.-S.P. 2018. Titanium
589 dioxide nanoparticles improve growth and enhance tolerance of broad bean plants under saline soil
590 conditions. Land Degrad. Dev. 29, 1065–1073.

591

592 Akhtar N., Ilyas N., Meraj T.A., Pour-Aboughadareh A., Sayyed R.Z., Mashwani Z.U.R., Poczai P.
593 2022. Improvement of Plant Responses by Nanobiofertilizer: A Step towards Sustainable Agriculture.
594 Nanomaterials. 12, 965.

595

596 An J., Hu P., Li F., Wu H., Shen Y., White J.C., Tian X., Li Z., Giraldo J.P. 2020. Molecular
597 mechanisms of plant salinity stress tolerance improvement by seed priming with cerium oxide
598 nanoparticles. *Environ. Sci: Nano.* 7, 2214.

599

600 Arora S., Sharma P., Kumar S., Nayan R., Khanna P.K., Zaidi M.G.H. 2012. Gold-nanoparticle
601 induced enhancement in growth and seed yield of *Brassica juncea*. *Plant Growth Regul.* 66, 303–
602 310.

603

604 Burello E., Worth A.P. 2015. A rule for designing safer nanomaterials: do not interfere with the
605 cellular redox equilibrium. *Nanotoxicology.* 9, 116-117.

606

607 Castillo-Michel H.A., Larue C., Pradas del Real A.E., Cotte M., Sarret G. 2017. Practical review on
608 the use of synchrotron based micro- and nano- X-ray fluorescence mapping and X-ray absorption
609 spectroscopy to investigate the interactions between plants and engineered nanomaterials. *Plant*
610 *Physiol Biochem.* 110, 13-32.

611

612 Castro B., Citterico M., Kimura S., Stevens D.M., Wrzaczek M., Coaker G. 2021. Stress-induced
613 reactive oxygen species compartmentalization, perception and signalling. *Nat. Plants* 7, 403–412.

614

615 Chebakova K.A., Dzidziguri E.L., Sidorova E.N., Vasiliev A.A., Ozherelkov D.Y., Pelevin I.A.,
616 Gromov A.A., Nalivaiko A.Y. 2021. X-ray Fluorescence Spectroscopy Features of Micro- and
617 Nanoscale Copper and Nickel Particle Compositions. *Nanomaterials.* 14, 11(9), 2388.

618

619 Chen L., Peng Y., Zhu L., Huang Y., Bie Z., Wu H. 2022. CeO₂ nanoparticles improved cucumber
620 salt tolerance is associated with its induced early stimulation on antioxidant system. *Chemosphere.*
621 299, 134474.

622

623 Chang C., Bowman J.L., Meyerowitz E.M. 2016. Field Guide to Plant Model Systems. Cell. 167,
624 325-339.

625

626 Chung I.M., Rekha K., Venkidasamy B., Thiruvengadam M. 2019. Effect of Copper Oxide
627 Nanoparticles on the Physiology, Bioactive Molecules, and Transcriptional Changes in *Brassica rapa*
628 ssp. *rapa* Seedlings. Water. Air. Soil Pollut. 230, 48.

629

630 d'Acapito F., Lepore G.O., Puri A., Laloni A., La Manna F., Dettona E., De Luisa A., Martin A. 2019.
631 The LISA beamline at ESRF. J. Synchrotron Rad. 26, 551-558.

632

633 Deng C., Wang Y., Cota-Ruiz K., Reyes A., Sun Y., Peralta-Videa J.R., Hernandez-Viezcas J.A.,
634 Turley R.S., Niu G., Li C., Gardea-Torresdey J.L. 2020. Bok choy (*Brassica rapa*) grown in copper
635 oxide nanoparticles-amended soils exhibits toxicity in a phenotype-dependent manner: Translocation,
636 biodistribution and nutritional disturbance. J Haz Mater, 398, 122978.

637

638 Deng C., Wang Y., Navarro G., Sun Y., Cota-Ruiz K., Hernandez-Viezcas J.A., Niu G., Li C., White
639 J.C., Gardea-Torresdey J. 2022a. Copper oxide (CuO) nanoparticles affect yield, nutritional quality,
640 and auxin associated gene expression in weedy and cultivated rice (*Oryza sativa* L.) grains. Sci. Total
641 Environ. 810, 152260.

642

643 Deng C., Wang Y., Navarro G., Sun Y., Cota-Ruiz K., Hernandez-Viezcas J.A., Niu G., Li C., White
644 J.C., Gardea-Torresdey J. 2022b. Copper oxide (CuO) nanoparticles affect yield, nutritional quality,
645 and auxin associated gene expression in weedy and cultivated rice (*Oryza sativa* L.) grains, Scie Tot
646 Environ, 810, 152260.

647

648 Deng C., Wang Y., Cantu J.M., Valdes C., Navarro G., Cota-Ruiz K., Hernandez-Viezcas J.A., Li
649 C., Elmer W.H., Dimkpa C.O., White J.C., Gardea-Torresdey, J.L. 2022c. Soil and foliar exposure
650 of soybean (*Glycine max*) to Cu: Nanoparticle coating-dependent plant responses, *NanoImpact*,
651 26,100406.

652

653 Dimkpa C.O., McLean J.E., Latta D.E., Manangón E., Britt D.W., Johnson W.P., Boyanov M.I.,
654 Anderson A.J. 2012. CuO and ZnO nanoparticles: Phytotoxicity, metal speciation, and induction of
655 oxidative stress in sand-grown wheat. *J. Nanoparticle Res.* 14, 1125.

656

657 Dimkpa C.O., Singh U., Bindraban P.S., Elmer W.H., Gardea-Torresdey J.L., White J.C. 2019. Zinc
658 oxide nanoparticles alleviate drought-induced alterations in sorghum performance, nutrient
659 acquisition, and grain fortification. *Sci. Total Environ.* 688, 926–934.

660

661 Dwivedi A.D., Yoon H., Singh J.P., Chae K.H., Rho S-C., Hwang D.S., Chang Y-S. 2018. Uptake,
662 Distribution, and Transformation of Zerovalent Iron Nanoparticles in the Edible Plant *Cucumis*
663 *sativus*. *Environ Sci Technol.* 52 (17), 10057-10066.

664

665 EFSA. 2021. Safety assessment of titanium dioxide (E171) as a food additive. *EFSA Journal.* 19(5),
666 6585.

667

668 Elmer W., De la Torre-Roche R., Pagano L., Majumdar S., Zuverza-Mena N., Dimkpa C., Gardea-
669 Torresdey, J.L., White J.C. 2018. Effect of Metalloid and Metal Oxide Nanoparticles on Fusarium
670 Wilt of Watermelon. *Plant Dis.* 102, 1394–1401.

