



Nanomaterials biotransformation: In planta mechanisms of action

Luca Pagano ^a, Riccardo Rossi ^{a,b}, Jason C. White ^c, Nelson Marmiroli ^{a,d}, Marta Marmiroli ^{a,e,*}

^a Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, 43124, Parma, Italy

^b Centro Interdipartimentale per L'Energia e L'Ambiente (CIDEA), University of Parma, 43124, Parma, Italy

^c The Connecticut Agricultural Experiment Station, New Haven, CT, 06504, USA

^d Consorzio Interuniversitario Nazionale per le Scienze Ambientali (CINSA), University of Parma, 43124, Parma, Italy

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

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ABSTRACT

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.

1. Engineered nanomaterial (ENM) biotransformation

1.1. ENMs: from exposure to biotransformation

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 behaviour 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

* Corresponding author. Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, 43124, Parma, Italy.

E-mail address: marta.marmiroli@unipr.it (M. Marmiroli).

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investigated (Keller & Lazareva, 2014; Mitrano et al., 2015). Considering the long-time span of use of select materials (1950–2050), 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 1000 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, 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 (Marmiroli 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).

1.2. Conceiving and studying the ENM biotransformation

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 Fig. 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). 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

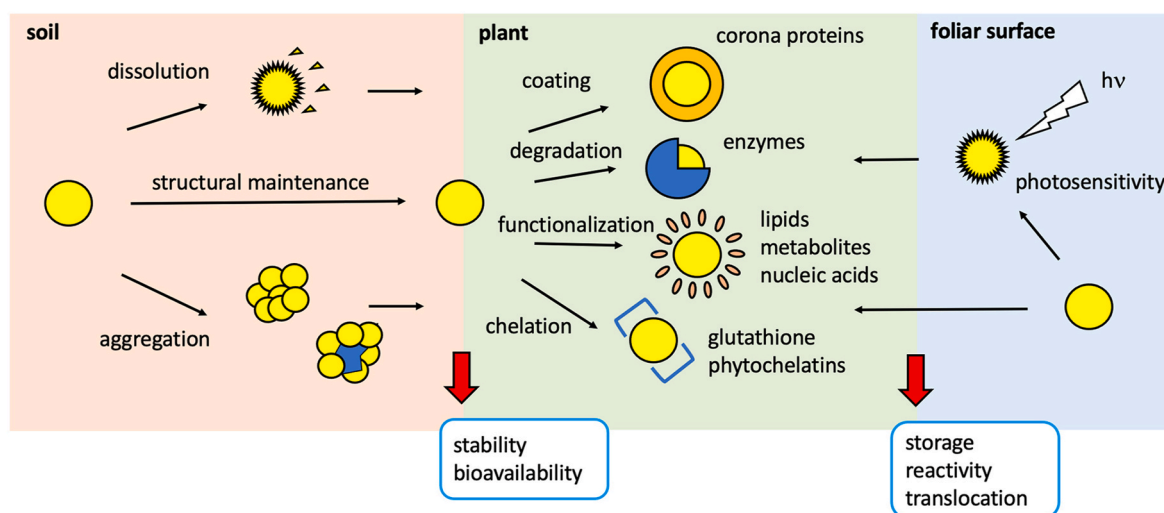


Fig. 1. Schematic representation of principal effects emerging after biotransformation of ENMs in soil, on foliar surface and within the plant tissues. ENMs in soil, due to particle and soil physico-chemical interactions, may remain unmodified, or may undergo to potential dissolution (with ions release), 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.

