Electronic Supplementary Information

Reply to the Comment on "Foliar application of nanoparticles: mechanisms of absorption, transfer, and multiple impacts" by S. Husted, P. Møs, S. Le Tougaard, A. Pinna and F. Minutello., *Environ Sci. Nano*, DOI: 10.1039/D1EN00630D

Jie Hong[a], Chao Wang[a], Dane C. Wagner[b], Jorge L. Gardea-Torresdey[c], Feng He[a*], Cyren M. Rico[b*]

[a]College of Environment, Zhejiang University of Technology, Hangzhou, Zhejiang, 310014, China

[b]Chemistry and Biochemistry Department, Missouri State University, Springfield, MO 65897

[c]Department of Chemistry and Biochemistry, The University of Texas, El Paso, TX 79968, United States

Contains two tables

Table S1. Advantages of foliar spray of NPs relative to control as foliar application of non-NPs or soil application of either NPs or non-NPs. Citations follow that of Hong et al. (2021); citations for additional new references are listed below.

Foliar application of NPs	Control	Advantages of foliar relative to control	Ref
Foliar application of copper nanoparticles (0.5, 1.0, and 1.5 g/L) Plant: Peppermint (*Metha piperita* L.)	Foliar application of copper sulfate (0.5, 1.0, and 1.5 g/L)	Copper nanoparticles (1.0 g/L) increased chlorophyll content and essential oil percentage by 35% and 20% higher than control, respectively. The copper sulfate (0.5 g/L) increased dry matter yield up to 58% higher than control. Copper nanoparticles (1.0 g/L) increased menthol, menthone and menthofuran content up to 15, 25 and 65% higher than in control, respectively. Results showed that copper nanoparticles fertilizer 0.5, 1.0 and 1.5 g/L significantly increased all forms of chlorophyll content of 25, 35 and 45% and essential oil percentage 10, 20 and 23% higher than that for control, respectively.	New Ref. 1
Foliar application of nano-iron chelate, nano-zinc Plant: *Zea mays*	Foliar application of chemical iron chelate, chemical zinc fertilizer	Application of nano-forms of fertilizers, compared to chemical forms of fertilizers, increased the phosphorus concentration, biomass, and crude protein and soluble carbohydrate concentration. Both forms of iron fertilizer increased leaf chlorophyll concentration, compared to other fertilizers and control. Nano-Zn treatment resulted in higher leaf chlorophyll concentration than that for the chemical form of zinc, water and control treatments. Plant height and total dry biomass (TDB) responses were generally greater in the nano-iron chelate treatment. Nano-zinc spraying increased plant height by 23.6 and TDB by 14.5%, respectively, compared with the control. The nano-iron treatment resulted in the highest crude protein, soluble carbohydrates and phosphorus concentration and lowest crude fiber content as compared with the other treatments. Zinc treatment increased the soluble carbohydrate concentration compared with the water and control treatment. Nano forms increased the phosphorus, TDB, crude protein and soluble carbohydrate concentration compared to chemical forms.	112

		The beneficial effects of nano zinc on corn plants were more pronounced than of chemical zinc.	
Foliar application of TiO_2 nanoparticles (0, 2, 4 and 6 ppm) Plant: Coriander (*Coriandrum sativum* L.)	No treatment	The results indicate that titanium dioxide (TiO_2) nanoparticles (NPs) lead to a significant increase in plant height, fruit yield and number of branches. It also caused an increase in amino acids, total sugars, total phenols, total indols and pigments. The contents of chlorophyll-a, chlorophyll-b and carotenoids were significantly increased with foliar application on coriander with varying TiO_2 nanoparticles concentrations (2, 4 and 6 mg/L). The foliar application of coriander with TiO_2 nanoparticles at 2, 4 and 6 mg/L had a significantly increased with total amino acids and total sugars content as compared with control ones.	115
Foliar application of SNPs nanoparticles (0, 100, 200, and 300 ppm) Plant: Tomato (*Solanum lycopersicum*)	No treatment	The obtained results revealed that the foliar spraying tomato leaves with 200 ppm sulfur nanoparticles are very beneficial to plant growth and produced healthy plant with greener leaves and high quality of tomato fruits compared with control. The plant height and root increase with increasing sulfur nanoparticles up to 200 ppm and then decrease with 300 ppm. This indicates that the organic materials in plant tissue needs proper quantity of SNPs to form organic-sulfur compounds, which helps the plant to grow healthy and to defend itself and has antimicrobial activity.	65
Foliar-sprayed with or without 100 µg Fe g^{-1} in the forms of Fe_3O_4 nanoparticles (NPs) and ethylene diamine-N,N-bis(2-hydroxyphenylacetic acid) Fe sodium complex (Fe-EDDHA) Plant: *Zea mays*	(Fe-EDDHA)	Iron treatments improved maize photosynthesis and hydrogen peroxide and superoxide anion scavenging capacity and lowered the rate of membrane lipid peroxidation. Iron treatment also accelerated vegetative growth and caused earlier entrance to the generative phase. Differences between ameliorative effects of Fe-EDDHA and Fe_3O_4 NPs were particularly noticeable in the generative growth phase. Improvement of calcium, Fe^{2+}, total Fe, and ferritin contents were more pronounced in Fe_3O_4 NPs treatments (164%, 200%, 300%, and 200% of the control, respectively).	113

Foliar application of Cu nanoparticles (50, 125, 250, 500 mg/L) Plant: Tomato (*Solanum lycopersicum*)		The application of the Cu nanoparticles induced the production of fruits with greater firmness. Vitamin C, lycopene, and the ABTS antioxidant capacity increased compared to the Control. In addition, a decrease in the ascorbate peroxidase (APX) and glutathione peroxidase (GPX) enzymatic activity was observed, while the superoxide dismutase (SOD) and catalase (CAT) enzymes showed a significant increase. The application of Cu NPs induced a greater accumulation of bioactive compounds in tomato fruits.	88
Foliar application of TiO_2 or ZnO NPs Plant: Tomato (*Solanum lycopersicum*)	root exposure	Aerosol mediated application was found to be more effective than the soil mediated application on the uptake of the nanoparticles was in plants. The results indicated that there is a critical concentration of TiO_2 and ZnO nanoparticles up to which the plant's growth and development are promoted; with no improvement beyond that. Overall, it was observed that foliar application induced more lycopene biosynthesis than soil application.	100
Foliar application and seed treatment with SNPs (30-100 mg/L) Plant: Tomato (*Solanum lycopersicum*)		Foliar application and seed treatment with SNPs (30−100 mg/L, 30 and 100 nm) suppressed pathogen infection in tomatoes, in a concentration- and size-dependent fashion in a greenhouse experiment. Foliar application with 100 mg/plant of 30 nm SNPs (30-SNPs) exhibited the best performance for disease suppression, significantly decreasing disease incidence by 47.6% and increasing tomato shoot biomass by 55.6% after 10 weeks application. Importantly, the disease control efficacy with 30-SNPs was 1.43-fold greater than the commercially available fungicide hymexazol. Mechanistically, 30-SNPs activated the salicylic acid-dependent systemic acquired resistance pathway in tomato shoots and roots, with subsequent upregulation of The expression of pathogenesis-related and antioxidase-related genes (upregulated by 11−352%) and enhancement of the activity and content of disease-related biomolecules (enhanced by 5−49%).	New Ref. 2