671

672 Eriksson P., Tal A.A., Skallberg A., Brommesson C., Hu Z., Boyd R.D., Olovsson W., Fairley N.,
673 Abrikosov I.A., Zhang X., Uvdal K. 2018. Cerium Oxide Nanoparticles with Antioxidant Capabilities
674 And Gadolinium Integration For MRI Contrast Enhancement. *Sci. Rep.* 8, 6999.
675

676 Faizan M., Yu F., Chen C., Faraz A., Hayat S. Zinc Oxide Nanoparticles Help to Enhance Plant
677 Growth and Alleviate Abiotic Stress: A Review. *Curr Protein Pept Sci.* 2021, 22(5), 362-375.
678

679 Gallo V., Zappettini A., Villani M., Marmioli N., Marmioli M. 2021. Comparative Analysis of
680 Proteins Regulated During Cadmium Sulfide Quantum Dots Response in *Arabidopsis thaliana* Wild
681 Type and Tolerant Mutants. *Nanomaterials.* 11, 615.
682

683 Gardea-Torresdey J.L., Rico C.M., White J.C. 2014. Trophic transfer, transformation, and impact of
684 engineered nanomaterials in terrestrial environments. *Environ. Sci. Technol.* 48, 2526–2540.
685

686 Giese B., Klaessig F., Park B., Kaegi R., Steinfeldt M., Wigger H., von Gleich A., Gottschalk F.
687 2018. Risks, Release and Concentrations of Engineered Nanomaterial in the Environment. *Sci Rep*
688 8, 1565.
689

690 Gohari G., Mohammadi A., Ak A., Panahirad S. 2020. Titanium dioxide nanoparticles (TiO₂ NPs)
691 promote growth and ameliorate salinity stress effects on essential oil profile and biochemical
692 attributes of *Dracocephalum moldavica*. *Sci. Rep.* 10, 912.
693

694 Goldstein J.I., Newbury D.E., Echlin P., Joy D.C., Lyman C.E., Lifshin E., Sawyer L., Michael J.R.
695 Editors. *Scanning Electron Microscopy and X-Ray Microanalysis*, Third Edition. 2003 Springer
696 Science+Business Media LLC, 233 Spring Street, New York, NY 10013 USA.
697

698 Gottschalk F., Sonderer T., Scholz R.W., Nowack B. 2009. Modeled Environmental Concentrations
699 of Engineered Nanomaterials (TiO₂, ZnO, Ag, CNT, Fullerenes) for Different Regions. *Environ. Sci.*
700 *Technol.* 43, 24, 9216–9222.

701

702 Gräfe M., Donner E., Collins R.N., Lombi E. 2014. Speciation of metal(loid)s in environmental
703 samples by X-ray absorption spectroscopy: A critical review. *Analytica Chimica Acta.* 822, 1-22.

704

705 Gui X., Rui M., Song Y., Yuhui M., Rui Y., Zhang P., He X., Li Y., Zhang Z., Liu L. 2017.
706 Phytotoxicity of CeO₂ nanoparticles on radish plant (*Raphanus sativus*). *Environ. Sci. Pollut. Res.*
707 24, 13775–13781.

708

709 Gurman S.J. 1995. Interpretation of EXAFS Data. *J. Synchr. Rad.* 2, (1), 56-63.

710

711 Hameed S., Baimanov D., Li X., Liua K., Wang L. 2022. Synchrotron radiation-based analysis of
712 interactions at the nano–bio interface. *Environ. Sci.: Nano.* Doi: 10.1039/D2EN00408A.

713

714 Hassanpouraghdam M.B., Mehrabani L.V., Bonabian Z., Aazami M.A., Rasouli F., Feldo M.,
715 Strzemski M., Dresler S. 2022. Foliar Application of Cerium Oxide-Salicylic Acid Nanoparticles
716 (CeO₂:SA Nanoparticles) Influences the Growth and Physiological Responses of *Portulaca oleracea*
717 L. under Salinity. *Int. J. Mol. Sci.* 23, 1–19.

718

719 Hernandez-Viezcas J.A., Castillo-Michel H., Andrews J.C., Cotte M., Rico C., Peralta-Videa J.R.,
720 Ge Y., Priester J.H., Holden P.A., Gardea-Torresdey J.L. 2013. In situ synchrotron X-ray
721 fluorescence mapping and speciation of CeO₂ and ZnO nanoparticles in soil cultivated soybean
722 (*Glycine max*). *ACS Nano.* 26, 7(2),1415-1423.

723

724 Holden P.A., Nisbet R.M., Lenihan H.S., Miller R.J., Cherr G.N., Schimel J.P., Gardea-Torresdey
725 J.L. 2013. Ecological Nanotoxicology: Integrating Nanomaterial Hazard Considerations Across the
726 Subcellular, Population, Community, and Ecosystems Levels. *Acc. Chem. Res.* 46, 813–822.
727

728 Hong J., Wang C., Wagner D.C., Gardea-Torresdey J.L., He F., Rico C.M. 2021. Foliar application
729 of nanoparticles: mechanisms of absorption, transfer, and multiple impacts. *Environ. Sci.: Nano*, 8,
730 1196-1210.
731

732 Howden R., Cobbett C.S. 1992. Cadmium-sensitive mutants of *Arabidopsis thaliana*. *Plant Physiol.*
733 99, 100–107.
734

735 Huang Y., Li W., Minakova A.S., Anumol T., Keller A.A. 2018. Quantitative analysis of changes in
736 amino acids levels for cucumber (*Cucumis sativus*) exposed to nano copper. *NanoImpact.* 12, 9–17.
737

738 Huangfu X., Xu Y., Liu C., He Q., Ma J., Ma C., Huang R. 2019. A review on the interactions between
739 engineered nanoparticles with extracellular and intracellular polymeric substances from wastewater
740 treatment aggregates. *Chemosphere.* 219, 766-783.
741

742 Imperiale D., Lencioni G., Marmiroli M., Zappettini A., White J.C., Marmiroli N. 2022. Interaction
743 of hyperaccumulating plants with Zn and Cd nanoparticles. *Sci Tot Environ.* 817, 152741.
744

745 Judy J.D., Unrine J.M., Rao W., Wirick S., Bertsch P.M. 2012. Bioavailability of gold nanomaterials
746 to plants: importance of particle size and surface coating. *Environ Sci Technol.* 7, 8467-8474.
747

748 Kah M., Kookana R.S., Gogos A., Bucheli T.D. 2018. A critical evaluation of nanopesticides and
749 nanofertilizers against their conventional analogues. *Nat. Nanotechnol.* 13, 677–684.

750

751 Kah M., Tufenkji N., White J.C. 2019. Nano-enabled strategies to enhance crop nutrition and
752 protection. *Nat. Nanotechnol.* 14, 532–540.