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

Micro-X-Ray Fluorescence (μ -XRF) and micro-X-Ray Adsorption Spectroscopy (μ -XAS) K-, L- or L_{III} -edge EXAFS and XANES spectra have been used to study the biotransformation of coated nanomaterials present in plants and soil (López-Moreno et al., 2010; Judy et al., 2012). μ -XRF is used for qualitative elemental analysis of heterogeneous biological samples. The interaction of the sample with high-energy X-ray radiation, which leads to X-ray absorption and emission of the fingerprint X-ray spectra for each element, is the key feature of this powerful analytical method. The absorption/excitation effect and relaxation process lead to atoms emitting fluorescence photons characterized by elemental specific energy (Chebakova et al., 2021). Extended X-ray absorption fine structure (EXAFS) is a technique that utilizes oscillations of the X-ray absorption coefficient on the high-energy side of an absorption edge. Such oscillations can reach up to 1000 eV above the edge and may have a magnitude of 10% or more. In addition, it is necessary to consider that atoms are not stationary. Thermal vibrations will obscure the EXAFS oscillations, and in the harmonic approximation, this can be accounted for by considering a Debye-Waller-type term. This led to a great improvement in the theoretical understanding of EXAFS and it is now established that a single scattering short-range order theory is adequate under most circumstances (Gurman, 1995). In addition, electrons that have undergone inelastic losses will not interfere in the process. This is considered by an exponential damping term. It is the limited range of the photoelectrons in the energy region of interest 50–1000 eV that allows for a short-range order description of EXAFS also in crystalline materials (Gurman, 1995). The region which includes the pre-edge, edge-jump and post-edge to approximately 30–50 eV is referred to as the X-ray Absorption Near Edge Structure (XANES), which describes the structural component of the X-ray absorption near-edge as an extension of the EXAFS, largely due to the long mean free path of the photoelectron and the dominance of high order multiple scattering contributions (Gräfe et al., 2014).

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 $m = 5.485 \cdot 10^{-4}$ 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).

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

Importantly, these powerful methodologies open the possibility to

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

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

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

The physiological behaviour and related molecular pathways of response are important to characterizing and understanding ENM biotransformation. An important part of this involves comparing the effects of a nanomaterial with that of the bulk and ionic counterparts, as well as by investigating different exposure times and by exposing different plant organs and tissues (Schwab et al., 2016; Marmiroli et al., 2021). 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). Detailed study of differential transcriptional regulation, protein abundance or metabolomic profiling (Huang et al., 2018; Majumdar et al., 2019; Gallo et al., 2021) are critical to demonstrating the nano-specificity of plant response. The study of organellar genome stability and the related stoichiometric variations during ENM treatment has also provided important mechanistic insight into plant to ENM exposure (Pagano et al., 2022). Advanced synchrotron-based techniques may either help to systematically understand the nano-bio interactions, with regard to physical and chemical reactions at the biomolecular surface: biomolecules may interact with ENMs, generating biomolecular corona, which change the ENM surface properties, and interfere with its functionality/reactivity (Hameed et al., 2022).

Regarding the physiological effects of ENMs on plants of agronomic interest, these studies have provided a better understanding of the specific properties of the ENMs that may enable sustainable use in the agrifood sector. 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). In addition, ENMs may act indirectly by protecting plants from biotic (e.g., nanopesticides) or abiotic stressors (e.g., wastewater and soil treatment) (Liu et al., 2015; Kah et al., 2018; Kumari et al., 2019). Due to the many variables involved, it is essential to obtain robust safety data regardless of the end use: ENM type, the modes and time of exposure, concentrations tested, and the plants used are all important considerations. Any recurring effects that occur under

these different conditions are of particular interest and are explored below (see Fig. 2 and Table 1). Table 2 summarizes the major outcomes related to metal based-ENM biotransformation in plants, including the principal mechanisms involved, and the major physiological and molecular insights observed from exposure.