		The oxidative stress in tomato shoots and roots, the root plasma membrane damage, and the growth of the pathogen in stem were all significantly decreased by SNPs. In this work, foliar application and seed treatment with SNPs effectively controlled the growth and damage from fusarium wilt in tomatoes, as determined by phenotype, biomass, photosynthetic parameters, and disease incidence.	
Foliar spray of TiO_2 nanoparticles (0, 100, 250 mg/L) Plant: *Zea mays*	Root application	The impact of TiO_2NPs (0, 100, 250 mg/L) and Cd (0, 50mM) co-exposure on hydroponic maize (*Zea mays* L.) was determined under two exposure modes. Results showed that root co-exposure to TiO_2NPs and 100 mg/L Cd significantly enhanced Cd uptake and produced greater phytotoxicity in maize than foliar exposure to TiO_2NPs. Meanwhile, plant dry weight and chlorophyll content showed a reduction of 45.3% and 50.5%, respectively, when compared with single Cd treatment. In addition, the accumulation of Ti in shoots and roots increased by 1.61 and 4.29 times, respectively when root exposure to 250 mg/L TiO_2NPs. By contrast, foliar exposure of TiO_2NPs could markedly decrease shoot Cd contents from 15.2% to 17.8%. Therefore, foliar spray of TiO_2NPs is considered as an effective and alternative way to protect maize seedling against Cd stress.	New Ref. 3
Foliar spray of Fe_2O_3 NPS (IONPs) Plant: Soybean (*Glycine max* L.)	Soil amendment	Furthermore, IONPs-Cit significantly enhanced photosynthetic parameters when sprayed foliarly at the eight-trifoliate leaf stage ($P<0.05$). The increases in photosynthetic rates following spraying were attributed to increases in stomatal opening rather than increased CO_2 uptake activity at the chloroplast level. A more pronounced positive effects of IONPs via foliar application than by soil treatment was observed. This study concluded that IONPs coated with citric acid at IONPs to citrate molar ratio of 1:3 can markedly improve the effectiveness of insoluble iron oxide for Fe foliar fertilization.	New Ref. 4
Foliar application of		Spraying with Si NPs could be used as a ration to	New Ref. 5

silicon nanoparticles (0 and 1.5 mM) Plant: Coriander (*Coriandrum sativum* L.)		reduce the harmful effects of Pb stress on coriander plants. Si NPs can adjust antioxidant enzyme activities and minimize the oxidative stress in plants. Treatments included four levels of Pb (0, 500, 1000, and 1500 mg/kg of soil), and two levels of Si NPs (0 and 1.5 mM) in all combinations. The Pb treatments alone decreased the plant biomass and vitamin C while increased the flavonoid, MDA, antioxidant enzyme activities, and Pb concentration in tissues depending upon the Pb treatments. The foliar-applied 1.5 mM Si NPs alleviated the adverse impacts of Pb on coriander plants which were due to the minimization of Pb concentration in plants and improvements in the plant defense system. Si NPs minimized accumulation of MDA in plant tissues and adjusted the activities of POD, CAT, and SOD in plants under Pb stress. Overall, Si NP foliar application might be a suitable approach in reducing the Pb concentrations in plants)	

New additional references for Table 1.

1. Z. N. Lafmejani, A. A. Jafari, P. Moradi, and A. L. Moghadam, Impact of foliar application of copper sulphate and copper nanoparticles on some morpho-physiological traits and essential oil composition of peppermint (Mentha piperita L.). *Herba Polonica*, 2018, **64**(2), 13-24.
2. X. Cao, C. Wang, X. Luo, L. Yue, and B. Xing, Elemental sulfur nanoparticles enhance disease resistance in tomatoes. *ACS Nano*, 2021 **15**(7), 11817-11827.
3. J. Lian, L. Zhao, J. Wu, H. Xiong, Y. Bao, A. Zeb, J. Tang, W. Liu, Foliar spray of TiO_2 nanoparticles prevails over root application in reducing cd accumulation and mitigating cd-induced phytotoxicity in maize (*Zea mays* L.). *Chemosphere, 239*, 124794-124794.
4. D. Alidoust, A. Isoda, Effect of γFe_2O_3 nanoparticles on photosynthetic characteristic of soybean (*Glycine max* (L.) Merr.): foliar spray versus soil amendment. *Acta Physiologiae Plantarum*, 2013, **35**(12), 3365-3375.
5. H. Fatemi, B. E. Pour, M. Rizwan, Foliar application of silicon nanoparticles affected the growth, vitamin C, flavonoid, and antioxidant enzyme activities of coriander (*Coriandrum sativum* L.) plants grown in lead (Pb)-spiked soil. *Environmental Science and Pollution Research*, 2021, **28**(2), 1417-1425.

Table S2. Reply to queries in the comments to Hong et al. (2021).

#	Statement [Paragraph] [reference number]	Comments	Reply
1	"NPs [...] can carry various types of functional groups that increase their cellular uptake" [Introduction] [ref. 6]	Ref. 6 is not a study of how functional groups increase the uptake of NPs, but rather a study of how chitosan NPs affect the growth of tomato inoculated w/wo mycorrhiza. NP uptake is not studied at the cellular level as indicated.	In Ref.6, the authors mentioned "The successful application of NPs is due to its nanometer size enabling uptake into plant cells, their large surface area, cationic nature, active functional groups, porosity and higher encapsulation efficiency [8]."
2	" […] increased shelf life of agricultural produce" [Introduction] [ref. 13]	Ref. 13 evaluated the effect of foliar application of PbO-NPs on Pb accumulation by spinach and associated biochemical changes and health hazards. There is no data presented which deals with the impacts of NPs on shelf life as listed in the sentence where the reference appears.	The correct citation should be Ref. 9. We sincerely apologize for the oversight.
3	" […] improved absorption and assimilation of foliar fertilizer"[Introduction] [ref. 14]	Ref. 14 is a poor choice to show that an essential plant nutrient (here Zn) is absorbed and assimilated in higher quantities when applied as NPs. No elemental analysis of Zn in tissue was performed in this study which is pivotal to support the claim. Vegetative biomass was significantly improved by NPs but differences in height and fruit yields are the same. Several papers exist where e.g. the Zn enrichment is analyzed for Zn applied as NP and as a simple salt and we wonder why some of these better suited studies have been omitted (e.g. Read et al. 2020). Read et al. (2020) Physiologia Plantarum 170: 384-397	In Ref. 14, the authors mentioned "These facts indicate that Zn availability and concentration by foliar fertilization with ZnO NPs during the main stages of vegetative growth were more effective and have important physiological functions that could improve fruit quality." "Results from this research suggest that foliar application of ZnO NPs at 1000 mg L 1 had a greater impact on plant growth and physiology than conventional Zn (ZnSO4) salts, probably due to greater capacity to be absorbed by the blade." The reference Read et al. (2020) Physiologia Plantarum 170: 384-397 was published when the manuscript had already been submitted for review.
4	Plants absorb foliar NPs usually through stomata, cracks or water pores, ion channels, protein carriers, endocytosis, stigma, wounds and	This sentence is more or less directly taken from the review ref. 9 NPs can enter plant cells and deliver nutrients by binding to carrier proteins, through aquaporins, ion channels, endocytosis or by binding to organic chemicals in plant tissues which is referring to	There are more information published about this part except Ref. 9. For example Ref. 21 "Furthermore, it is also reported that, nanoparticles have the ability to enter different plant tissues through either root tissues (via soil application) or aboveground

