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Interaction of Copper-Based Nanoparticles to Soil, Terrestrial, and Aquatic Systems: Critical Review of the State of the Science and Future Perspectives

Vishnu Rajput, Tatiana Minkina, Bilal Ahmed, Svetlana Sushkova, Ritu Singh, Mikhail Soldatov, Bertrand Laratte, Alexey Fedorenko, Saglara Mandzhieva, Eliza Blicharska, et al.

► To cite this version:

Vishnu Rajput, Tatiana Minkina, Bilal Ahmed, Svetlana Sushkova, Ritu Singh, et al.. Interaction of Copper-Based Nanoparticles to Soil, Terrestrial, and Aquatic Systems: Critical Review of the State of the Science and Future Perspectives. *Reviews of Environmental Contamination and Toxicology*, 252, Springer New York, pp.51-96, 2019. hal-02353826

HAL Id: hal-02353826

<https://hal.science/hal-02353826>

Submitted on 7 Nov 2019

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8 *This is an author produced version of a paper published in:*

9
10
11 **Reviews of Environmental Contamination and Toxicology (ISSN: 0179-5953)**

12
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15
16
17 ***Citation for the published paper:*** Rajput V, Minkina T, Ahmed B, Sushkova S, Singh R, Soldatov M, Laratte B, Fedorenko
18 A, Mandzhieva S, Blicharska E, Musarrat J, Saquib Q, Flieger J, Gorovtsov A. (2019). "Interaction of Copper Based Nanoparticles to Soil,
19 Terrestrial and Aquatic Systems: *Critical Review of the State of the Science and Future Perspectives*". Reviews of Environmental
20 Contamination and Toxicology https://doi.org/10.1007/398_2019_34

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23
24
25 **Notice:** This paper has been peer reviewed but does not include the final publisher proof-
26 corrections or pagination. When citing this work, please refer to the original publication.

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28 (-article starts on next page-)

29 **Interaction of Copper Based Nanoparticles to Soil, Terrestrial and Aquatic Systems:**

30 *Critical Review of the State of the Science and Future Perspectives*

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61 Abstract

62 In the past two decades, increased production and usage of metallic nanoparticles (NPs) has inevitably increased
63 their discharge into the different compartments of the environment, which ultimately paved the way for their
64 uptake and accumulation in various trophic levels of the food chain. Due to these issues, several questions have
65 been raised on the usage of NPs in everyday life and has become a matter of public health concern. Among the
66 metallic NPs, Cu-based NPs have gained popularity due to their cost-effectiveness and multifarious promising
67 uses. Several studies in the past represented the phytotoxicity of Cu-based NPs on plants. However,
68 comprehensive knowledge is still lacking. Additionally, the impact of Cu-based NPs on soil organisms such as
69 agriculturally important microbes, fungi, mycorrhiza, nematode, and earthworms are poorly studied. This review
70 article critically analyses the literature data to achieve a more comprehensive knowledge on the toxicological
71 profile of Cu-based NPs and increase our understanding of the effects of Cu-based NPs on aquatic and terrestrial
72 plants as well as on soil microbial communities. The underlying mechanism of biotransformation of Cu-based
73 NPs and the process of their penetration into plants has also been discussed herein. Overall, this review could
74 provide valuable information to design rules and regulations for the safe disposal of Cu-based NPs into a
75 sustainable environment.

76

77 **Keywords:** Bioaccumulation; Bioavailability; Biotransformation; Cellular; Copper; Cytotoxic; Effects;
78 Emissions; Exposure; Fate; Freshwater; Genotoxicity; Nanoparticles; Nanotechnology; Microorganism;
79 Permissible levels; Phytotoxicity; Sediments; Soil; Sources; Sub-cellular; Techniques; Toxicity mechanism;
80 Trophic transfer; Ultrastructure