2.1. Lanthanides based ENMs

Cerium Oxide (nCeO₂) has shown significant potential for agricultural applications, largely due to its properties as an ROS scavenger (Ma et al., 2015; Servin et al., 2017b). While CeO₂ as a bulk crystal mainly consists of Ce(IV), the reduction to nCeO₂ significantly enhances the relative amount of Ce(III), resulting in a higher catalytic effects comparable to the capabilities of a biological antioxidant (Eriksson et al., 2018). Servin et al. (2017b) used μ -XRF and μ -XANES to analyze the interactions between nCeO₂ and different biochars in soil, observing that much of the Ce remained in nCeO₂ form within the plant tissues. The dissolution rate of the nanoform can increase in acidic environments to generate Ce(III), as reported by Hernandez-Viezcas et al. (2013) who analyzed in *Glycine max* L. the effects of nCeO₂ (1000 mg L⁻¹, 48d exposure). Results have been confirmed by Rui et al. (2015), who used XANES on exposed cucumber (*Cucumis sativus* L.) tissues (2000 mg L⁻¹, 21d exposure) to observe nCeO₂ association with phosphate. These properties highly impact not only reactivity but also nCeO₂ translocation. In zucchini (*Cucurbita pepo* L.), treated with 500 mg L⁻¹ of nCeO₂, the nanoform is mainly present in the roots and stems, with limited translocation to the leaves (Pagano et al., 2016). However, co-contamination with other ENMs (e.g., CdS QDs) under same experimental conditions resulted in increased translocation to the shoots from 1000 to 3000 mg kg⁻¹ (Pagano et al., 2017). Similar results have been reported in several plant species: for example, Rossi et al. (2017) nCeO₂ under co-exposure with ionic Cd in soybean (*Glycine max* L.) showed an altered (1–2 fold increased) translocation to the shoots. Interestingly, bulk CeO₂ translocation resulted similar to the nano-form, whereas ionic Ce was translocated in greater amounts to the shoots (Pagano et al., 2016). This analysis was supplemented with molecular data; the transcriptional profiles were evaluated in *C. pepo* and *S. lycopersicum* as a function of nCeO₂, nLa₂O₃ and nCuO exposure and were compared with bulk and ionic forms using a set of 38 genes based on the *A. thaliana* orthologs as potential biomarkers of exposure/effects (Marmioli et al., 2014). The responses observed were generally different in term of up- or down-regulation as a function of Ce form (Pagano et al., 2016). Of particular interest are impacts on the chloroplast are the *PetL* and *PSBN* genes, which encode for a structural component of the cytochrome b₆f complex and low molecular weight protein located on thylakoid membrane as a component of the photosystem II (PSII), respectively (Fig. 2, Table 1). These two chloroplastic genes were differentially expressed across nano-, bulk, and ionic forms. A similar trend was also evident for nLa₂O₃ and nCuO exposure scenarios. Interestingly, by analyzing the effects on chloroplast and mitochondrial genomes in *A. thaliana* in terms of copy number, the effects of nCeO₂ and CeCl₃ exposure were rather limited as compared to the untreated control, which agrees with the limited translocation to the shoots (Pagano et al., 2022). With regard to proteomic analysis, Majumdar et al. (2015) conducted a quantitative proteomic analysis of kidney beans (*Phaseolus vulgaris* L.) seeds after nCeO₂ exposure and reported that the major seed proteins associated with nutrient storage (phaseolin) and carbohydrate metabolism (lectins) were significantly reduced by nCeO₂ (62.5–500 mg kg⁻¹, 50d exposure) in a dose dependent manner. Interesting, the plants did not exhibit overt toxicity.

In fact, at the physiological level cerium-based nanoparticles generally do not produce phytotoxicity (Ma et al., 2015; Rui et al., 2015; Lizzi et al., 2020; Rodrigues et al., 2021), though some have highlighted a positive impact on biomass and on physiological indicators such as chlorophyll and photosynthesis at selected doses (Rossi et al., 2017; Gui et al., 2017). Another important aspect of nCeO₂ seems to involve

enhance tolerance to saline stress, leading to improved phenotypic and enzymatic performances and ROS elimination in seeds priming (An et al., 2020; Liu et al., 2021; Hassanpouraghdam et al., 2022; Chen et al., 2022).

Compared to nCeO₂, nanoscale lanthanum oxide (nLa₂O₃) exhibits lower stability, increased ion dissolution, greater translocation from roots and shoots, all of which seems to lead to higher phytotoxicity. 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).

Interestingly, co-contamination with nCeO₂ strongly reduces the uptake of nLa₂O₃ (Pagano et al., 2017). The different behaviour of the two ENMs was evident in the transcriptomic profile: only 7 out of 38 genes were commonly modulated between nCeO₂ and nLa₂O₃; these genes were involved in primary metabolic functions, protein synthesis and stress response (Pagano et al., 2016). Several publications using different model plants were compared, and the reported effects due to nLa₂O₃ exposure in soil include reduction in root and leaf biomass (Ma et al., 2015), decreased transpiration (Yue et al., 2019), decreased photosynthesis (Xiao et al., 2021) and reduced pigment concentration (Neves et al., 2019). The decrease in photosynthetic activity is also reflected by altered root morphology, including root cracking (Xiao et al., 2021) and the presence of apoplastic barriers (Yue et al., 2019). Interestingly, the adverse effect of nLa₂O₃ on plant biomass was alleviated under binary exposure combinations with ENMs such as nCuO and nZnO (Pagano et al., 2017). As ideal case study, information on RedOx state and potential translocation of lanthanide-based nanoforms within plant tissues become fundamental in the mechanistic understanding on the physiological and molecular effects, with regard to application in the agrifood sector.