753

754 Karny A., Zinger A., Kajal A., Shainsky-Roitman J., Schroeder A. 2018. Therapeutic nanoparticles
755 penetrate leaves and deliver nutrients to agricultural crops. *Sci Rep.* 8(1), 7589.

756

757 Kaveh R., Li Y.S., Ranjbar S., Tehrani R., Brueck C.L., Van Aken B. 2013. Changes in *Arabidopsis*
758 *thaliana* gene expression in response to silver nanoparticles and silver ions. *Environ. Sci. Technol.*
759 47 (18), 10637–10644.

760

761 Keller A.A., McFerran S., Lazareva A., Suh S. 2013. Global life cycle releases of engineered
762 nanomaterials. *J. Nanopart. Res.* 15, 1692–1709.

763

764 Keller A.A., Lazareva A. Predicted Releases of Engineered Nanomaterials: From Global to Regional
765 to Local. *Environ. Sci. Technol. Lett.* 2014, 1, 1, 65–70.

766

767 Kumar V., Guleria P., Kumar V., Yadav S.K. 2013. Gold nanoparticle exposure induces growth and
768 yield enhancement in *Arabidopsis thaliana*. *Sci. Total Environ.* 461–462, 462–468.

769

770 Kumari P., Alam M., Siddiqi W.A. 2019. Usage of nanoparticles as adsorbents for wastewater
771 treatment: An emerging trend. *Sustain. Mater. Technol.* 22, e00128.

772

773 Lahuta L.B., Szablińska-Piernik J., Głowacka K., Stałanowska K., Railean-Plugaru V., Horbowicz
774 M., Pomastowski P., Buszewski B. 2022. The Effect of Bio-Synthesized Silver Nanoparticles on

775 Germination, Early Seedling Development, and Metabolome of Wheat (*Triticum aestivum* L.).
776 *Molecules*. 27, 2303.
777

778 Landa P., Prerostova S., Petrova S., Knirsch V., Vankova R., Vanek T. 2015. The Transcriptomic
779 Response of *Arabidopsis thaliana* to Zinc Oxide: A Comparison of the Impact of Nanoparticle, Bulk,
780 and Ionic Zinc. *Environ. Sci. Technol.* 49 (24), 14537–14545.
781

782 Larue C., Castillo-Michel H., Sobanska S., Cécillon L., Bureau S., Barthès V., Ouerdane L., Carrière
783 M., Sarret G. 2014. Foliar exposure of the crop *Lactuca sativa* to silver nanoparticles: Evidence for
784 internalization and changes in Ag speciation. *J Haz Mat.* 264, 98-106.
785

786 Li M., Zhang P., Adeel M., Guo Z., Chetwynd A.J., Ma C., Bai T., Hao Y., Rui Y. 2021. Physiological
787 impacts of zero valent iron, Fe₃O₄ and Fe₂O₃ nanoparticles in rice plants and their potential as Fe
788 fertilizers. *Environ. Pollut.* 269, 116134.
789

790 Liu W., Tian S., Zhao X., Xie W., Gong Y., Zhao D. 2015. Application of Stabilized Nanoparticles
791 for In Situ Remediation of Metal-Contaminated Soil and Groundwater: a Critical Review. *Curr.*
792 *Pollut. Reports.* 1, 280–291.
793

794 Liu Y., Persson D.P., Li J., Liang Y., Li T. 2021. Exposure of cerium oxide nanoparticles to the
795 hyperaccumulator *Sedum alfredii* decreases the uptake of cadmium via the apoplastic pathway. *J.*
796 *Hazard. Mater.* 417, 125955.
797

798 Lizzi D., Mattiello A., Piani B., Fellet G., Adamiano A., Marchiol L. 2020. Germination and early
799 development of three spontaneous plant species exposed to nanoceria (nCeO₂) with different
800 concentrations and particle sizes. *Nanomaterials.* 10, 1–16.

801

802 Lopez-Lima D., Mtz-Enriquez A.I., Carrión G., Basurto-Cereceda S., Pariona N. 2021. The
803 bifunctional role of copper nanoparticles in tomato: Effective treatment for Fusarium wilt and plant
804 growth promoter. *Sci. Hortic.* 2, 277.

805

806 López-Moreno M.L., de la Rosa G., Hernández-Viezcas J.A., Castillo-Michel H., Botez C.E., Peralta-
807 Videa J.R., Gardea-Torresdey J.L. 2010. Evidence of the Differential Biotransformation and
808 Genotoxicity of ZnO and CeO₂ Nanoparticles on Soybean (*Glycine max*) Plants. *Environ. Sci.*
809 *Technol.* 44, 19, 7315–7320.

810

811 Lowry G.V., Avellan A., Gilbertson L.M. 2019. Opportunities and challenges for nanotechnology in
812 the agri-tech revolution. *Nat. Nanotechnol.* 14, 517–522.

813

814 Lv J., Zhang S., Luo L., Zhang J., Yang K., Christie P. 2015. Accumulation, speciation and uptake
815 pathway of ZnO nanoparticles in maize. *Environ. Sci.: Nano.* 2, 68.

816

817 Lv J., Christie P., Zhang S. 2019. Uptake, translocation, and transformation of metal-based
818 nanoparticles in plants: recent advances and methodological challenges. *Environ. Sci.: Nano.* 6, 41-
819 59.

820

821 Lv Z., Sun H., Du W., Li R., Mao H., Kopittke P.M. 2021. Interaction of different-sized ZnO
822 nanoparticles with maize (*Zea mays*): Accumulation, biotransformation and phytotoxicity. *Sci Tot*
823 *Environ.* 796, 148927.

824

825 Ma C., White J.C., Zhao J., Zhao Q., Xing B. 2018. Uptake of Engineered Nanoparticles by Food
826 Crops: Characterization, Mechanisms, and Implications. *Annu. Rev. Food Sci. Technol.* 9, 129–153.

827

828 Ma C., Borgatta J., Hudson B.G., Tamijani A.A., De La Torre-Roche R., Zuverza-Mena N., Shen Y.,
829 Elmer W., Xing B., Mason S. E., Hamers R. J., White J.C. 2020. Advanced material modulation of
830 nutritional and phytohormone status alleviates damage from soybean sudden death syndrome. Nat.
831 Nanotechnol. 15, 1033–1042.

832

833 Ma Y., Zhang P., Zhang Z., He H., Li Y., Zhang J., Zheng L., Chu S., Yang K., Zhao Y., Chai Z.
834 2015. Origin of the different phytotoxicity and biotransformation of cerium and lanthanum oxide
835 nanoparticles in cucumber, Nanotoxicology. 9(2), 262–270.

