Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Critical evaluation of selected references used in the review by Hong *et al.* 2021		
#	**Statement** [Paragraph] [reference number]	**Comments**
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.
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.
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
4	*"Plants absorb foliar NPs usually through stomata, cracks or water pores, ion channels, protein carriers, endocytosis, stigma, wounds and trichomes"* [Introduction] [refs. 9, 21-24]	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 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. Rico *et al.* (2011), *J Agric Food Chem*, 59:3485–3498
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.
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.

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.
8	*" […]and organic NPs can serve as nanocarriers of nutrient elements (e.g., iron and magnesium) to treat acute malnutrition in crops"* [par 2.1] [ref. 48]	The use of ref. 48 is misleading as they evaluated the potential of SiNPs 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.
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.
10	*"Its structure is also suitable for encapsulating metal ions; chitosan NPs have been shown to increase the antibacterial efficacy of metal ions."* [par. 2] [ref. 56]	This paper is a poor choice as reference for this statement. In ref. 56 chitosan complexed Zn carriers for 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. $ZnSO_4$) 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)). Kopittke *et al.* (2019), Environ. Sci.: Nano 2019, 6 (12), 3513−3524.
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.
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.
13	*"Researchers have shown that slow-release nanofertilizers enhanced plant uptake of nitrogen, phosphorus, and potassium"* [par. 2.2] [ref. 65]	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 support for the statement that slow release nanofertilizers enhance the uptake of these plant nutrients.

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 As^{III} 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
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.
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.
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. Sun *et al.* (2014) *Plant Cell Rep.* 33:1389–402
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.
19	*"Studies have shown that negatively charged particles may be transported through vascular tissues, while 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]	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 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.
20	*"A report showed that surface coating material prevents blocking*	We could not find this information in ref. 97.

	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]	
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.
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 (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”*. Raliya *et al.* (2016), *Front. Plant Sci.* 7:1288 Schwab *et al.* (2014) *Nanotoxicology* 10:257-278
24	*“It is noteworthy that foliar spray of NPs improved elemental contents in plants: ZnO NPs enhanced phosphorus 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 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 SiO_2 NPs (spherical particles, 97.8 ± 2.8 nm) significantly (...) reduced Cd*	Ref. 58 is a review, which contains a reference to a study where application of SiO_2 NPs has been found to alleviate Cd toxicity in rice – however, the SiO_2 NPs used in the study are approximately 60 nm and thus not the same

	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]	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 SiO_2 NPs. Ref. 108 is a study about application of Mn_3O_4 "nanozymes" to cucumbers and is not related to SiO_2 NPs.
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 has significant effects on gene expression[79]"* [par. 4.1.2] [ref. 79]	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.
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*".
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 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.
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 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. Passam et al (2007), *Eur. J. Plant Sci. Biotechnol.*, 1:1 – 21
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.
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 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*".
33	"*Foliar application and root pathways can work together to reduce plant damage and improve utilization of nutrient*s.[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 ^{141}Ce and then fed to snails. However, the study does not relate to the topic of nutrient utilization or phytotoxicity/plant damage.

	[par. 4.2.1] [refs. 81, 102]	Ref. 102 is a study where γ-Fe_2O_3 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-Fe_2O_3 NPs had no translocation from roots to shoots. Therefore, either foliar spray or root supply of γ-Fe_2O_3 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.
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. 4.2.1] [refs. 35, 39 and 89]	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 healthy-looking 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 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
36	*"Foliar application of NPs can promote growth, biomass production, and yield in some agricultural crops[22,41] and can cause nutrient deficiency, retard root elongation, and delay flowering in others.[14,94]"* [par. 4.3] [refs. 22, 14 and 94]	Ref. 22 does not report promotion of growth, but phytotoxicity and concerns for public health. 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.
37	*"NPs can affect plant growth by releasing toxic ions, hindering biochemical processes, and inducing imbalance in reactive oxygen species (ROS).[130] An*	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).

	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 129]	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
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
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. Ref. 90 reports negative effects on photosynthesis through degradation of chlorophyll in wheat after Fe_2O_3 NP application, but did not study chloroplast membrane damage, nor gas exchange.
40	*"and destroyed chlorophyll machineries that resulted in leaf chlorosis, necrosis, and senescence.[35,135]"* [par. 4.3] [refs. 35 and 135]	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 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 TiO_2 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 TiO_2 NPs. Ref. 27 contains no mention of TiO_2, but studies Ag@$CoFe_2O_4$ NPs.
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*	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."*

	defense molecules including glutathione.[134]*"* [par. 4.3] [ref. 134]	
43	*"In addition, the application of NPs may change the activities of starch-degrading enzyme, starch phosphorylase, and sucrose phosphate synthase in plants, thus inducing the change of carbohydrate content in plants.*[9,133]*"* [par. 4.3] [refs. 9 and 133]	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, 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."*
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.
45	*"Tyrosine and phenylalanine are precursors of alkaloids, glucosinolates and other secondary metabolites; when these two amino acids are up-regulated they can be an indicator of activated defense response.*[134]*"* [par. 4.3] [ref. 134]	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, was disturbed at the dose of 40 mg of AgNPs*".
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 membrane composition, instead of physically damaging it", thus the opposite of Hong *et al.*'s statement.