2.2. Titanium oxide ENM

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

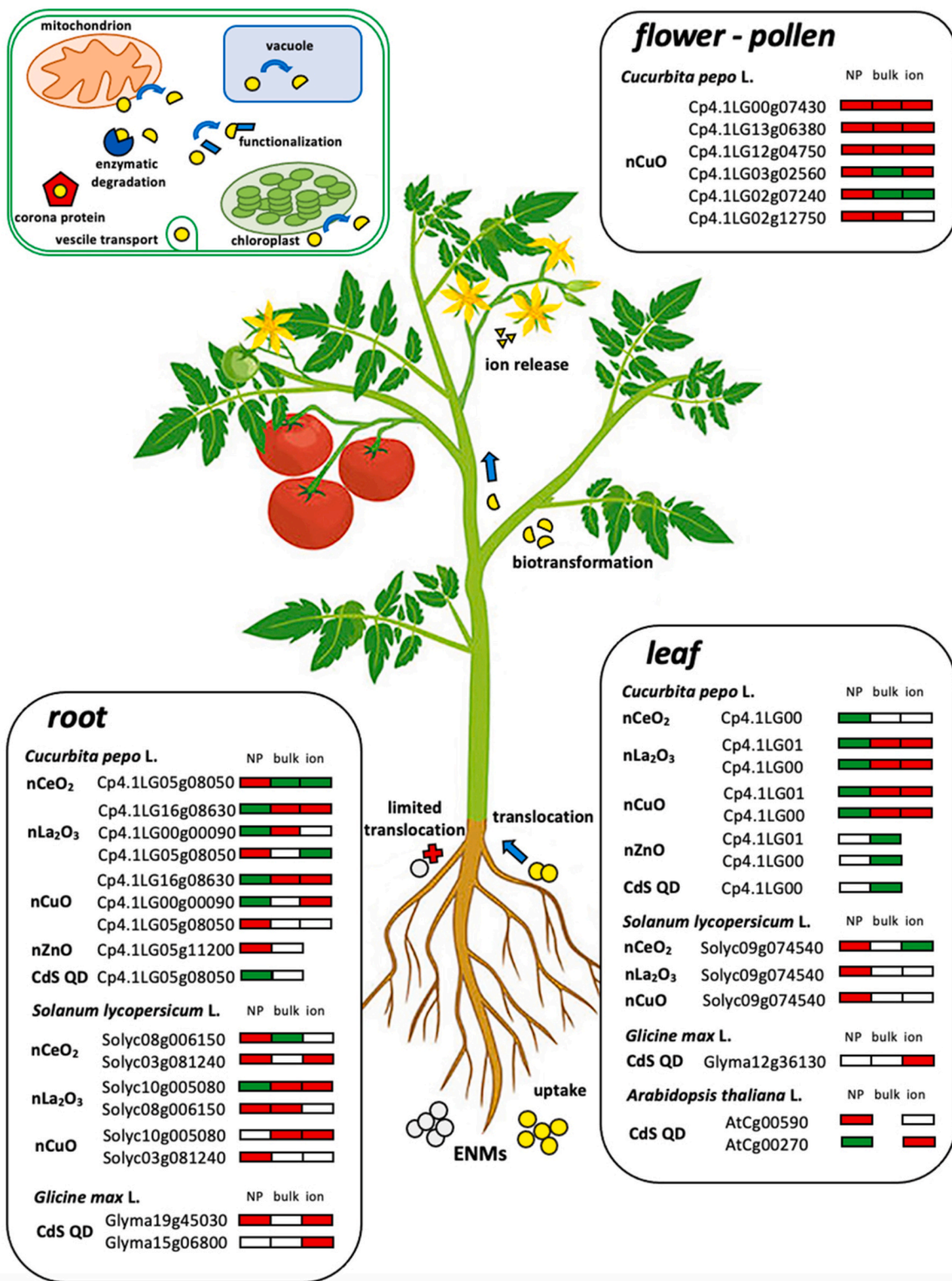


Fig. 2. Principal effects of ENM biotransformation in plant and relevant biomarkers observed in different plant species from model organisms (*A. thaliana*) to crops (*C. pepo*; *S. lycopersicum*; *G. max*) and different tissues (roots, leaves and flowers/pollen). Relevant ENM parameters such as size,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

Table 1

Genes as potential biomarkers of exposure/effect observed in roots, leaves and pollen, in different plant species (reported in Fig. 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 Fig. 2).