836

837 Majumdar S., Almeida I.C., Arigi E.A., Choi H., VerBerkmoes N.C., Trujillo-Reyes J., Flores-
838 Margez J.P., White J.C., Peralta-Videa J.R., Gardea-Torresdey J.L. 2015. Environmental Effects of
839 Nanocerium on Seed Production of Common Bean (*Phaseolus vulgaris*): A Proteomic Analysis.
840 Environ. Sci. Technol. 49, 22, 13283–13293.

841

842 Majumdar S., Pagano L., Wohlschlegel J.A., Villani M., Zappettini A., White J.C., Keller A.A. 2019.
843 Proteomic, Gene And Metabolite Characterization Reveal The Uptake And Toxicity Mechanisms Of
844 Cadmium Sulfide Quantum Dots In Soybean Plants. Environ. Sci.: Nano. 6, 3010–3026.

845

846 Manzoor N., Ahmed T., Noman M., Shahid M., Mudassir M., Ali L., Alnusaire T.S., Li B., Schulin
847 R., Wang G. 2021. Science of the Total Environment Iron oxide nanoparticles ameliorated the
848 cadmium and salinity stresses in wheat plants, facilitating photosynthetic pigments and restricting
849 cadmium uptake. Sci. Total Environ. 769, 145221.

850

851 Marmiroli M., Pagano L., Savo Sardaro M.L., Villani M., Marmiroli N. 2014. Genome-wide
852 approach in *Arabidopsis thaliana* to assess the toxicity of cadmium sulfide quantum dots. Environ.
853 Sci. Technol. Environ. Sci. Technol. 48, 5902–5909.

854

855 Marmiroli M., Imperiale D., Pagano L., Villani M., Zappettini A., Marmiroli N. 2015. The Proteomic
856 Response of *Arabidopsis thaliana* to Quantum Dots, and Its Correlation with the Transcriptomic
857 Response. Front. Plant Sci. 6, 1104.

858

859 Marmiroli M., Lepore G.O., Pagano L., d’Acapito F., Gianoncelli A., Villani M., Lazzarini L., White
860 J.C., Marmiroli N. 2020. The fate of CdS Quantum Dots in plants as revealed by Extended X-ray
861 Absorption Fine Structure (EXAFS) analysis. Environ. Sci. Nano 7, 1150–1162.

862

863 Marmiroli M., Pagano L., Rossi R., De La Torre-Roche R., Lepore G.O., Ruotolo R., Gariani G.,
864 Bonanni V., Pollastri S., Puri A., Gianoncelli A., Aquilanti G., d’Acapito F., White J.C., Marmiroli
865 N. 2021. Copper Oxide nanomaterial fate in plant tissue: Nanoscale impacts on reproductive tissues.
866 Environ Sci Technol. 55, 15, 10769–10783.

867

868 Mattiello A., Filippi A., Poscic F., Musetti R., Salvatici M.C., Giordano C., Vischi M., Bertolini A.,
869 Marchiol L. 2015. Evidence of Phytotoxicity and Genotoxicity in *Hordeum vulgare* L. Exposed to
870 CeO₂ and TiO₂ Nanoparticles. Front. Plant Sci. 6, 1043.

871

872 Milosevic A., Romeo D., Wick P. 2020. Understanding Nanomaterial Biotransformation: An Unmet
873 Challenge to Achieving Predictive Nanotoxicology. Small. 1907650.

874

875 Mitrano D.M., Motellier S., Clavaguera S., Nowack B. 2015. Review of nanomaterial aging and
876 transformations through the life cycle of nano-enhanced products, *Environment International*, 77,
877 132-147.

878

879 Morrissey J., Guerinot M.L. 2009. Iron uptake and transport in plants: the good, the bad, and the
880 ionome. *Chem. Rev.* 109 (10), 4553–67.

881

882 Mustafa H., Ilyas N., Akhtar N., Iqbal N., Zainab T. 2021. Biosynthesis and characterization of
883 titanium dioxide nanoparticles and its effects along with calcium phosphate on physicochemical
884 attributes of wheat under drought stress. *Ecotoxicol. Environ. Saf.* 223, 112519.

885

886 Nasir Khan M. 2016. Nano-titanium Dioxide (Nano-TiO₂) Mitigates NaCl Stress by Enhancing
887 Antioxidative Enzymes and Accumulation of Compatible Solutes in Tomato (*Lycopersicon*
888 *esculentum* Mill.). *J. Plant Sciences.* 11, 1-11.

889

890 Neves V.M., Heidrich G.M., Rodrigues E.S., Enders M.S.P., Muller E.I., Nicoloso F.T., Carvalho
891 H.W.P. De, Dressler V.L. 2019. La₂O₃ Nanoparticles: Study of Uptake and Distribution in *Pfaffia*
892 *glomerata* (Spreng.) Pedersen by LA-ICP-MS and μ - XRF. *Environ. Sci. Technol.* 53, 10827–10834.

893

894 Oh E., Liu R., Nel A, Boeneman Gemill K., Bilal M., Cohen Y., Medintz I.L. 2016. Meta-analysis of
895 cellular toxicity for cadmium-containing quantum dots. *Nature Nanotech.* 11, 479–486.

896

897 Pagano L., Servin A.D., De La Torre-Roche R., Mukherjee A., Majumdar S., Hawthorne J.,
898 Marmiroli M., Maestri E., Marra R.E., Isch S.M., Dhankher O.P., White J. C., Marmiroli N. 2016.
899 Molecular Response of Crop Plants to Engineered Nanomaterials. *Environ. Sci. Technol.* 50 (13),
900 7198–7207.

901

902 Pagano L., Pasquali F., Majumdar S., De La Torre-Roche R., Zuverza-Mena N., Villani M.,
903 Zappettini A., Marra R.E., Isch S.M., Marmiroli M., Maestri E., Dhankher O.P., White J.C.,
904 Marmiroli N. 2017. Exposure of *Cucurbita pepo* to binary combinations of engineered nanomaterials:
905 Physiological and molecular response. *Environ. Sci.: Nano.* 4, 1579–1590.

906

907 Pagano L., Maestri E., White J.C., Marmiroli N., Marmiroli M. 2018. Quantum dots exposure in
908 plants: Minimizing the adverse response. *COESH.* 6, 71–76.

909

910 Pagano L., Rossi R., Paesano L., Marmiroli N., Marmiroli M. 2021. miRNA regulation and stress
911 adaptation in plants. *Env. Exp. Bot.* 104369.

912

913 Pagano L., Marmiroli M., Villani M., Magnani J., Rossi R., Zappettini A., White J.C., Marmiroli N.
914 2022. Engineered nanomaterial exposure affects organelle genetic material replication in *Arabidopsis*
915 *thaliana*. *ACS Nano.* 16, 2249–2260.