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125 **Introduction**

126

127 In recent years, potential effects of engineered nanoparticles (ENPs), and more so of metallic and metal-oxide
128 NPs, on aquatic and terrestrial systems have received increased attention due to their wide applications and
129 consequential release into the environment. Metallic NPs possess unique properties for potential use in the
130 rapidly growing nanotechnology industry (Ali et al. 2015; Arruda et al. 2015; Saleem et al. 2017). Various
131 products containing NPs are currently in the marketplace, and many are still being added to the list (Ahmed et al.
132 2018b; Rajput et al. 2018c; Vance et al. 2015). The Global Market for Metal Oxide Nanoparticles indicates that
133 the metal oxide NPs production could increase from 0.27 million tons (2012) to 1.663 million tons by 2020 (The
134 Global Market for Metal Oxide Nanoparticles to 2020). Among them, Cu-based NPs have wide applications in
135 the field of metallurgy, electronics, automotive, fuel, transportation, machinery etc. The annual production of Cu
136 was approximately 18.7 million metric tons in 2015 (Keller et al. 2017), out of which a small fraction of
137 approximately 200 tons was comprised of Cu-based NPs (Keller and Lazareva 2013). Since then, the use of Cu-
138 based NPs has been rapidly escalating into applications such as solar cells, sensor development, catalysts,
139 hydrogen production, drug delivery, catalysts for typical C-N cross-coupling reactions and light emitting diodes
140 (Keller et al. 2017; Rajput et al. 2017b). Due to their antimicrobial and antifungal properties, Cu-based NPs are
141 suitable for biomedical applications and are also used in water treatment (Ben-Sasson et al. 2016), textile
142 industries (Sedighi and Montazer 2016), food preservation, and agricultural practices (Montes et al. 2016;
143 Ponmurugan et al. 2016; Ray et al. 2015). The rapid production and multifarious applications of Cu-based NPs
144 in various industries have necessitated the assessment of their impacts on the environment (Ahmed et al. 2018b,
145 c).

146 Copper (Cu) is a naturally occurring ubiquitous element present in the environment with a concentration
147 around 60 g per ton in the Earth's crust (Ojha et al. 2017) and essential micronutrient for plant growth at certain
148 concentrations and is known to play important roles in mitochondrial respiration, hormone signalling, cell wall
149 metabolism, iron mobilization, and electron transport (Yruela 2009). However, at higher concentrations, Cu is
150 generally toxic to plants and other organisms including algae, mussels, crustaceans, and fish (Aruoja et al. 2009;
151 Braz-Mota et al. 2018; Katsumiti et al. 2018; Ruiz et al. 2015). While there is no data available on the
152 concentration of CuO-NPs in the soil total Cu could range from 2-100 mg kg⁻¹ in unpolluted soils (Nagajyoti et
153 al. 2010). Soil receives Cu-based NPs from direct application of agricultural nano-products and industrial wastes
154 (Adeleye et al. 2016; Rajput et al. 2017b, 2018b). The toxic action of pesticides specifically Cu-based NPs and
155 Cu-based nano pesticides (e.g., Kocide 3000) makes them appropriate to be used for the control of plant
156 pathogens and pests (Anjum et al. 2015; Shahid and Khan 2017). Cu-based fungicides have been used for more

157 than a century contributing to soil contamination based on their Cu^{2+} content, allowing them to function as a
158 reducing or oxidizing agent in biochemical reactions. Terrestrial species can have more interactions with NPs
159 because up to 28% of the total NPs production ends into soils (Keller and Lazareva 2013). Substantially
160 increased production of Cu-based NPs in the last decade emphasizes the need of thorough and systematic
161 investigation of nano-Cu release, environmental fate, bioavailability, dissolution of $\text{Cu}^+/\text{Cu}^{2+}$ ions from Cu-based
162 NPs, exposure routes, and their toxic impacts on non-target organisms (Keller et al. 2017).

163 Plants are one of the most important entities and provide a very large surface area for NPs exposure via
164 roots and above ground parts (Dietz and Herth 2011). For instance, the air-dispersed NPs may penetrate and
165 transport via the stomatal openings (Pullagurala et al. 2018; Raliya et al. 2016). Different plants exhibit specific
166 behaviours towards excess metal present in the growth medium. In particular, metal-tolerant plants could limit
167 the uptake of NPs into photosynthetic tissues by restricting the transport of metals across the root endodermis
168 and storing them in the root cortex; hyperaccumulating plants could compile excess NPs in the harvestable
169 tissues (Manceau et al. 2008). The exact mechanism of plant defence towards NPs toxicity is not fully
170 understood.

171 At present, inadequate information is available on how Cu-based NPs affect the soil organisms, for
172 instance, agriculturally important microbes, fungi, nematodes and earthworms. The NPs may affect soil flora
173 directly by inducing changes in the bioavailability of other toxins and nutrients or indirectly via interactions with
174 natural organic compounds possible interactions with toxic organic compounds which may increase or decrease
175 the toxicity of NPs (Haris and Ahmad 2017).