flower & pollen					
plant	gene	function	pathway	ENM	reference
<i>Cucurbita pepo</i> L.	Cp4.1LG00g07430	Beta-galactosidase	primary metabolism	nCuO	Marmioli et al., (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	Pagano et al., (2016) Pagano et al., (2017)
	Cp4.1LG00(*)	PetL, component of Cytochrome b6f	chloroplast electron transport	nCuO nZnO	
<i>Solanum lycopersicum</i> L.	Solyc09g074540	PetL, component of Cytochrome b6f	chloroplast electron transport	CdS QD nCeO2 nLa2O3 nCuO	Pagano et al., (2016)
<i>Glicine max</i> L.	Glyma12g36130	PetL, component of Cytochrome b6f	chloroplast electron transport	CdS QD	Majumdar et al., (2019)
<i>Arabidopsi thaliana</i> L.	AtCg00590	PetL, component of Cytochrome b6f	chloroplast electron transport	CdS QD	Marmioli et al., (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	Pagano et al., (2016) Pagano et al., (2017)
	Cp4.1LG00g00090	GPT2, glucose-6-phosphate/phosphate translocator	primary metabolism	nLa2O3 nCuO nZnO	
	Cp4.1LG05g08050	RPS12, ribosomal protein S12A	protein synthesis	CdS QD	
	Cp4.1LG05g11200	PLP2, phospholipase	biotic/abiotic stress response		
<i>Solanum lycopersicum</i> L.	Solyc08g006150	ChaC-like family protein	glutathione degradation	nCeO2	Pagano et al., (2016)
	Solyc03g081240	PRR5, pseudo-response regulator 5	biotic/abiotic stress response	nLa2O3 nCuO	
<i>Glicine max</i> L.	Solyc10g005080	LHY1, Homeodomain-like superfamily protein	stress response	CdS QD	Majumdar et al., (2019)
	Glyma19g45030	LHY1, Homeodomain-like superfamily protein	stress response		
	Glyma15g06800	PR1, pathogenesis-related gene 1	biotic/abiotic stress response		

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).

2.3. Gold and silver nanoparticles

Similar to nTiO₂, gold nanoparticles (nAu) are highly stable in plants: nAu remained mostly as Au⁰ within the plant tissues (*Nicotiana tabacum* L. cv. Xanthi nc.), even if accumulated and translocated (Sabo-Attwood et al., 2011). Specifically, XANES analyses demonstrated that nAu maintained its nanoparticle structure without any biotransformation or ionic release. There are no actual uses for gold nanoparticles and plants, it just used as a tool to study NP-plant interactions. nAu levels in bio-solids would ever be high enough to be considered phytotoxic. It is known that nAu toxicity depends on concentration, particle size and shape: nAu with a smaller particle size (3.5 nm, concentration of 48 mg L⁻¹) were evenly biodistributed across the plant in comparison with the 18.5 nm nAu (in a concentration of 76 mg L⁻¹), even leading to the formation of necrotic leaf lesions and plant death after 30 days

(Sabo-Attwood et al., 2011). Other studies have shown that nAu exposure improved radical scavenging and antioxidant enzymatic activities and modulated miRNA expression implicated plant abiotic stress response (miR398, miR408). In particular, the regulation of superoxide dismutase (SOD) led to an increased ROS scavenging activity, root elongation, seedling growth, and seed yield (Arora et al., 2012; Kumar et al., 2013; Siddiqi & Husen, 2017).

Given the widespread commercial utilization and environmental relevance (e.g., wastewater treatment; fertilization) of silver nano-formulations (nAg), the effect on plant species has been a topic of robust study. Stegemeier et al. (2015) analyzed the nAg and nAg₂S speciation in *Medicago sativa* L., demonstrating that nAg accumulates in the root elongation area but that nAg₂S remains adhered to the root surface; Ag ions accumulate more uniformly throughout the root tissues. Notably, the Ag accumulation in the root apoplast was determined by XRF. The presence of nAg in root cell walls demonstrated the uptake of partially dissolved nAg and translocation along the apoplast. Larue et al. (2014) localized and determined nAg speciation in *L. sativa* after foliar spray treatment through μ -XRF and μ -XAS techniques; the authors reported that nAg was able to cross the foliar cuticle, penetrating in the leaf tissue through the stomata. Moreover, nAg biotransformed through oxidation and complexation with thiol-containing molecules such as glutathione (GSH). These findings correlated well with the transcriptomics analyses of *A. thaliana* exposed to different types of nAg: plant response included