916

917 Prado de Moraes A.C., Ribeiro L. da S., de Camargo E.R., Lacava P.T. 2021. The potential of
918 nanomaterials associated with plant growth-promoting bacteria in agriculture. *3 Biotech.* 11, 1–17.

919

920 Proux O., Lahera E., Del Net W., Kieffer I., Rovezzi M., Testemale D., Irar M., Thomas S., Aguilar-
921 Tapia A., Bazarkina E.F., Prat A., Tella M., Auffan M., Rose J., Hazemann J.L. 2017. High-Energy
922 Resolution Fluorescence Detected X-Ray Absorption Spectroscopy: A Powerful New Structural Tool
923 in Environmental Biogeochemistry Sciences. *J Environ Qual.* 46, 1146-1157.

924

925 Puri A., Lepore G.O., d'Acapito F. 2019. The New Beamline LISA at ESRF: Performances and
926 Perspectives for Earth and Environmental Sciences. *Condensed Matter.* 4, 12.

927

928 Rawat S., Pullagurala V.L.R., Adisa I.O., Wang Y., Peralta-Videa J.R., Gardea-Torresdey J.L. 2018.
929 Factors affecting fate and transport of engineered nanomaterials in terrestrial environments, COESH,
930 6, 47-53.

931

932 Rincon A.M. 2019. Chapter 6 - Presence of nanomaterials on consumer products: food, cosmetics,
933 and drugs. pp. 165-181. Marmiroli N., White J.C., Song J., Eds: In Micro and Nano Technologies,
934 Exposure to Engineered Nanomaterials in the Environment, Elsevier, Amsterdam, The Netherlands.

935

936 Rizwan M., Ali S., Ali B., Adrees M., Arshad M., Hussain A., Zia ur Rehman M., Waris A.A. 2019.
937 Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and
938 cadmium concentration in wheat. Chemosphere. 214, 269–277.

939

940 Rodrigues E.S., Montanha G.S., de Almeida E., Fantucci H., Santos R.M., de Carvalho H.W.P. 2021.
941 Effect of nano cerium oxide on soybean (*Glycine max* L. Merrill) crop exposed to environmentally
942 relevant concentrations. Chemosphere. 273, 128492.

943

944 Rossi L., Zhang W., Schwab A.P., Ma X. 2017. Uptake, Accumulation, and in Planta Distribution of
945 Coexisting Cerium Oxide Nanoparticles and Cadmium in *Glycine max* (L.) Merr. Environ. Sci.
946 Technol. 51, 12815–12824.

947

948 Roubeau Dumont E., Elger A., Azéma C., Castillo H., Surble S., Larue C. 2022. Cutting-edge
949 spectroscopy techniques highlight toxicity mechanisms of copper oxide nanoparticles in the aquatic
950 plant *Myriophyllum spicatum*. Sci. Total Environ. 803, 150001.

951

952 Rui Y., Zhang P., Zhang T., Ma Y., He X., Gui X., Li Y., Zhang J., Zheng L., Chu S., Guo Z., Chai
953 Z., Zhao Y., Zhang Z. 2015. Transformation of ceria nanoparticles in cucumber plants is influenced
954 by phosphate, *Environmental Pollution*. 198, 8-14.

955

956 Ruotolo R., Maestri E., Pagano L., Marmiroli M., White J.C., Marmiroli N. 2018. Plant response to
957 metal-containing engineered nanomaterials: an omics-based perspective. *Environ Sci Technol*. 52, 5,
958 2451-2467.

959

960 Sabo-Attwood T., Unrine J.M., Stone J.W., Murphy C.J., Ghoshroy S., Blom D., Bertsch P.M.,
961 Newman L.A. 2011. Uptake, distribution and toxicity of gold nanoparticles in tobacco (*Nicotiana*
962 *xanthi*) seedlings. *Nanotoxicology*. 6(4), 353-360.

963

964 Sarret G., Pilon Smits E.A.H., Castillo Michel H., Isaure M.P., Zhao F.J., Tappero R. 2013. Chapter
965 One - Use of Synchrotron-Based Techniques to Elucidate Metal Uptake and Metabolism in Plants,
966 *Adv. Agron*. 119, 1–82.

967

968 Schwab F., Zhai G., Kern M., Turner A., Schnoor J.L., Wiesner M.R. 2016. Barriers, pathways and
969 processes for uptake, translocation and accumulation of nanomaterials in plants – Critical review.
970 *Nanotoxicology*. 10, 257–278.

971

972 Semida W.M., Abdelkhalik A., Mohamed G.F., Abd El-Mageed T.A., Abd El-Mageed S.A., Rady
973 M.M., Ali E.F. 2021. Foliar application of zinc oxide nanoparticles promotes drought stress tolerance
974 in eggplant (*Solanum melongena* L.). *Plants*. 10, 1–18.

975

976 Servin A.D., Castillo-Michel H., Hernandez-Viezcas J.A., Corral Diaz B., Peralta-Videa J.R.,
977 Gardea-Torresdey J.L. 2012. Synchrotron Micro-XRF and Micro-XANES Confirmation of the

978 Uptake and Translocation of TiO₂ Nanoparticles in Cucumber (*Cucumis sativus*) Plants. Environ.
979 Sci. Technol. 46, 14, 7637–7643.

980

981 Servin A.D., Morales M.I., Castillo-Michel H., Hernandez-Viezcas J.A., Munoz B., Zhao L., Nunez
982 J.E., Peralta-Videa J.R., Gardea-Torresdey J.L. 2013. Synchrotron Verification of TiO₂
983 Accumulation in Cucumber Fruit: A Possible Pathway of TiO₂ Nanoparticle Transfer from Soil into
984 the Food Chain. Environ. Sci. Technol. 47, 20, 11592–11598.

985

986 Servin A.D., Pagano L., Castillo-Michel H., De la Torre-Roche R., Hawthorne J., Hernandez-Viezcas
987 J.A., Loredó-Portales R., Majumdar S., Gardea-Torresday J.L., Dhankher O.P., White J.C. 2017a.
988 Weathering in soil increases nanoparticle CuO bioaccumulation within a terrestrial food chain.
989 Nanotoxicology. 11, 98–111.

990

991 Servin A.D., De la Torre-Roche R., Castillo-Michel H., Pagano L., Hawthorne J., Musante C.,
992 Pignatello J., Uchimiya M., White J.C. 2017b. Exposure of agricultural crops to nanoparticle CeO₂
993 in biochar-amended soil. Plant Physiology and Biochemistry. 110, 147-157.

994

995 Siddiqi K.S., Husen A. 2017. Engineered Gold Nanoparticles and Plant Adaptation Potential.
996 Nanoscale Res Lett. 11, 400-409.

997

998 Song U., Jun H., Waldman B., Roh J., Kim Y., Yi J., Lee E.J. 2013. Functional analyses of
999 nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon*
1000 *esculentum*). Ecotoxicol. Environ. Saf., 93, 60-67.