	trichomes [Introduction] [refs. 9, 21-24]	another review by Rico et al. 2011. In this review several of these pathways are proposed, but no papers with experiments data are listed as reference to support the proposal, which is misleading as it should be noted that e.g. aquaporins and ion channels not yet have been experimentally shown to be pathways for NP uptake.	organs (via foliar application) including trichomes, stomata, cuticles and stigma, as well as through wounds and root junctions (Shukla et al. 2016; Ruttkay Nedecky et al. 2017; Tripathi et al. 2017; Zuverza-Mena et al. 2017). In general, when different nanoparticles could be applied on plant leaf surfaces, they can enter through the stomata pores (openings) or through trichome bases moving towards various plant tissues (Hatami et al. 2016)." Ref. 22 "It is well known that the common symptoms of NPs phytotoxicity appear as the clogging of pores and barriers in the apoplastic stream resulting in reduced uptake of nutrients and hydraulic transfer (Dietz and Herth, 2011; Aken Van, 2015), ..." Ref. 23 "Stomatal and cuticular uptake pathways are two potential routes of entrance into the leaves.""Our results indicate that uptake of PVP-coated AuNPs occurs at least partly via disruption and/or diffusion through the cuticle layer, and possibly through stomata as well" Ref. 24 "Studies indicate that plants can absorb metal(loid)s via stomata, lenticels, cuticle, cracks, and aqueous pores (Schreck et. 2013)" Rico et al. 2011 only discussed what was proposed in earlier studies as to how NPs enter the plants. Those studies were published much early on when so little was understood about NP-plant interaction.
5	the effect of NPs is complex and affected by many factors, such as [...] rhizosphere and foliage microorganisms [Introduction] [ref. 29]	Ref. 29 is irrelevant as it is not dealing with NPs. There is no reference to effects on the interaction between NPs, rhizosphere and microorganisms on the foliage.	Ref. 29 mentioned that "plant microbiota, are an integral part of plant life" and "The role of microbiota in plant growth promotion (PGP) and protection against various stresses is well known." Thus, it is possible that NPs

			adsorption on plants surface or absorption into plants tissues, and their positive or negative effects on plants, could be affected by microbes' activities.
6	Spraying the proper amount of micro/macronutrients on foliage can mitigate damage caused by traditional soil root application methods [par 2] [ref. 21]	In ref. 21 it is correctly stated that foliar fertilization is generally recommended for supplying additional nutrients like nitrogen (N), magnesium (Mg) and micronutrients as well as P, K and sulphur (S) to improve plant nutritional status as well as increase the crop yield and its quality. But in the ref. 21 nothing is mentioned about mitigating damaging effects caused by traditional soil application methods such as over-fertilization.	In Ref. 21, the authors mentioned "the more soluble nutrients such as nitrogen (N) are easily leached down the soil profile. What is lost through leaching reaches the aquifer and pollutes the groundwater (El-Ramady 2014; El-Ramady et al. 2014a, b, 2015, 2016a)." In addition, there was a statement that mentioned "The most important use of foliar nutrition is the application of micronutrients in small amounts as well as macronutrients (e.g., nitrogen, phosphorus, or potassium) without causing any phytotoxicity (Oosterhuis and Weir 2010)." Avellan et al. (ES&T 2021, 55, 13417-13431) also discussed this concern: "Yet, current use of agrochemicals is highly inefficient. It has been estimated that up to 50%[6] of the nutrients and >95% of pesticides[7] that are applied on crops never reach their target and are wasted, causing soil pollution,[8] antibiotic resistance,[9] and runoff that degrades the ecosystems.[10]"
7	[...] carbon-based NPs can be used as coatings for slow-release nanofertilizers to improve plant biomass in agriculture [par 2.1] [ref. 46]	The use of ref. 46 is misleading as it is not documented that NPs were produced (diameter less than 100 nm) and particles were not applied as foliar fertilizers but applied to soil in a greenhouse set-up.	Ref. 46 mentioned "The resulting fertilizers produced by incorporate NPK into the nanoparticles presents put with an apparatus described previously by EL-Aila *et al*. (2001) to produce slow-release fertilizers with nanoparticles." So, the particles used in the Ref. 46 were nano-size. Section 2.1 discussed types of nanoparticles, and it should not matter whether these NPs were applied to leaves or roots.
8	[...]and organic NPs can serve as nanocarriers of	The use of ref. 48 is misleading as they evaluated the potential of SiNPs	In Hong et al. (2021), this sentence was supported by Ref. 47 and Ref. 48. Iron

	nutrient elements (e.g., iron and magnesium) to treat acute malnutrition in crops [par 2.1] [ref. 48]	for delivering proteins in tomato to control insect pests. There is no focus on NP as nanocarriers for nutrient elements as listed in the sentence where the reference is used.	and magnesium were mentioned in Ref. 47. In Ref. 48, the authors mentioned "Nanoparticle based formulations have been effective in delivering growth factors –naphthalene acetic acid; chemical pesticides – cyhalothrin, avermectin; biopesticides – microbial extracts, essential oils; essential compounds – nitric oxide, zinc DNA and siRNA to plants." These essential compounds were not listed in the review but they were supposed to be part of nutrient elements.
9	Chitosan NPs also adsorb easily on leaves so that it can be used as a coating for slow-release fertilizers and pesticides. [par 2] [ref. 55]	This paper is not dealing with foliar application of chitosan coated NPs for fertilizer delivery. In the introduction section it is stated that chitosan NPs easily gets adsorbed by plant surfaces, and that nanoencapsulation is used for the controlled release of micronutrients (which is true) but none of the references listed in ref. 55 have this as an experimental focus point. The listed references deal with herbicides, hormones and parasitic control.	Ref. 55 has several statements supporting this opinion. "CSNPs are one of the best nano-carriers (Pereira et al., 2017), which can accomplish the slow-and-steady release of target compounds and thus increases their bioavailability." "Chitosan in the form of NPs gets easily adsorbed to plant surfaces (e.g. leaf and stems) and helps in prolonged contact time between agrochemicals and the target surface. Chitosan nanoparticles (CSNPs) were effectively used in the delivery of insulin (Fernández-Urrusuno, Calvo, Remuñán-López, Vila-Jato, & Alonso, 1999). While, chitosan/alginate and chitosan/tripolyphosphate NPs have been used for sustained release of agrochemicals and phytohormones (Grillo, Pereira et al., 2014; Pereira, Silva, Oliveira, Oliveira, & Fraceto, 2017)." "Previously CSNPs have demonstrated improved molecular bioavailability of active ingredients by penetrating through the cell membrane to provide enhanced absorption (Nagpal, Singh, & Mishra, 2010)."
10	Its structure is also suitable for encapsulating metal	This paper is a poor choice as reference for this statement. In ref. 56 chitosan complexed Zn carriers for	This statement is not only based on Ref. 56, but also from the authors' summary of previous studies "Chitosan shows

	ions; chitosan NPs have been shown to increase the antibacterial efficacy of metal ions. [par. 2] [ref. 56]	foliar application of durum wheat (NP size >200 nm) were produced. The study found that foliar application of chitosan complexed Zn to plants grown in soil increased the Zn content of leaves, but no comparison to simple Zn salts (e.g. ZnSO4) of similar concentration were used and consequently the potential role of chitosan cannot be evaluated which is a major shortcoming of the paper (see also comments on this work in review by Kopittke et al. (2019)).	surface adherence (Zeng & Luo, 2012) and exhibits antibacterial properties. In addition, thiolated derivatives of chitosan showed enhanced antibacterial activity than pristine chitosan (Croce, Conti, Maake, & Patzke, 2016). Similarly, loading of metal ions on chitosan nanoparticles lead to increased antibacterial action (Du, Niu, Xu, Xu, & Fan, 2009)."
11	Due to rainfall and adsorption on soil complexes, the utilization rate of soil-applied fertilizers by plants is low, resulting in increased application of chemical fertilizers which leads to eutrophication. [par 2.2] [ref. 62]	This paper is not dealing with eutrophication caused by soil applied fertilizers, but is a study focusing on NP uptake in water melon. The reference lists eutrophication as a challenge in soil fertilization in the introduction section of the paper, but obviously should not be used as a relevant reference in a scientific paper as it is not the topic. Several quality studies on this important topic have been produced in well reputed journals and it is striking that none of these are cited.	Ref. 62 mentioned "Chemical fertilizer uptake efficiency in plants is low due to fixation of nutrients with other soil composites or run off due to precipitation leading to a growing anthropogenic eutrophication issue."
12	Compared with traditional soil applied fertilizers, foliar applied nanofertilizers have the advantages of being quickly absorbed by plants, being more cost-effective, and minimally impacting soil health [par 2.2] [ref. 64]	This paper contains no data to support the statement that foliar applied NPs are taken up more quickly, more cost-effective and with minimal impact on soil relative to traditional soil-applied fertilizers. The paper shows that B containing NPs relative to a conventional B salt leads to the same B concentration in the shoot tissue, but a significantly higher biomass was obtained when B containing NPs are applied.	Ref. 64 summarized this information from other studies "The advantage of using nano-fertilizers is that application can be performed in smaller amounts than regular fertilizers, whose efficiency is about 30-50% (Fageria et al., 2009). Therefore, nano-fertilizers may be more efficient, decreasing soil pollution and other environmental risks that may occur when using regular chemical fertilizers and/or conventional foliar sprays (Liu and Lal, 2015)."
13	Researchers have shown that slow-release nanofertilizers enhanced plant uptake	This is a study of foliar application of sulfur containing NPs on tomato. There is no experimental data on N, P and K in the paper and certainly no	The authors of Ref. 65 mentioned that potassium and nitrogen were increased under SNPs treatment: "Foliar sprayed with SNPs was found acting on