176 In order to get more in-depth knowledge of Cu-based NPs, this review critically assessed the literature
177 data present over effects of Cu-based NPs on terrestrial and aquatic ecosystems, the interaction of soil microbial
178 communities with Cu-based NPs, the bioaccumulation of Cu-based NPs in plants and their toxicity mechanism,
179 and their biotransformation in soil (Figure1).

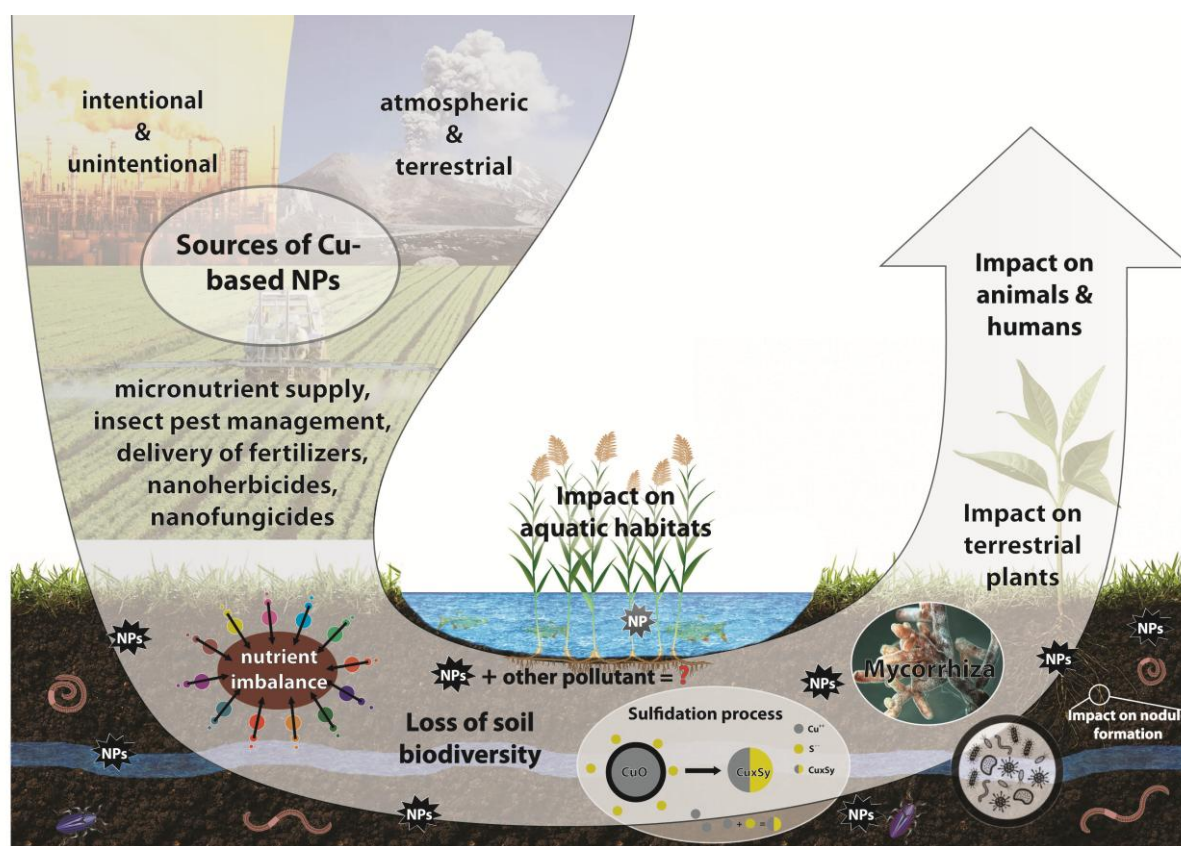
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181 **2 Sources, variants and fate of Cu-based NPs in the environment**

182

183 Owing to diverse applications of Cu-based NPs in the nanotechnology industry, the release of nanoscale-Cu in a
184 different sphere of the environment is expected (Qiu and Smolders 2017). Sources of NPs include both the point
185 and non-point sources. Point sources are comprised of production and storage units, research laboratories,
186 disposal of nanomaterial-containing consumer products and wastewater treatment plants etc., whereas Cu
187 discharge through non-point sources occurs through wear and tear of Cu-based NPs containing paints, cosmetic
188 products, and cleaning agents (Rajput et al. 2018b). The Cu-NPs have potential to enter water, soil, and