1001

1002 Stegemeier J.P., Schwab F., Colman B.P., Webb S.M., Newville M., Lanzirotti A., Winkler C.,
1003 Wiesner M.R., Lowry G.V. 2015. Speciation Matters: Bioavailability of Silver and Silver Sulfide
1004 Nanoparticles to Alfalfa (*Medicago sativa*). Environ Sci Technol. 49 (14), 8451-8460.
1005
1006 Syu Y., Hung J.H., Chen J.C., Chuang H. 2014. Impacts of size and shape of silver nanoparticles on
1007 Arabidopsis plant growth and gene expression. Plant Physiol. Biochem. 83, 57-64.
1008
1009 Tamez C., Hernandez-Molina M., Hernandez-Viezcas J.A., Gardea-Torresdey J.L. 2019. Uptake,
1010 transport, and effects of nano-copper exposure in zucchini (*Cucurbita pepo*). Sci. Total Environ. 665,
1011 100-106.
1012
1013 Verma K.K., Song X.-P., Joshi A., Tian D.-D., Rajput V.D., Singh M., Arora J., Minkina T., Li Y.-
1014 R. 2022. Recent Trends in Nano-Fertilizers for Sustainable Agriculture under Climate Change for
1015 Global Food Security. Nanomaterials. 12, 173.
1016
1017 Wang J., Koo Y., Alexander A., Yang Y., Westerhof S., Zhang Q., Schnoor J.L., Colvin V.L., Braam
1018 J., Alvarez P.J.J. 2013. Phytostimulation of poplars and Arabidopsis exposed to silver nanoparticles
1019 and Ag⁺ at sublethal concentrations. Environ. Sci. Technol. 47, 5442–5449.
1020
1021 Wang Y., Lin Y., Xu Y., Yin Y., Guo H., Du W. 2019. Divergence in response of lettuce (var. ramosa
1022 Hort.) to copper oxide nanoparticles/microparticles as potential agricultural fertilizer. Environ. Pollut.
1023 Bioavailab. 31, 80–84.
1024
1025 Wang Y., Deng C., Cota-Ruiz K., Tan W., Reyes A., Peralta-Videa J.R., Hernandez-Viezcas J.A., Li
1026 C., Gardea-Torresdey J.L. 2021a. Effects of different surface-coated nTiO₂ on full-grown carrot
1027 plants: Impacts on root splitting, essential elements, and Ti uptake. J Haz Mater, 402, 123768.

1028

1029 Wang Y., Deng C., Cota-Ruiz K., Peralta-Videa J.R., Hernandez-Viezcas J.A., Gardea-Torresdey
1030 J.L. 2021b. Soil-aged nano titanium dioxide effects on full-grown carrot: Dose and surface-coating
1031 dependent improvements on growth and nutrient quality. *Scie Tot Environ*, 774, 145699.

1032

1033 Wang Y., Deng C., Elmer W.H., Dimkpa C.O., Sharma S., Navarro G., Wang Z., LaReau J., Steven
1034 B.T., Wang Z., Zhao L., Li C., Parkash Dhankher O., Gardea-Torresdey J.L., Xing B., White J.C.
1035 2022. Therapeutic Delivery of Nanoscale Sulfur to Suppress Disease in Tomatoes: In Vitro Imaging
1036 and Orthogonal Mechanistic Investigation. *ACS Nano*, 16, 7, 11204–11217.

1037

1038 White J.C., Zuverza-Mena N., Elmer W.H. 2022. From nanotoxicology to nano-enabled agriculture:
1039 Following the science at the Connecticut Agricultural Experiment Station (CAES). *Plant Nano*
1040 *Biology*, 1, 100007. Doi: 10.1016/j.plana.2022.100007.

1041

1042 Willett W., Rockström J., Loken B., Springmann M., Lang T., Vermeulen S., Garnett T., Tilman D.,
1043 DeClerck F., Wood A., Jonell M., Clark M., Gordon L.J., Fanzo J., Hawkes C., Zurayk R., Rivera
1044 J.A., De Vries W., Sibanda L.M., Afshin A., Chaudhary A., Herrero M., Agustina R., Branca F.,
1045 Lartey A., Fan S., Crona B., Fox E., Bignet V., Troell M., Lindahl T., Singh S., Cornell S.E., Reddy
1046 K.S., Narain S., Nishtar S., Murray C.J.L. 2019. Food in the Anthropocene: the EAT–Lancet
1047 Commission on healthy diets from sustainable food systems. *Lancet*. 393, 447–492.

1048

1049 Xiao Z., Yue L., Wang C. Chen F., Ding Y., Liu Y., Cao X., Chen Z., Rasmann S., Wang Z. 2021.
1050 Downregulation of the photosynthetic machinery and carbon storage signaling pathways mediate
1051 La₂O₃ nanoparticle toxicity on radish taproot formation. *J. Hazard. Mater.* 411, 124971.

1052

1053 Xiong Z., Zhang T., Xian Y., Kang Z., Zhang S., Dumat C., Shahid M., Li S. 2021. Foliar uptake,
1054 biotransformation, and impact of CuO nanoparticles in *Lactuca sativa* var. *ramosa* Hort. Environ.
1055 Geochem. Health. 43(1), 423-439.
1056

1057 Xu T., Wang Y., Aytac Z., Zuverza-Mena N., Zhao Z., Hu X., Ng K.W., White J.C., Demokritou P.
1058 2022. Enhancing Agrichemical Delivery and Plant Development with Biopolymer-Based Stimuli
1059 Responsive Core–Shell Nanostructures. ACS Nano 16, 4, 6034–6048.
1060

1061 Yang J., Jiang F., Ma C., Rui Y., Rui M., Adeel M., Cao W., Xing B. 2018. Alteration of Crop Yield
1062 and Quality of Wheat upon Exposure to Silver Nanoparticles in a Life Cycle Study. J. Agric. Food
1063 Chem. 66, 2589–2597.
1064

1065 Yin L., Cheng Y., Espinasse B., Colman B.P., Au M., Wiesner M., Rose J., Liu J., Bernhardt E.S.
1066 2011. More than the Ions: The Effects of Silver Nanoparticles on *Lolium multiflorum*. Environ. Sci.
1067 Technol. 45, 2360–2367.
1068

1069 Yue L., Chen F., Yu K., Xiao Z., Yu X., Wang Z., Xing B. 2019. Early development of apoplastic
1070 barriers and molecular mechanisms in juvenile maize roots in response to La₂O₃ nanoparticles. Sci.
1071 Total Environ. 653, 675–683.
1072

1073 Zafar S, Perveen S, Kamran Khan M, Shaheen MR, Hussain R, Sarwar N, Rashid S., Nafees M.,
1074 Farid G., Alamri S., Shah A.A., Javed T., Irfan M., Siddiqui M.H. 2022. Effect of zinc nanoparticles
1075 seed priming and foliar application on the growth and physio-biochemical indices of spinach
1076 (*Spinacia oleracea* L.) under salt stress. PLoS ONE, 17(2), e0263194.
1077

1078 Zhong Z., Zhu L., Young S. 2020. Approximation Framework of Embodied Energy of Safety:
1079 Insights and Analysis. *Energies*. 13, 4230.