	of nitrogen, phosphorus, and potassium [par. 2.2] [ref. 65]	support for the statement that slow release nanofertilizers enhance the uptake of these plant nutrients.	increasing the potassium content in fruits of tomato compared with control." and "These observations is a result of interaction of SNPs absorbed by leaves with organic compounds of tomato tissues forming organic sulfur compounds, which help in building the chlorophyll and nitrogen content of the leaves." It was a mistake to include phosphorous.
14	[...] the vacuole and cell wall serve as the main accumulation sites of NPs. [par. 3][refs. 74 and 75]	Ref. 75 does not show NP accumulation into cell vacuoles. Also, ref. 74 only discusses vacuole sequestration of heavy metal ions (not NPs) through specific ion transporters. The only example provided in ref. 74 refers to Kachenko et al. (2010), a study about AsIII vacuole sequestration in As hyperaccumulating fern Pityrogramma calomelanos. Therefore, the references chosen provide no evidence of NP accumulation in vacuoles. Kachenko et al. (2010) Environ. Sci. Technol. 44 (12), 4735-4740	Ref. 75 does not show NP accumulation into cell vacuoles but it shows accumulation in cell wall, which supports the statement. In Ref. 75, the authors mentioned that NP could accumulate in cell walls. "Indeed, Lan et al. (2019) found that more than 90% of the internalized Cd was located in the cell wall, which explained why *Microsorum pteropus* is a Cd hyperaccumulator (Lan et al., 2019). Similar results were also found in *Canna indica* L., as the majority of internalized Cd was blocked by the cell walls, and this Cd proportion increased with increasing Cd exposure levels (Dong et al., 2019)." Ref. 74 is a review about heavy metals, and the authors mentioned many times about metal nanoparticles (nano-size heavy metals). The statement was based on their general comment "These mechanisms include reduced metal uptake and transport, induction of specific heavy metal transporters, limiting accumulation in sensitive tissue or sequestration in tolerant organs(vacuoles)……"
15	For example, the cuticle contains a large amount of pectin which promotes NP penetration. [par. 3.1][ref. 85]	Ref. 85 is a critical review from 1986 which only studies foliar uptake of inorganic ions. NPs are never mentioned in the text.	Ref. 85 mentioned that "The cuticle contains large fractions of pectins which have properties of swelling and shrinking with the absorption or loss of water and, therefore, are likely to facilitate the permeability."

			This part of the review discusses cuticle composition and the cuticle permeability which is suitable for NPs absorption in leaves.
16	Once inside the leaves, NPs could accumulate in the vacuole to slow down absorption and transfer in plants. [par. 3.1][refs. 74 and 89]	Vacuoles are never mentioned in ref. 89. Also, as explained above, ref. 74 only discusses vacuole sequestration of heavy metal ions (not NPs) through specific ion transporters. Therefore, the references chosen provide no evidence of NP accumulation in vacuoles.	This comment has been addressed in query #14. It is a mistake to include Ref. 89 here.
17	Lastly, the Casparian strip serves as the ultimate barrier that could hinder the penetration of NPs into the xylem. [par. 3.1][ref. 36	From ref. 36: Sun et al. (2014) used fluorescently labeled mesoporous silica NPs to visualize SiNP transport in plants and reported on the important role of the Casparian strip in minimizing NP penetration into the xylem vessels. The statement above refers to NPs applied to plant roots (and not to leaves), as clearly stated in the abstract of Sun et al. (2014). Hong et al. may have misunderstood this statement, as the Casparian strip is not present in leaves of higher plants (with rare exceptions, of little or no relevance to modern agriculture). The plant species tested in Sun et al. (2014) are lupin, wheat and maize, which do not possess a Casparian strip in leaves.	There is a reference (Lersten, N.R. Occurrence of endodermis with a casparian strip in stem and leaf. *Bot. Rev* **63,** 265–272 (1997).) that discussed about Casparian strip occurring in plant stem and leaf. It is unfortunate that there was a mistake in the citation.
18	A report showed that light (which affects photosynthetic efficiency) and root temperature influence leaf surface absorption of NPs. [par. 3.1][ref. 85]	As explained above, ref. 85 is a critical review from 1986 which only studies foliar uptake of inorganic ions. NPs are never mentioned in the text.	As mentioned in Ref. 85: “Light, temperature, and relative humidity affect the leaf growth and the cuticular development”, factors that could probably affect absorption of NPs.
19	Studies have shown that negatively charged particles may be transported through vascular tissues, while	This sentence contains a number of statements based on the work from several studies. In our opinion, it would be easier for the reader if Hong et al. cited the original works. In this	This statement was based on many studies that had been summarized by the authors from Ref. 72. It was an oversight not to clarify that statement was a summary from the cited reference

	positively charged particles may cross the cell membrane by endocytosis. Studies also showed that negative charge is more favorable for transport, while positive or neutral charge is more favorable for accumulation on the plant vascular system and therefore not transported. [par. 3.1][ref. 72]	particular instance, finding the original sources is quite difficult and time demanding. To give an example, we could not find in ref. 72 any sentence about positively-charged NPs crossing cell membranes by endocytosis.	(i.e., Ref 72).
20	"A report showed that surface coating material prevents blocking of stomata by reducing the excessive accumulation of NPs, thus increasing the probability of NPs being absorbed in the leaves. [par. 3.1][ref. 97]	We could not find this information in ref. 97.	In Ref. 97, the authors mentioned "PVP coatings have been demonstrated to prevent NP aggregation through steric hindrance effects". And "Once NPs enter plants, steric repulsive interactions between NPs and conducting tube surfaces are predicted to facilitate NP transport throughout the plant. Compared to PVP and Ct, GA was highly effective in inhibiting the aggregation of NPs in synthetic sap and enhancing the mobility of NPs in trees."
21	For example, hydroxyapatite can be applied to modify the NPs' surface to reduce their aggregation and increase leaf absorption. [par. 3.1][ref. 95]	Ref. 95 is about Zn NPs in plants, hydroxyapatite is never mentioned here.	This is a mistake. It should be humic acid, not hydroxyapatite.
22	Leaf pores have a diameter of about 100 nm but waxy hydrophobic stomata have a smaller pore size, which can block large particles [par. 3.2][ref. 72]	This sentence does not reflect what is stated in ref. 72 par. 2.1, namely: This waxy hydrophobic cuticle has very small pores (<5.0 nm),40 which prevent the uptake of all but the smallest nanomaterials.41 In addition to these nanopores, plant leaves have larger pores, known as stomata	This statement should have been leaf pores having a diameter of about 10 μm or more but waxy hydrophobic cuticle has a smaller pore size, which can block large particles. It was another mistake.