189 sediments during and at the end of their life cycle (Keller et al. 2013; Slotte and Zevenhoven 2017). Soil can
 190 receive NPs through various channels, for example, agricultural amendments of sewage sludge, atmospheric
 191 deposition, landfills, or accidental spills during industrial production (Simonin and Richaume 2015). The Cu-
 192 based NPs are available with various morphologies like Cu, CuO, Cu₂O, Cu₃N exhibiting various oxidation
 193 states, for instance, Cu⁰, Cu^I, Cu^{II}, and Cu^{III}, Cu⁺ (Cu₂O) or Cu²⁺ (CuO) (Ojha et al. 2017). In soil, nanoscale-Cu
 194 might be present in various forms like complexes with soil organic matters such as natural organic matter, humic
 195 acid, fulvic acid etc., Cu-NPs containing pesticides including Kocide 3000 [nCu(OH)₂], as complex with other
 196 metal components/plant exudates etc. (Conway et al. 2015; Gao et al. 2018; Peng et al. 2017; Servin et al.
 197 2017a).



198

199 Fig 1. Schematic of CuO NPs sources to environment and their effects on different ecosystems

200

201 Due to their high density, Cu-NPs tend to settle rapidly from nano to micro scale. The Cu-NPs, both in
 202 the presence and absence of organisms may undergo micro scale aggregation with high polydispersity in water
 203 and simple salt solutions (Adeleye et al. 2014; Conway et al. 2015; Griffitt et al. 2007). In a study by Adeleye et
 204 al. (2014), only 20% Cu-NPs was detected after 6 h at pH 7.0 in NaCl (10mM) which suggested rapid
 205 aggregation of Cu-NPs leading to sedimentation. On the other hand, natural organic matter released in the
 206 environment may reduce the Cu-NPs sedimentation; for instance, approximately 40% of Cu-NPs remained

207 stabilized by organic matter released by fish even after 48 h (Griffitt et al. 2007). Indeed, the dissolution of CuO-
208 NPs in aqueous medium is too slow; so much so that a within concentration range of 0.01-10 mg L⁻¹, CuO-NPs
209 showed as little as ≤1% dissolution after weeks in freshwater and after a month in seawater (Adeleye et al. 2014;
210 Atha et al. 2012; Buffet et al. 2013; Conway et al. 2015; Hanna et al. 2013). A month after soil contaminated by
211 CuO-NPs, an increase in labile fraction of the Cu was noted, which had negative effects on the *T. aestivum*
212 growth (Gao et al. 2018).

213 Thus, once entered into the environment, nanoscale-Cu is expected to undergo a series of
214 transformations and partitioning that ultimately decides its fate and bioavailability to organisms.

215

216 **3 Biotransformation of Cu-based NPs in soil**

217

218 Being a less dynamic component of the biosphere, the soil system has a relatively high potential for pollutants
219 accumulation in comparison to the atmosphere and hydrosphere. Soil not only acts as a depot for pollutants but
220 also serves as a source of contaminant input into food chains. Additionally, the soil matrix is considered
221 abundant in natural occurring NPs which exist in both forms; as primary particles and as
222 agglomerates/aggregates. The natural organic matter of soil influences the bioavailability of NPs through a
223 variety of mechanisms like electrostatic interactions, ligand-exchange, hydrophobic effect, hydrogen-bonding
224 and complexation (Philippe and Schaumann 2014). The various soil processes such as homo/hetero-aggregation,
225 oxidation, dissolution, sulfidation, sedimentation may impact NPs toxicity (Adeleye et al. 2016; Conway et al.
226 2015; Garner and Keller 2014; Lowry et al. 2012; Miao et al. 2015; Torres-Duarte et al. 2016). Aggregation and
227 dissolution of NPs are generally influenced by a range of environmental factors such as pH, organic matter, ionic
228 species and colloids. A passivation process frequently occurring under various environmental conditions is the
229 sulfidation of CuO-NPs (Gogos et al. 2017; Ma et al. 2014). This process is expected to alter the speciation and
230 properties of CuO-NPs significantly and might increase its apparent solubility resulting in increased
231 bioavailability and thus eco-toxicity attributed to toxic Cu²⁺ (Ma et al. 2014).