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</i> L.	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 modified redox state, from Ce(IV) to Ce(III) low translocation from roots to shoots	Rui et al., (2015)
	<i>Lactuca sativa</i> L. <i>Cucurbita pepo</i> L. <i>Zea mays</i> L. <i>Glycine max</i> L.	biochar 600 °C was largely unaffected	–	SEM μ-XRF μ-XANES		Servin et al., 2017a
nCeO2 nZnO	<i>Glycine max</i> L.	–	–	μ-XRF μ-XANES		Hernandez-Viezcas et al., (2013)
nCeO2 nLa2O3	<i>Cucumis sativus</i> L.	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	Ma et al., 2018
nTiO2	<i>Cucumis sativus</i> L.	at all concentrations, nTiO2 significantly increased root length (average > 300%)	–	μ-XRF μ-XANES	high stability low translocation from roots to shoots	Servin et al., (2012)
	<i>Cucumis sativus</i> L.	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		Servin et al., (2013)
nAu	<i>Nicotiana tabacum</i> L.	leaf necrosis was observed after 14 days of exposure to 3.5 nm nAu	–	μ-XRF	high stability no changes in Au valence	Sabo-Attwood et al., 2011
nAg	<i>Lolium multiflorum</i> L.	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	Yin et al., (2011)
	<i>Lactuca sativa</i> L. <i>Cucurbita pepo</i> L. <i>Zea mays</i> L. <i>Glycine max</i> L. <i>Medicago sativa</i> L.	fresh foliar biomass was unchanged. Chlorophyll a, chlorophyll b, carotenoid and pheophytin contents were not affect	–	SEM μ-XRF XANES		Larue et al., 2014
		–	–	TEM XRF		Stegemeier et al., (2015)
nZVI	<i>Cucumis sativus</i> L.	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	Dwivedi et al., (2018)
ENM	plant	physiological response	molecular response	techniques	biotransformation	reference
nZnO	<i>Zea Mays</i> L.	–	–	μ-XRF XANES	low stability high translocation from roots to shoots	Lv et al., (2015)
	<i>Zea Mays</i> L.	By the 7th day, the treatment of 9 nm nZnO and ZnSO4 significantly reduced the dry weight of roots by 44% and 58% respectively, compared to the unexposed control plants. In general, ZnSO4 treatment had the greatest effect on root biomass, followed by 9 nm nZnO and finally 40 nm nZnO	–	μ-XRF		Lv et al., (2021)
nCuO	<i>Nicotiana tabacum</i> L.	When exposed to equivalent weight of Cu, nCu2O 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	Dimkpa et al., 2019
	<i>Lactuca sativa</i> L.	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	modified redox state, from Cu(II) to Cu (I)	Servin et al., 2017a

(continued on next page)

Table 2 (continued)

ENM	plant	physiological response	molecular response	techniques	biotransformation	reference
	<i>Cucurbita pepo</i> L.	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		Marmioli et al., (2021)
CdS QD	<i>Arabidopsis thaliana</i> L.	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	Marmioli et al., (2020)

defensin-like proteins, plant thionin, β -glucosidases, cytochrome P450 proteins, and glutathione-S-transferase (GST) members (Kaveh et al., 2013). Although some studies point out that the morphological and physiological effects of nAg exposure were strictly dependent on particle size and concentration and that sublethal concentrations may have also beneficial effects (Wang et al., 2013; Syu et al., 2014), most of reports demonstrated reduced root elongation and shoot biomass, together with decreased levels of chlorophyll, pigments, micronutrients, and increased level of ROS and activity of enzymes involved in the oxidative stress response (Yin et al., 2011; Zuverza-Mena et al., 2016; Yang et al., 2018; Lahuta et al., 2022).

2.4. Iron-based ENMs

Iron-based nanomaterials, including iron oxides (nFeOx) and zero valent iron (nZVI), have been investigated in plant systems and the reports highlight two major routes of entry: i) a reductive and proton-promoted process able to modify the structure of the ENM or ii) through the secretion of plant transporters (e.g., phytosiderophores) with a high affinity for Fe (III) (Morrissey & Guerinot, 2009). Dwivedi et al. (2018) investigated nZVI exposure in *C. sativus* and reported that transformed nZVI was stored in the root cell membrane and vacuoles of the leaf parenchyma. XAS identified ferric citrate and iron (oxyhydr) oxides as the main transformation products in roots and shoots, albeit in different proportions. The major pathways of nZVI biotransformation involve interaction with low molecular weight organic acid ligands and on the dissolution/precipitation of the mineral products. Transcriptional analyses performed on H⁺-ATPase genes (*CsHA1*, *CsHA2*) showed an upregulation of these genes upon nZVI exposure (and relative root acidification), indicating that the plant-promoted transformation of nZVI can be driven by protons released by the roots.