		(which can occupy up to 5% of the total leaf surface area) that are used to regulate water and gas exchange with the environment; these stomata have sizes that run in the 10's of microns .	
23	Large particles (50 200 nm) are mainly transported through the apoplast, while small particles (10 50 nm) are transported mostly via the symplast. [par. 3.2][ref. 58]	This is a very confident statement, which doesn't fully address the current disagreements and uncertainties on NP size exclusion limits in plant biology. Furthermore, ref. 58 (review article) has taken these numbers (e.g. 50-200 nm) from another article (Raliya et al. (2016)), which in turn cites another review (Schwab et al. (2014)), which does not report these exact numbers, and further states: The available literature provides no definite answer whether NPs prefer transport through the apoplast or symplast. However, to date, most data support transport through the apoplast.	In Ref. 58, the authors mentioned "Transport of NPs (between 10 and 50 nm) is favored through the symplastic route (through the cytoplasm of adjacent cells) whereas translocation of larger NPs (between 50 and 200 nm) occurs via the apoplast (in between the cells)." They made this statement based on four other references not only from Raliya et al. (2016). NPs have been found both inside and outside the cell suggesting both their apoplastic and symplastic movement inside plants.
24	It is noteworthy that foliar spray of NPs improved elemental contents in plants: ZnO NPs enhanced P and zinc uptake in tomato,18,100 (...) [par. 4.1.1] [refs. 18 and 100]	Ref. 18 is a study examining the effects of foliar application of ZnO NPs to tomato plants in relation to reducing negative effects of cadmium toxicity but there is no elemental analysis of nutrient accumulation or uptake or any other form of documentation of improved P or Zn uptake because of foliar NP application. In addition, the study had no control treatment with foliar application of a conventional zinc fertilizer in combination with cadmium or a control with soil-applied ZnO NPs. Ref. 100 is a study of foliar vs. soil application of ZnO NPs to tomatoes, which found enhanced Zn accumulation in the leaves of the foliar treatment compared to soil application. However, soil	Regrettably, this is an example mistakenly included in the wrong list.

		application enhanced Zn accumulation in the shoot compared to foliar application and overall the effects on plant growth and quality from the different treatments was varying. Furthermore, there is no documentation of P uptake. With reference to another study that found increased phytase and phosphatase activity after ZnO NP application, the authors in ref. 100 hypothesize that the ZnO NPs could increase P availability, but there is no evidence to support this in the study.	
25	In the same manner, foliar SiO2 NPs (spherical particles, 97.8 2.8 nm) significantly (...) reduced Cd toxicity in rice,58 (...) prolonged the storage time of grains and fruits,88 reduced disease rate in harvested crops and improved the quality of fruits.108 [par. 4.1.2] [refs. 58, 88, 108]	Ref. 58 is a review, which contains a reference to a study where application of SiO2 NPs has been found to alleviate Cd toxicity in rice however, the SiO2 NPs used in the study are approximately 60 nm and thus not the same size as written in the Hong et al. (2021) paper. Ref. 88 is a study of foliar applied Cu NPs to tomatoes and does not use or discuss the application of SiO2 NPs. Ref. 108 is a study about application of Mn3O4 nanozymes to cucumbers and is not related to SiO2 NPs.	This statement is supposed to be the effects of different foliar NPs, but information got deleted during review and revisions.
26	The potential hazards of food safety should be taken seriously especially since there are reports showing that NPs could induce cancer and genotoxicity in human cells.79 [par. 4.1.2] [ref. 79] Foliar application of high concentrations of metal NPs to vegetables	Ref. 79 is a study that examines the toxicity of micro-sized metal-rich particles in cabbage plants, and therefore cannot be used as a report on the effect of nanoparticles in relation to genotoxicity or cancer in human cells, or for the effects of NPs on gene expression in vegetables, since it does not involve nano-sized particles.	Ref. 79 used CuO particle with size <50 nm as given in the supporting information.

	has significant effects on gene expression79 [par. 4.1.2] [ref. 79]		
27	Although humans may naturally digest and excrete NPs,81 their accumulation in the human body and their toxic side effects cannot be ignored.63 [par. 4.1.2] [ref. 63]	Ref. 63 is a review on the latest (from 2016) R&D in foliar nanofertilizers, and the topic is not bioaccumulation or toxicity to humans. Toxicity as a topic is limited to this sentence about ecotoxicity: In-depth and long-term field trials are required globally to observe the practical environmental behaviour and ecotoxicity of nanoparticles.	Ref. 63 discussed foliar nanofertilizer and they proposed that evaluation system including ecotoxicity is an important part of future perspectives.
28	Foliar NPs may cause unknown toxicity which limits their use in agriculture.103,123 [par. 4.2] [refs. 103, 123]	Ref. 103 is a study of the potential of β-D-glucan nanoparticles (a biopolymer) for protection of turmeric plants against rhizome rot disease by increasing the activity of the plant s own defense mechanisms. This reference is in the section about Adverse effects of foliar NPs on plants but it does not relate to toxicity of foliar NPs (neither towards humans, crops or the environment) rather it finds that the NPs used in the study was beneficial to the plants. The only mentioning of toxicity of NPs and how that might affect its use in agriculture is as part of the introduction to the study, as a justification for their use of a biopolymer: The possible environmental toxicity due to unpredicted nature of metal nanoparticles has raised serious questions of their application in crops. Therefore, the selection of nanomaterial for application in field may be critical as materials which are non-toxic, biocompatible and biodegradable are desirable. Ref. 123 is a review of production and application of silver nanoparticles in	This statement is supposed to mean that NPs used as foliar fertilizer may cause unknown toxicity. Although Ref. 103 is a study about NPs' benefits to the plants, the statement from Ref. 103 ("But the possible environmental toxicity due to unpredicted nature of metal nanoparticles has raised serious questions of their application in crops.") supports the opinion in the review. In Ref. 123, the authors pointed out that silver NPs cause health and environmental concerns in the abstract. In addition, they discussed may times the Silver NPs toxicity to human and environment. "There are various literatures that suggest that the nanoparticles can cause various environmental and health problems, though there is a need for more studies to be conducted to conclude that there is a real problem with silver nanoparticles." "…also looks at the chances of these particles to induce toxicity in humans and the environment as a whole." "…which may pose potential environmental and biological risks." "However, there are studies and reports that suggest that nanosilver can

		medicine. It mentions that there are a few studies of silver NPs toxicity and that Nanosilver with its antimicrobial activity can hinder the growth of many friendly bacteria in the soil. By showing toxic effects on denitrifying bacteria, silver can disrupt the denitrification process, which involves the conversion of nitrates into nitrogen gas which is essential for the plants. However, they also write: Though these studies tend to suggest that nanosilver can induce toxicity to living beings, it has to be understood that the studies on nanosilver toxicity were done in in vitro conditions which are drastically different from in vivo conditions and at quite high concentrations of nanosilver particles. Hence, it is imperative that more studies be carried out to assess the toxicity effect nanosilver has in vivo before a conclusion on its toxicity is reached. There is no mention of foliar applied NPs or any use of Ag NPs in agriculture it therefore seems to be an unsuitable reference to support the statement that the use of foliar NPs in agriculture is limited by their unknown toxic effects.	allegedly cause adverse effects on humans as well as the environment." "Nanosilver with its antimicrobial activity can hinder the growth of many 'friendly' bacteria in the soil."
29	Copper deficiency can cause young leaf dysplasia, but excessive use can cause toxicity to plants.37 [par. 4.2] [ref. 37]	Ref. 37 is a study of biosynthesized silver and copper NPs as foliar biological control of bird's eye spot disease in tea plants. The study does not mention or investigate the adverse effects of high doses of NPs on plants, and the only mention of Cu toxicity is this: Cu deficiency may become more prevalent in coming future, increased use of nitrogenous fertilisers will lead to severity of Cu deficiency. However, higher concentration than optimum showed toxicity in uptake of	In Ref. 37, the authors mentioned that "Cu deficiency in plants is an important factor as it results in yield losses, with little evidence of the characteristic symptoms." "Higher concentration than optimum showed toxicity in uptake of nutrients." The discussion in the review is regarding high concentration of elements, and the statement is used to support negative effects if high amount of Cu is used.