232 Additionally, colloidal stability of particle is one of the critical factors controlling their fate and effects
233 (Lowry et al. 2012). The toxicity and bioavailability of Cu changes according to the Cu speciation including
234 ionic-Cu, Cu-NPs, complexed-Cu, bulk-Cu, oxidation states and environmental factors such as pH, soil, water,
235 sedimentation, organic matter, redox potential, plant species, and growth phase (Cornelis et al. 2014; Garner and
236 Keller 2014; Zhang et al. 2018)

237 In soil, NPs either interact with each other forming homoaggregates or interact with different NPs and
238 natural colloids forming heteroaggregates (Cornelis et al. 2014; del Real et al. 2018). The process of NPs

239 aggregation mainly impacts their colloidal stability which is among the key factors controlling NPs fate and
240 impact (Bundschuh et al. 2018). The extent of aggregation correlates well with the ionic strength of the medium
241 but not with the sedimentation rate (Conway et al. 2015). The major controlling factor for Cu-based NPs
242 sedimentation includes phosphate and carbonate content in the matrix and the oxidation state of Cu. The
243 dissolution of Cu-based NPs is majorly hindered by sulfidation which is often regarded as passivation process
244 for Cu/CuO-NPs. It increases the solubility of Cu/CuO-NPs resulting in enhanced bioavailability and toxicity
245 (Ma et al. 2014). The transformation of Cu-based NPs is further influenced by geochemical properties of soil. In
246 line with this, low translocation of Cu-NPs was observed in organic-rich soil, whereas high translocation was
247 noticed in sandy clay soil. The highest rate for transformation to Cu ions and adsorption complexes was detected
248 in acidic soils (Shah et al. 2016). Under slightly acidic conditions, CuO-NPs may combine with the hydrogen
249 ions of soil and release Cu^{2+} or $\text{Cu}(\text{OH})^+$. Under long-term exposure, CuO-NPs and Cu in combination with
250 humic acid get transformed to Cu_2S , and Cu goethite complex (Peng et al. 2017).

251 Moreover, Wang et al. (2013) investigated the transformations of CuO-NPs in biological and
252 environmental media and their effect over Cu-bioavailability, redox activity, and toxicity. The authors revealed
253 that CuO-NPs underwent sulfidation process via sequential dissolution and re-precipitation mechanism to
254 generate complex secondary aggregates of copper sulfide (CuS) NPs which are considered as active catalysts for
255 bisulfide oxidation. Although the sulfidation is considered as a natural detoxification mechanism for heavy
256 metals, the authors suggested that it may not permanently detoxify copper as CuS-NPs but also show redox
257 activity through the release of Cu(I) or Cu(II) by H_2O_2 oxidation. In another study, wheat crop was exposed to
258 CuO-NPs in a sand growth matrix and similar transformation of CuO to Cu (I)-sulphur complexes was noticed
259 (Dimkpa et al. 2012). Significant reduction of CuO-NPs to Cu_2S and Cu_2O was also shown in maize during root-
260 shoot-root translocation of CuO-NPs (Wang et al. 2012). The reason behind the transformation of Cu(II) to Cu(I)
261 in plants may be ascribed to the presence of reducing sugars which get transported from leaf cells to roots
262 (Huang et al. 2017; Servin et al. 2017a).

263 The leaching and mobilization of nano-Cu ions from the source material followed by their complexation
264 with humic acids or organic acids when secreted by fungi and contained in the plant root exudates influence the
265 biotransformation. Although CuO-NPs are often considered as insoluble materials, the presence of organic acids
266 such as citric and oxalic acid in the environment enhances the dissolution of Cu and CuO-NPs which in turn
267 increases their mobility and bioavailability to plants and animals. In addition, the nature of the organic acids also
268 affects NPs dissolution significantly (Mudunkotuwa et al. 2012). Other factors affecting NPs dissolution includes
269 pH, dissolved organic matter, biomolecular ligands, ionic strength etc. (Yu et al. 2018). All these factors
270 determine the toxicity of Cu-based NPs by influencing the total dissolved concentration of Cu in the concerned

271 media. Among these factors, the pH has an inverse relationship with dissolution. The CuO-NPs have good
272 solubility at lower pH which is turn down as the pH increases. However, the presence of ligands including those
273 with amine functional groups, induce solubility of CuO-NPs at neutral pH (Wang et al. 2013). Recently,
274 Kovacec et al. (2017) investigated potential efficacy of two phytopathogenic fungi namely *Botrytis cinerea* and
275 *Alternaria alternata* for biotransformation of Cu^{2+} ions, micro and nanoparticulate forms of Cu and CuO. The
276 study revealed that *B. cinerea* could transform micro and nanoparticulate forms of Cu and CuO into Cu-oxalate
277 complex.