1080

1081 Zulficar F., Navarro M., Ashraf M., Akram N.A., Munné-Bosch S. 2019. Nanofertilizer Use For
1082 Sustainable Agriculture: Advantages And Limitations. *Plant Science*. 289, 110270.

1083

1084 Zuverza-Mena N., Armendariz R., Peralta-Videa J.R., Gardea-Torresdey J.L. 2016. Effects of silver
1085 nanoparticles on radish sprouts: Root growth reduction and modifications in the nutritional value.
1086 *Front. Plant Sci.* 7, 1–11.

1087

1088 Zuverza-Mena N., Martínez-Fernandez D., Du W., Hernandez-Viezcas J.A., Bonilla-Bird N., Lopez-
1089 Moreno M.L., Komarek M., Peralta-Videa J.R., Gardea-Torresdey J.L. 2017. Exposure of engineered
1090 nanomaterials to plants: insights into the physiological and biochemical responses-A review. *Plant*
1091 *Physiol. Biochem.* 110, 236-264.

1092

1093

1094 **Figure captions and Tables**

1095

1096 Figure 1. Schematic representation of principal effects emerging after biotransformation of ENMs in
1097 soil, on foliar surface and within the plant tissues. ENMs in soil, due to particle and soil physico-
1098 chemical interactions, may remain unmodified, or may undergo to potential dissolution (with ions
1099 release), or undergo homo-/hetero-aggregation, which may highly influence particle stability and
1100 potential bioavailability (and consequently their uptake). On the foliar surface, temperature and light
1101 may also affect particle stability before uptake into the leaf tissues. Once within the plant, ENMs
1102 pristine, or modified, can interact with several biomolecule types (peptides, sugars, lipids, nucleic
1103 acids, secondary metabolites) leading to phenomena such as coating, enzymatic degradation,

1104 chelation or functionalization, which may influence the biotransformed particle at level of
1105 translocation, storage or reactivity. These parameters may also influence the interaction within the
1106 plant cell, triggering differential responses (*e.g.*, toxicity, oxidative stress, ROS production), which
1107 may be indirectly measured by physiological and molecular assays, but directly observed through
1108 physical strategies, including synchrotron-based methods.

1109

1110 Figure 2. Principal effects of ENM biotransformation in plant and relevant biomarkers observed in
1111 different plant species from model organisms (*A. thaliana*) to crops (*C. pepo*; *S. lycopersicum*; *G.*
1112 *max*) and different tissues (roots, leaves and flowers/pollen). Relevant ENM parameters such as size,
1113 stability, dissolution may influence the translocation from roots to shoots. Potential biotransformation
1114 mechanisms that may occur within plant tissues are also reported: enzymatic degradation, protein
1115 functionalization, functionalization at the level of cytoplasm and organelles (organic acids, thiol-
1116 containing compounds, aminoacids, sugars, secondary metabolites). In this scenario, chloroplast
1117 become not only a in important actor in the energy production but also one of the key targets and
1118 main regulators involved in the ENM exposure and response. Details on the biomarkers generated
1119 are reported in Table 1.

1120

1121

1122

1123

1124 Table 1. Genes as potential biomarkers of exposure/effect observed in roots, leaves and pollen, in
1125 different plant species (reported in Figure 2). Genes reported cannot be considered only as modulated
1126 in the different plant organs by the different type of ENMs, but also they showed a nano-specificity
1127 during the ENM response. It is also important to observe how, depending on the ENM type,
1128 biotransformation, and as indirect consequence, the transcriptomic response can be convergent
1129 between different forms (nano, bulk, ion) of the same element (see Figure 2).

flower & pollen					
plant	gene	function	pathway	ENM	reference
<i>Cucurbita pepo</i> L.	Cp4.1LG00g07430	Beta-galactosidase	primary metabolism	nCuO	Marmiroli <i>et al.</i> , 2021
	Cp4.1LG13g06380	Pectinesterase	primary metabolism		
	Cp4.1LG12g04750	Phosphatidylinositol 3-/4-kinase family protein	primary metabolism		
	Cp4.1LG03g02560	Delta-1-pyrroline-5-carboxylate synthetase	primary metabolism		
	Cp4.1LG02g07240	Leucine-rich repeat family protein	signaling, stress response		
	Cp4.1LG02g12750	Protein EFR3 like	signaling		

leaf					
plant	gene	function	pathway	ENM	reference
<i>Cucurbita pepo</i> L.	Cp4.1LG01(*)	PSBN, photosystem II reaction center protein N	chloroplast electron transport	nCeO2 nLa2O3	Pagano <i>et al.</i> , 2016 Pagano <i>et al.</i> , 2017
	Cp4.1LG00(*)	PetL, component of Cytochrome b6f	chloroplast electron transport	nCuO nZnO CdS QD	
<i>Solanum lycopersicum</i> L.	Solyc09g074540	PetL, component of Cytochrome b6f	chloroplast electron transport	nCeO2 nLa2O3 nCuO	Pagano <i>et al.</i> , 2016
<i>Glicine max</i> L.	Glyma12g36130	PetL, component of Cytochrome b6f	chloroplast electron transport	CdS QD	Majumdar <i>et al.</i> , 2019
<i>Arabidopsi thaliana</i> L.	AtCg00590	PetL, component of Cytochrome b6f	chloroplast electron transport	CdS QD	Marmiroli <i>et al.</i> , 2014
	AtCg00270	PSBN, photosystem II reaction center protein N	chloroplast electron transport		

root					
plant	gene	function	pathway	ENM	reference
<i>Cucurbita pepo</i> L.	Cp4.1LG16g08630	BIP3, Heat shock protein 70 family protein	protein folding, stress response	nCeO2 nLa2O3 nCuO nZnO CdS QD	Pagano <i>et al.</i> , 2016 Pagano <i>et al.</i> , 2017
	Cp4.1LG00g00090	GPT2, glucose-6-phosphate/phosphate translocator	primary metabolism		
	Cp4.1LG05g08050	RPS12, ribosomal protein S12A	protein synthesis		
	Cp4.1LG05g11200	PLP2, phospholipase	biotic/abiotic stress response		
<i>Solanum lycopersicum</i> L.	Solyc08g006150	ChaC-like family protein	glutathione degradation	nCeO2 nLa2O3 nCuO	Pagano <i>et al.</i> , 2016
	Solyc03g081240	PRR5, pseudo-response regulator 5	biotic/abiotic stress response		
	Solyc10g005080	LHY1, Homeodomain-like superfamily protein	stress response		
<i>Glicine max</i> L.	Glyma19g45030	LHY1, Homeodomain-like superfamily protein	stress response	CdS QD	Majumdar <i>et al.</i> , 2019
	Glyma15g06800	PR1, pathogenesis-related gene 1	biotic/abiotic stress response		

1130

1131

1132

1133

1134 Table 2. Principal evidence of the ENM biotransformation in plant observed by physiological,
 1135 molecular and synchrotron-based analyses.