		nutrients (Passam et al (2007). Passam et al. (2007) is a review paper about tomato nutrition and fruit quality which does not mention nanoparticles anywhere.	
30	NPs induce the accumulation of ROS, causing damage to lipids and proteins.126 [par. 4.2.1] [ref. 126]	Ref. 126 is a study of the effect of Fe NPs (as a proxy for industrial emissions) on a bryophyte in which the authors did not find any impact on plant health (measured by ATP generation) or any significant disturbance in ROS generation. They also did not find any significant increase in malondialdehyde levels and no damage to cell membranes. It is therefore incorrect to use this reference to make the general statement that NPs induce the accumulation of ROS and cause damage to lipids and proteins.	In Ref. 126, the authors did find ROS increase. "We did not observe significant changes in the quantities of ROS/RNS except for a significant increase ($p<0.05$) after 3 days of exposure at 50 ng and 50 mg/plant (Fig. 3)." "We observed ROS/RNS over production in P. patens for both the longest time period and the highest doses." Although in their study they did not find damage to cell membranes, they discussed this phenomenon found in other studies.
31	NPs can undergo chemical changes in plants, such as redox and valence transformation, which can cause damage to plants. 9,128 [par. 4.2.1] [refs. 9, 128]	Ref. 9 is a review on the benefits of using NPs to fertilize fruit crops, where they have included just one case of damage /negative effects of NP application to a fruit crop (Negative effects of NPs in different fruit tree species may occur at high concentrations, but since there is limited knowledge on this topic, no definite conclusion can be made.). This reference does not mention chemical changes to NPs in plants like redox or valence transformations. Ref. 128 is a study of broad beans cultivated in a soil amended with varying levels of cadmium sulfide NPs. It examines how the metabolic, phenotypic and biochemical response of the plants change as a result of heavy-metal induced stress and if the outcome is toxicity or detoxification,, but it does not engage with any use of foliar NPs or their chemical	Based on statement from Ref. 9 "After penetrating the leaf or root cuticle tissue, NPs move through different pathways (apoplastic, symplastic, lipophilic and hydrophilic), which influence their effectiveness, final fate and may also change their properties and therefore their reactivity, delivery and translocation inside plant tissues, which may result in various responses of different plant parts to the same NP." Ref. 128 was cited by mistake.

		transformation in plants.	
32	The interaction between NPs and cells may cause some mechanical damage to the cell structure, such as blocking the ducts, cell wall pores, and stomata, resulting in obstruction of nutrient uptake and transport.5,87 [par. 4.2.1] [ref. 5]	Ref. 5 is study of Ag NPs uptake and translocation in lettuce after foliar and root exposure, where they observe phytotoxicity but there is no evidence (such as microscopy data) of the mechanical damage to the cell structure that Hong et al. (2021) refers to, i.e. "blocking the ducts, cell wall pores, and stomata". There is only speculation, as can be seen here: "The action chain of toxicity of particulate Ag was induced by the penetration of AgNPs into cells, followed by the translocation to various organs and by suggested blocking of internal trafficking, thus resulting in biomass reduction".	Based on the statement in Ref. 5 "After uptake and accumulation of $AgNPs_{(total)}$, particles can deposit and/or aggregate in plasmodesmata and in the cell wall, which might cause mechanical damage and/or the blockage of intercellular communication. This could affect nutrient uptake and translocation, and the regulation of plasma membrane receptors, as well as plasma membrane recycling and signaling in plants."
33	Foliar application and root pathways can work together to reduce plant damage and improve utilization of nutrients.81,102 [par. 4.2.1] [refs. 81, 102]	Ref. 81 is a study of a simulated trophic transfer of cerium, where lettuce is subjected to both root and foliar exposure of 141Ce and then fed to snails. However, the study does not relate to the topic of nutrient utilization or phytotoxicity/plant damage. Ref. 102 is a study where γ-Fe2O3 nanoparticles were foliar applied to citrus. There was no evidence of increased root activity or promotion of plant growth following application, so this reference cannot be used to support the statement. Supposedly, this section from ref. 102 is the reason for the choice of reference: We observed the uptake of iron into shoots but no difference of iron content in C. maxima roots between all treatments, suggesting that no downward transport of iron occurred in C. maxima plants. In our previous study, we observed that root-applied γ-Fe2O3 NPs had no	It is a mistake to cite Ref. 81. Based on the statement in Ref. 102 "Moreover, in real applications, foliar sprayed γ-Fe2O3 NPs may be utilized together with soil supplied γ-Fe2O3 NPs to alleviate chlorosis and improve the iron use efficiency."

		translocation from roots to shoots. Therefore, either foliar spray or root supply of γ-Fe2O3 NPs alone cannot meet the requirement of the whole plants. A combination of both application methods may improve the effectiveness of iron fertilization in agricultural and horticultural production.	
34	Furthermore, it is worth noting that the accumulation of heavy metals, pesticides, and antibiotics in plants is significantly reduced when metalbased or carbon-based NPs are applied.36 [par. 4.2.1] [ref. 36]	Ref. 36 is a food science review on uptake of nanoparticles and writes: metal- or carbon based NPs can significantly reduce the accumulation of heavy metals, pesticides, and antibiotics in plants, suggesting that nanotechnology for soil remediation may be an efficient and sustainable approach to recovering land for agricultural use (emphasis added). The reference considers the specific context of NP application to contaminated soils. When Hong et al. writes applied in a review on foliar application it would be natural to assume they are not mentioning results from soil application, and the reference is thus misleading if not clarified further.	Even though this review is focused on foliar application, the effects of NPs dropping down into soil by wind or rainfall after foliar application should not be ignored.
35	Therefore physical barriers, such as plastic greenhouses to reduce the adsorption of atmospheric particulate matter by plants and cultivating tall shrubs and plants that can block and accumulate pollutants in highly polluted areas can be alternative mitigating measures to reduce potential accumulation and risks of NPs in plants.35,39,89 [par.	The referenced papers do not directly concern physical barriers to protect against NPs, or evidence that this has an effect. They do, however, find that deposition of NPs on foliage can be a health concern. Ref. 35 finds that NPs from urban areas can be carcinogenic in healthylooking new lettuce leaves. Ref. 39 finds that atmospheric deposition of NPs is a health concern, but no mentions of physical barriers were found in the paper. Ref. 89 studies adhesion of NPs to foliage. One sentence in the discussion is relevant for the	Ref. 35 and Ref. 39 had statements about plants accumulating pollutants from atmospheric aerosols and atmospheric deposition. Ref. 89 contains suggestion about physical barriers. Ref.35 "Over the last few years, studies concerning foliar metal transfer have demonstrated that the leaves can accumulate heavy metals from atmospheric aerosols and may reduce PM amount in the atmosphere, i.e., foliar dust." "Finally, plant leaves are effective particulate interceptors and have high potential of transferring airborne trace elements to the food chain."