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

Given their widespread application in soil and water remediation, a primary concern with iron nanoparticles is a potential toxicity from excessive accumulation in the environment. However, several studies have shown that plant exposure to this type of nanoparticle does not result in phytotoxicity. For example, Dwivedi et al. (2018) evaluated the potential environmental impact of nZVI on *C. sativus* in soil and in hydroponic culture, and reported no instances of reduced plant biomass even at the highest doses (from 250 to 1000 mg L⁻¹) and for *O. sativa*, the low doses (50–500 mg L⁻¹) of nZVI and nFe₃O₄ improved plant

growth (Li et al., 2021). The use of this nanomaterial as a soil conditioner for remediation of metal-contaminated soils is confirmed by the demonstration of improved plant growth in Cd-contaminated soils (Rizwan et al., 2019; Manzoor et al., 2021); mechanistically, this involves limiting cadmium translocation and the promotion of antioxidant activity.

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

2.5. Zinc-based ENMs

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

In recent years other forms of Zn-based nanomaterials have been tested for a potential plant remediation purpose, such as ZnS QDs (Imperiale et al., 2022). An analysis of the effect of ZnS QDs and ionic Zn exposure on mitochondrial and plastid genome copy number demonstrates that both increase by 1 to 3-fold, but that ZnS QDs dissolution alone does not explain the phenomenon; this suggests that ZnS QDs biotransformation may occur within the plant tissues and organs to a form more similar to ionic than nanoscale Zn (Pagano et al., 2022). Zinc-based nanomaterials have also shown interesting properties as nanofertilizers, including mitigating abiotic and biotic stress (e.g., salt stress, infections), regulating micronutrient uptake, improving water use efficiency, and promoting detoxification of heavy metals (Akhtar et al., 2022; Zafar et al., 2022). Under drought conditions, the nZnO (5 mg kg⁻¹) significantly increased grain yield in sorghum (*Sorghum vulgare*

Moench) and fruit yield in eggplant (*Solanum melongena* L.), respectively by 22–183% and 12–23% (Dimkpa et al., 2019; Semida et al., 2021).

2.6. Copper oxide

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). nCuO dissolution within the plant tissues has been demonstrated (in *C. pepo*), and this was shown to depend not only on uptake, and translocation, but also on the interaction with important biomolecules (Tamez et al., 2019; Marmioli et al., 2021).

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

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

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

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). For example, exposure of nCuO to *Solanum lycopersicum* increased root and stem length, leaf number, and chlorophyll content, and also inhibited the mycelial growth of *Fusarium oxysporum* sp. *Lycopersici* (Lopez-Lima et al., 2021). Conversely, some authors report toxic and inhibitory effects on the growth in plants such