ENM	plant	physiological response	molecular response	techniques	biotransformation	reference
nCeO2	<i>Cucumis sativus L.</i>	nCeO2 exposure had no significant effects on the biomass production under both the +P and -P conditions. However, the uptake of Ce in the plants is different under the two conditions	-	TEM XANES NEXAFS	high stability	<i>Rui et al., 2015</i>
	<i>Lactuca sativa L.</i> <i>Cucurbita pepo L.</i> <i>Zea mays L.</i> <i>Glycine max L.</i>	biomass in the agricultural soil amended with biochar 600°C was largely unaffected	-	SEM μ-XRF μ-XANES	modified redox state, from Ce(IV) to Ce(III) low translocation from roots to shoots	<i>Servin et al., 2017</i>
nCeO2 nZnO	<i>Glycine max L.</i>	-	-	μ-XRF μ-XANES		<i>Hernandez-Viezcas et al., 2013</i>
nCeO2 nLa2O3	<i>Cucumis sativus L.</i>	nCeO2 had no phytotoxicity to cucumber at all tested concentrations, while nLa2O3 showed significant inhibition on root elongation, shoot elongation, root biomass, and shoot biomass, as well as induced more reactive oxygen species and cell death in roots	-	μ-XRF XAS	higher dissolution compared to nCeO2 moderate translocation from roots to shoots	<i>Ma et al., 2014</i>
nTiO2	<i>Cucumis sativus L.</i>	at all concentrations, nTiO2 significantly increased root length (average >300%)	-	μ-XRF μ-XANES	high stability	<i>Servin et al., 2012</i>
	<i>Cucumis sativus L.</i>	In nTiO2 treated plants, the chlorophyll content in leaves increased as the external concentration of NPs increased. nTiO2 treatments increased CAT activity in leaves.	-	μ-XRF μ-XANES FTIR	low translocation from roots to shoots	<i>Servin et al., 2013</i>
nAu	<i>Nicotiana tabacum L.</i>	leaf necrosis was observed after 14 days of exposure to 3.5 nm nAu	-	μ-XRF	high stability no changes in Au valence	<i>Sabo-Attwood et al., 2012</i>
nAg	<i>Lolium multiflorum L.</i>	nAg and ionic silver significantly reduced growth, resulting in shorter shoots and roots and lower biomass. The growth inhibition from nAg was stronger than that from AgNO3. Higher concentrations of AgNPs caused broken epidermis and rootcap. Cell structures were unaltered in AgNO3 treated roots.	-	μ-XRF XANES	low stability high translocation from roots to shoots	<i>Yin et al., 2011</i>
	<i>Lactuca sativa L.</i> <i>Cucurbita pepo L.</i> <i>Zea mays L.</i> <i>Glycine max L.</i>	fresh foliar biomass was unchanged. Chlorophyll a, chlorophyll b, carotenoid and pheophytin contents were not affect	-	SEM μ-XRF XANES		<i>Larue et al., 2013</i>
	<i>Medicago sativa L.</i>	-	-	TEM XRF		<i>Stegemeier et al., 2015</i>
nZVI	<i>Cucumis sativus L.</i>	nZVI treatments did not affected the biomass of plants in hydroponic or soil systems. Only nZVI treated plant shoots grown under hydroponic conditions exhibited increased biomass (15%). Chlorosis observed in the leaves of the control plants but not in the plants treated with nZVI	ATPase isoforms increased their expression in the roots of plant exposed to nZVI.	EXAFS	low stability limited translocation from roots to shoots modified particle structure	<i>Dwivedi et al., 2018</i>

1136 Table 2 continue in the next page ...

1137
1138

ENM	plant	physiological response	molecular response	techniques	biotransformation	reference
nZnO	<i>Zea Mays L.</i>	-	-	μ -XRF XANES	low stability high translocation from roots to shoots	<i>Lv et al., 2015</i>
	<i>Zea Mays L.</i>	By the 7th day, the treatment of 9 nm nZnO and ZnSO ₄ significantly reduced the dry weight of roots by 44% and 58% respectively, compared to the unexposed control plants. In general, ZnSO ₄ treatment had the greatest effect on root biomass, followed by 9 nm nZnO and finally 40 nm nZnO	-	μ -XRF		<i>Lv et al., 2021</i>
nCuO	<i>Nicotiana tabacum L.</i>	When exposed to equivalent weight of Cu, nCu ₂ O exhibited higher toxicity than nCuO, implying that the transformation may elevate the toxicity upon nCuO exposure	-	TEM XANES	low stability high translocation from roots to shoots consistent with an increased ion release modified redox state, from Cu(II) to Cu (I)	<i>Dai et al., 2019</i>
	<i>Lactuca sativa L.</i>	Cu exposure had limited impacts on lettuce biomass. For the unweathered exposures, only the root biomass of NP-exposed plants was less than in bulk treatment; no other tissue- specific differences were evident. In the W exposure, the total biomass ranged from 8.2 g (W NP) to 9.5 g (unexposed control); nCuO and ion-treated plant biomass was significantly less than the unexposed controls. With regard to individual tissues in the W exposure, there were no differences of significance in the root biomass.	The expression level of nine genes involved in Cu transport shows that the mechanisms of nCuO and bulk CuO response-accumulation are different from ionic Cu	μ -XRF XANES		<i>Servin et al., 2017</i>
	<i>Cucurbita pepo L.</i>	no impact on zucchini biomass, photosynthetic activity or cellular respiration.	RNA-seq analyses on vegetative and reproductive tissues highlighted common and nanoscale-specific components of the response. Mitochondrial and chloroplast functions were uniquely modulated in response to ENM exposure as compared with bulk and salt forms	μ -XRF XANES EXAFS		<i>Marmioli et al., 2021</i>
CdS QD	<i>Arabidopsis thaliana L.</i>	treatment with CdS QDs caused a slight stress that increased the biomass in the mutants, but not in the wt, while CdSO ₄ caused modest phytotoxicity to both the wt and mutants	-	EXAFS	high stability limited ion release high translocation modification in bonds distance	<i>Marmioli et al., 2020</i>