	4.2.1] [refs. 35, 39 and 89]	statement, referencing Song et al. (2015), which is a study on urban trees effects in mitigation of airborne particulate matter. Song et al. (2015), Atmos. Environ., 105:53–60	"Indeed, vegetation can effectively adsorb and reduce particulates in the air by capturing the airborne PM on their leaves." In Ref. 39, "These results showed that heavy metals accumulation in pakchoi shoots exposed to high deposition areas was not only from the root transfer of the original heavy metals in soils but also from atmospheric deposition. Previous research also showed that the majority of Ni and Cu found in birch foliage in the heavily contaminated site was resulted from atmospheric deposition." "Therefore, some solutions of reducing the effects from atmospheric deposition should be taken, such as plastic greenhouse planting in slightly polluted soil near the smelter [47]. However, the seriously contaminated soil were not recommended for planting vegetables due to high health risks of consumption, even excluding the effects of atmospheric deposition. Some hyperaccumulators or high biomass plants (Elsholtzia Splendens, Pennisetum sp., and Sedum plumbizincicola) were recommended and planted in seriously contaminated soil for phytoremediation [39]." In Ref. 89, "Since NPs are not removed by washing only with water, strategies to limit human consumption of metallic NPs from atmospheric deposits and agricultural foliar sprays should focus on the removal of outer peels and leaves and implementation of physical barriers, such as the use of greenhouses and the cultivation of tall bushes and trees on garden perimeters."
36	Foliar application of NPs can promote growth, biomass	Ref. 22 does not report promotion of growth, but phytotoxicity and concerns for public health.	In Ref. 22, the authors summarized that "Application of some of NPs become an active ingredient for plant

	production, and yield in some agricultural crops22,41 and can cause nutrient deficiency, retard root elongation, and delay flowering in others.14,94 [par. 4.3] [refs. 22, 14 and 94]	Ref. 14 found both positive or negative growth in habanero peppers depending on the amount applied. No clear findings of the effects mentioned by Hong et al. In ref. 94 they studied the response of wheat to salinity stress, and found that foliar application of FeO NPs increased growth.	micronutrients and increase nutrient availability by the developing crop. Reports show the positive impacts from metal/metal oxide NP on crop growth and pathogen inhibition, such as Ag, ZnO, TiO2, CuO. The increase in crop growth/yield might occur simply by the result of anti-pathogenic activity of the NP itself, or indirectly through the induction of key defensive pathways and metabolites within the plant." Ref.14 should been cited in the first half sentence, together with Ref. 22 and 41. In Ref. 94, the authors stated that "Adequate Zn can accelerate the wheat growth, tillers, and anthesis, while excessive Zn content in the environment will inhibit the growth of wheat seedlings." These four references talked about both positive and negative effects of foliar applied NPs, and should have been cited together at the end of the sentence.
37	"NPs can affect plant growth by releasing toxic ions, hindering biochemical processes, and inducing imbalance in reactive oxygen species (ROS).130 An appropriate amount of ROS plays a key role in plant development, cell division, and gene expression.133 However, excessive production of ROS in plants will cause the reduction of protein content, DNA damage, and lipid peroxidation and lead to plant death eventually.12,129 [par. 4.3] [refs. 130, 12 and	In this section, they do not reference directly to papers studying ROS, even though several well-established reviews on ROS production, signaling and defense systems can be found, e.g. Gill and Tuteja (2010) or Apel and Hirt (2004). 130, 12 and 129: Are all studies of NP application and development of antioxidants. None is directly investigating ROS effects, although the effects of ROS are mentioned in introductions and discussions. Gill and Tuteja (2010), Plant Physiol Biochem. 48(12):909-30 Apel and Hirt (2004), Annual Review of Plant Biology. 55:373-399	There is more than enough information in Ref. 130, 12 and 129 to support our statement. The two papers (Gill and Tuteja (2010) or Apel and Hirt (2004) mentioned are not related to nanoparticles' effects. In Ref. 130 "The indirect effects of NPs are caused inter alia by the release of toxic ions (e.g., metal ions), enhancement of the bioavailability of some toxic compounds, or by causing overproduction of reactive oxygen species (ROS) [1,2,6]." "Most studies have demonstrated that an excess of metal-based NPs can cause negative effects like reduced germination, dry weight, biomass, and transpiration, disturbances in the photosynthetic process, chlorophyll degradation, protein reduction, DNA damage, nutrient displacement, and

	129]		others [8,9]. NPs trigger an oxidative burst by interfering with the electron transport chain and the production of ROS [1]." In Ref 12. "Ceria NPs are reported to have both beneficial and toxic effects on biological systems (Yokel et al., 2014; Walkey et al., 2015). The particles can exert a pro-oxidative effect by producing reactive oxygen species (ROS) which damage lipids, proteins, DNA, and cause cell death. On the other hand, ceria NPs have been shown to be excellent free radical scavengers and therefore protecting cells from oxidative damages (Dahle and Arai, 2015)." In Ref. 129, "Spraying the iron oxide NPs significantly increased the leaf area in the plants under salt stress conditions. Total phenolic, flavonoid and anthocyanin content, as well as the activity of guaiacol peroxidase, ascorbate peroxidase, catalase and glutathione reductase enzymes were enhanced in the shoot and root of the plants treated with 100 mM of NaC1 solution." "Reactive oxygen species (ROSs) produced during oxidative stress react with lipids, proteins, nucleic acids and cell enzymes and induce planned cell death pathway, ultimately leading to plant damage (Gill and Tuteja, 2010). Under natural conditions, there is a balance between the amount of ROSs production and scavenging. However, under severe environmental stresses, the balance is disturbed and the oxidative stress is produced in plants cells accordingly (Hussain et al., 2016)." "In previous studies, iron oxide NPs

			increased the antioxidant enzymes activity in wheat plants under salinity stress (Babaei et al., 2017), and improved antioxidant activity (Rizwan et al., 2018)."
38	Malondialdehyde (MDA) is the end product of polyunsaturated fatty acid oxidation, which directly reflects the degree of lipid damage caused by oxidative stress.99 [par. 4.3] [ref. 99]	Ref. 99 is not a study on the effects of MDA, but a study on the effects of iron sulfide NP application on growth in B. juncae, where MDA contents was used as a proxy for membrane damage by lipid peroxidation. However, this sentence was found almost word-for-word in Hong et al. s reference no. 134, Zhang et al. (2018), which was not referenced for this statement. Zhang et al. writes the following: MDA is an end product of polyunsaturated fatty acid oxidation, which directly reflects the extent of lipid damage induced by oxidative stress. Emphasis added to the differences between Hong et al. and the quote from Zhang et al. Zhang et al. (2018) Environ. Sci. Technol., 52:8016-8026	In Ref. 99, the authors had this statement "Malondialdehyde is produced as a result of lipid peroxidation which is an index of membrane damage (Sharma et al., 2012b)."
39	Some studies have shown that the photosynthetic related processes of plants are inhibited after foliar application of NPs, which includes decreased photosynthetic activity, damaged chloroplast membrane, decreased gas exchange capacity,9,90 [par. 4.3] [refs. 9 and 90]	Ref. 9 is a review focusing on the positive aspects of foliar NP application in fruit crops. No mentions of negative impacts on photosynthesis, chloroplast membranes or gas exchange capacity could be found.	This was a mistake, it should be only Ref. 90.
40	and destroyed chlorophyll machineries that resulted in leaf	Ref. 35 is a study of foliar transfer of metals in lettuce which found necrotic leaves after exposure to CdO NPs. Speculated causes were: metal	The statement should not have included "destroyed chlorophyll machineries". Ref. 135 was cited by mistake. It should be only Ref. 35.