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

2.7. Quantum dots

Cadmium-based nanomaterials, and cadmium sulfide quantum dots (CdS QDs) in particular, have been used as a model material to elucidate physiological mechanisms and molecular pathways involved in the response plant response to exposure (Marmioli et al., 2014; Imperiale et al., 2022). A Systems biology approach gave a complete picture of the targets in both model (*A. thaliana*) and crop (*C. pepo*) species (Marmioli et al., 2014; Marmioli et al., 2015; Pagano et al., 2017; Gallo et al., 2021; Marmioli et al., 2020; Pagano et al., 2022). In *A. thaliana*, CdS QDs tolerant mutants were used to establish *in vitro* inhibition concentrations for growth (80 mg L⁻¹) in an attempt to elucidate the mechanisms involved in the plant response; the results largely implicated metabolic functions and chloroplast energy production as sensitive targets (Marmioli et al., 2014). The results demonstrate that CdS QDs and ionic Cd were exploiting different pathways in the plant, highlighting that the tolerance to CdS QDs did not overlap with the tolerance to CdSO₄. Conversely, Cd sensitive mutants of Arabidopsis (Howden & Cobbett, 1992) that were exposed to CdS QDs did not exhibit differences in growth as compared to the wild type line (Marmioli et al., 2014). A transcriptomic analysis and proteomic comparison between wild type and tolerant mutants highlighted that only a few genes were commonly modulated upon ionic Cd and CdS QDs treatment (Marmioli et al., 2015; Gallo et al., 2021). Marmioli et al., (2020) used EXAFS to investigate the cadmium environment in planta and showed that the spectra were compatible with a mixed O/S coordination; while Cd–S distances did not show relevant variations, Cd–O distances varied in samples grown with QDs compared with those grown with CdSO₄. The number of Cd–S bonds in plants grown with QDs was higher than Cd–O bonds. This EXAFS analysis demonstrated that CdS QDs were biotransformed after uptake: the QD original structure was modified but not completely absent within the plant cell, and Cd atoms were not released as Cd ions. Interestingly, CdS QDs showed a relatively high stability; once accumulated by the plant, the QD may go through different stages in the response pathways: i) exposure: explained by the different genetic mechanisms behind the physiological/molecular response between the wild type and tolerant phenotypes; ii) reactivity/biotransformation: explained by a transition phase in which the structure of CdS QDs is modified to decrease particle reactivity, and this can be detected by XANES and EXAFS analyses; iii) effects/detoxification: transcriptomic, proteomic and metabolomic response related to the physico-chemical forms after QDs biotransformation. Additionally, the effects on organelle genomes (ptDNA and mtDNA) demonstrate how QDs biotransformation may modify the genomes stoichiometry or sub-stoichiometry, likely through potential morpho-functional adaptive response triggered by modifications in the bioenergetic redox balance, or a reduction of photosynthesis or cellular respiration rates after QD exposure (Pagano et al., 2022).

Similarly to what was observed in *A. thaliana*, CdS QDs induced analogous effects other plant species of agricultural interest: Pagano et al. (2017) analyzed the effects of the CdS QDs in a context of ENMs binary co-contamination, highlighting a similar response as in *A. thaliana*; specific and common biomarkers were involved between CdS QDs and other the ENMs tested (nCeO₂, nLa₂O₃, nCuO, nZnO).

Majumdar et al. (2019) investigated the effect of differently functionalized CdS QDs in *G. max*; the authors used proteomic and metabolomic endpoints to demonstrate how the transmembrane proteins involved uptake and related genes including *NRAMP6* and *HMA8* were differently regulated in CdS QDs and ion treated plants. In addition, ATP-dependent ion transporters in the membranes presented feedback mechanisms in the soybean roots to restrict the uptake of CdS QDs and simultaneously to alter the mineral acquisition. Moreover, CdS QDs altered major metabolic functions, including glutathione metabolism, the tricarboxylic acid cycle, glycolysis, fatty acid oxidation and phenylpropanoid and amino acids biosynthesis. Physiologically, CdS QDs, induced oxidative stress, decreased biomass, reduced chlorophyll and carotenoids content, and damaged primary roots (Majumdar et al., 2019; Pagano et al., 2022).

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

ENMs have been rather extensively tested in recent years, with data indicating that several physico-chemical parameters are fundamental to explaining their behaviour during exposure, including composition, stability, surface charge, and functionalization. These ENM properties become biologically relevant and mediate subsequent biotransformation processes, including: i) the possibility to be translocated within organs, tissues, and cells; ii) the ability to interact with the biologically active environment within the plant (e.g., phospholipids, nucleic acids, proteins, secondary metabolites, reactive oxygen species); iii) the dissolution rate and the consequent ion release. Importantly, to fully comprehend the plant response to ENMs exposure, one must consider the biologically modified ENMs forms that are indicative of the highly complex interactions between plants and ENMs interaction. 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.

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

The importance of in planta ENM biotransformation is corroborated indirectly at molecular level by “omic” analyses that can describe the effects on the plant at genetic and epigenetic level (including genome stability) by measuring transcriptional modulation, protein abundance and metabolite synthesis, as well as on physiological (phenotypic) level by observing the plant redox state, ROS production, photosynthetic activity, and cellular respiration rate in response to stress (Marmioli et al., 2020; Gallo et al., 2021). The direct measurement of changes upon ENM biotransformation within the plant tissues by synchrotron-based techniques (μ -XRF, μ -XANES, and XAS) provide critical information in terms of distribution, atomic redox state, and atomic local structure, and add critical knowledge necessary to understand the ENM-plant interactions. 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).

Author contributions

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.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nelson Marmioli reports financial support was provided by European Union.

Data availability

The manuscript is a review

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