	chlorosis, necrosis, and senescence. 35,135 [par. 4.3] [refs. 35 and 135]	uptake which could affect metabolism, and metal aggregates on the surface which could interfere with gas exchange, but not a destruction of chlorophyll machinery. Ref. 135 studies erythromycin in algae, and did not apply nanomaterials.	
41	On the other hand, some literature reports have shown that foliar spray of TiO2 NPs can increase the photosynthetic rate by stimulating enzyme activity and accelerating the photolysis of water.17,27 [par. 4.3] [refs. 17 and 27]	Ref. 17 contains no mention of photolysis, but does report an increase of stress enzymes and chlorophylls in cowpea after foliar application with TiO2 NPs. Ref. 27 contains no mention of TiO2, but studies Ag@CoFe2O4 NPs.	Ref. 17 cited for enzyme activity and water photolysis is from Ref. 117: "For example, TiO_2 NPs could promote the light absorption by the chloroplast in Arabidopsis, regulate the distribution of light energy from PSI to PSII, and accelerate the transformation from light energy to electric energy, water photolysis, and oxygen evolution (Ze et al. 2011)." The citation should be Ref. 17 & 117, not 17 & 27.
42	A study showed that glycine and serine are two essential amino acids which are formed during photorespiration, and their ratio is usually used as an indicator for photorespiration activity and leaf senescence. Glycine can also be used to synthesize a wide range of defense molecules including glutathione.134 [par. 4.3] [ref. 134]	The statements cannot be directly supported by the reference, which merely refers to others: Ref. 134 states: Gly/Ser ratio is commonly used as an indicator of photorespiratory activity. and Wingler et al. suggested that the photorespiration pathway may provide additional protection against oxidative damage under high light-induced stress by supplying glycine, which can be used for synthesis of the broad defense molecule glutathione.	Ref. 134 discussed: "Glycine (Gly) and serine (Ser) are two essential amino acids formed during photorespiration, and the Gly/Ser ratio is commonly used as an indicator of photorespiratory activity." "Wingler et al.37 suggested that the photorespiration pathway may provide additional protection against oxidative damage in under high light induced stress by supplying glycine, which can be used for the synthesis of the broad defense molecule glutathione. Earlier reports also demonstrated that the Gly/Ser ratio is a sensitive biomarker of leaf senescence, changing significantly even prior to senescence symptoms."
43	In addition, the application of NPs may change the activities of starch-degrading enzyme, starch phosphorylase, and	Ref. 9 contains no mention of starch, sucrose or carbohydrate. Ref. 133 is a paper about As toxicity. It mentions changes in enzyme activity as a response to As, but not as a response to NP application. Here,	In Ref. 9, there is a discussion about sugar change based on NPs treatment: "Treatment with N-containing NFs at 0.25(N1) and 0.50 (N2) g/L also improved the quality of pomegranate fruits by increasing aril juice (mL/100 g

	sucrose phosphate synthase in plants, thus inducing the change of carbohydrate content in plants. 9,133 [par. 4.3] [refs. 9 and 133]	the authors state: "Additionally, a strong inhibition of the activities of starch degrading enzymes, i.e., α- and β-amylase, and starch phosphorylase has been reported as a result of As-induced plant toxicity. (…) Furthermore, the upregulation in activities of sucrose-hydrolyzing enzymes, namely acid invertase and sucrose synthase, was investigated along with the suppression of activity of sucrose phosphate synthase, under in-situ As toxicity."	arils) (control: 62.5; N1: 63.3; N2: 68.3), total sugars (g/100 g juice) (control: 14.18; N1: 14.56; N2:15.54) and TA (%) (control: 1.74; N1: 1.84; N2: 1.89) of fruits. 41 An increase in N concentration improved turgor pressure 50, carbohydrate supply 45 and translocation of organic acids 51." In Ref. 133, the authors discussed that enzyme activities could affect starch and sugar content. So these two references were cited and to dicuss that "the application of NPs may…"
44	However, the application of NPs on leaves may result in the oxidation of several amino acids (such as lysine, methionine, proline, threonine, etc.) to form free carbonyl groups, which will inhibit the activity of protein.127,133 [par. 4.3] [refs. 127 and 133]	Ref. 127 studies oxidative stress response after application of Ag NPs, with no mentions of amino acid oxidation nor the formation of carbonyl groups. Ref. 133 is a study on As uptake and toxicity. There is no mention of NPs. The authors might have read the following statements regarding ROS, and included it because NPs have been shown to induce ROS production: "The ROS produced in response to As stress can modify proteins, thereby delivering carbonyls. The amino acids, particularly Arg, His, Lys, Pro, Thr, and Trp, of any protein become oxidized and form free carbonyl groups, which may inhibit or alter the protein activities. These references are not a sufficient basis to confirm the stated effects of NP application.	Ref. 127 discussed "ROS generation and scavenging during SNPs" discussed about ROS generated under NPs treatment. In Ref. 133, the authors pointed out that protein are oxidized by ROS. In Ref. 133: "In addition to hydrolysis, like lipid moieties, proteins are also susceptible to ROS attack [239] (Figure 3). These ROS-induced modifications in proteins may be initiated by leakage of electrons during metal ion-dependent reactions and auto-oxidation of both carbohydrate and lipids [198]. The ROS produced in response to As stress can modify proteins, thereby delivering carbonyls [239]. The amino acids, particularly Arg, His, Lys, Pro, Thr, and Trp, of any protein become oxidized and form free carbonyl groups, which may inhibit or alter the protein activities [240,241]."
45	Tyrosine and phenylalanine are precursors of alkaloids, glucosinolates and other secondary metabolites; when these two amino acids are up-	Ref. 134 contains no mention of tyrosine. Phenylalanine was only mentioned in this statement: Biological pathway analysis also reveals that phenylalanine metabolism, which is a stress response-related biological pathway,	This part was based on statement from Ref. 137: "As mentioned before, phenylalanine and tyrosine are precursors of defense related secondary metabolites. Their up-regulation in whole plant tissues indicates the activation of defensive systems."

	regulated they can be an indicator of activated defense response. 134 [par. 4.3] [ref. 134]	was disturbed at the dose of 40 mg of AgNPs .	“Tyrosine and phenylalanine are precursor compounds for a variety of secondary metabolites such as phenylpropanoids, alkaloids and glucosinolates,36 the up-regulation of these two amino acids is a likely indicator of leaf activated defense response.” Ref. 134 cited by mistake, it should be 137.
46	Also, when the contents of linolenic acid, which is one of the main components of the plasma membrane, decrease significantly it indicates that the cell membrane is destroyed.134,136 [par. 4.3] [refs. 134 and 136]	Based on the references, this statement is highly speculative. Ref. 134 finds a downregulation of the synthesis of linolenic acid as a response to Ag NPs. However, this study does not investigate the effects of such downregulation on the intactness of the membrane. They speculate that it either indicates a lipid peroxidation and damage, or that it is caused by a change in membrane composition as an acclimation to the NPs, to rebuild membrane integrity; Clearly, one potential reason for the observed up- or downregulation of fatty acid metabolites is the result of lipid peroxidation. Another possibility is that plants adjust the membrane composition to rebuild membrane integrity and to restrict Ag ion permeation into cells. However, this is not confirmed in the study. Ref. 136 studies effects of C60 fullerol NPs, and finds a decrease in linolenic acid following foliar NP application. However, they merely state that this is an indication of an altered cell membrane composition, not a sign of membrane destruction. Their results even indicate that no cell membrane disruption occurred upon exposure to both doses of C60 fullerols. This suggests that C60 fullerols may possibly alter the	In Ref. 134, the authors mentioned clearly that “All of these metabolite changes are indicative of Ag-induced disruption to the composition and integrity of lipid membranes.” Also, their results showed that “The malondialdehyde (MDA) content in cucumber leaves exposed to 4 and 40 mg AgNPs significantly increased (28.6% and 44.93%, respectively p 0.05) as compared to the control.” “Here, the MDA increase indicates potentially significant membrane damage as a function of AgNPs exposure.” So, linolenic acid definitely related to cell membrane damage. In Ref. 136, the authors stated “Linolenic acid, a major component of the plasma membrane” which we used to support the statement “…linolenic acid, which is one of the main components of the plasma membrane…”

		membrane composition, instead of physically damaging it , thus the opposite of Hong et al. s statement.	