278 Furthermore, the waterlogged conditions as in the case of paddy fields, may influence NPs dissolution,
279 mobility, bioavailability, accumulation, translocation and transformation. Peng et al. (2017) studied
280 bioavailability and speciation of CuO-NPs in the paddy soil and transformation of CuO-NPs in the soil-rice
281 system. Experimental findings showed that CuO-NPs significantly reduce the redox potential of the soil and
282 alleviate the electrical conductivity at the maturation stage of paddy. The bioavailability of CuO-NPs showed a
283 declining trend with rice growth, but an increase was noticed after drying-wetting cycles. Most of the Cu present
284 in the root, shoot and leaves of the plant was found in the form of Cu-citrate. Nearly 1/3rd of the Cu(II) was
285 transformed to Cu(I)-cysteine while 15.7% was present as Cu_2O in roots and 19% as Cu(I)-acetate in shoot
286 section. In chaff, about 30% of Cu was found as Cu-citrate and Cu(I)-acetate but no CuO was reported to
287 reached polished rice. In another study, a higher content of Cu in the form of Cu(I) in rice grain was found in the
288 presence of sulphur (Sun et al. 2017). It was suggested that sulphur fertilization decreases the Cu content in the
289 root, leaf, and husk of the plant yielding higher biomass but showed higher amounts of Cu in rice grains in the
290 form of Cu(I)-cysteine and Cu(I)-acetate.

291 Therefore, the mechanism of biotransformation of Cu-based NPs includes series of chemical and
292 biochemical reactions with soil components and living organisms.

293

294 **4 Interaction of Cu-based NPs with soil organisms**

295

296 Deliberate administration of NPs into soils might have a significant impact on the living entities, as they are
297 extremely resistant to degradation and have the potential to accumulate in the soil. The effect of NPs may also vary
298 with varying concentration, soil properties, and enzymatic activity. Soil properties, such as pH, texture, structure,
299 and organic matter content influence the structure of soil microbial community and the ability of pollutants to exert
300 toxic effects on microorganisms (Simonin and Richaume 2015). As NPs have the ability to mobilize soil pollutants,
301 comparison of the toxicity of the NPs in various soil types is much required. In order to understand the influence of

302 soil physicochemical properties on Cu-based NPs toxicity, a number of predictive models have been developed;
303 however, these models are not always effective for other region soils (Duan et al. 2016).

304 The toxic effect of Cu-based NPs has been shown for beneficial soil microbes such as nitrifying bacteria,
305 nitrogen-fixing bacteria, *Arbuscular mycorrhiza* and other *Rhizobacteria*; however, it also influences other
306 microorganisms. You et al. (2017) suggested that the soil types could play an important role in determining NPs
307 toxicity over soil bacterial community composition and size. Recent studies showed that NPs might affect enzymatic
308 and metabolic activities, nitrification potential, colony count and abundance of soil bacterial diversity (Colman et al.
309 2013; Ge et al. 2011; He et al. 2016).

310 Copper ions released from the Cu-NPs can be toxic to both the pathogenic and beneficial bacteria (Lofts et al.
311 2013). The study conducted on CuO-NPs toxicity to *Saccharomyces cerevisiae* showed increased toxicity over time
312 due to increased dissolution of Cu ions from CuO (Kasemets et al. 2009). Furthermore, Concha-Guerrero et al.
313 (2014) have shown that CuO-NPs were very toxic for native soil bacteria, as the formation of cavities, holes,
314 membrane degradation, blebs, cellular collapse, and lysis in the cells of soil bacterial isolates were observed.
315 Pradhan et al. (2011) investigated the effect of CuO-NPs on leaf microbial decomposition and found a decrease in
316 leaf decomposition rate. The bacteria from *Sphingomonas* genus and Rhizobiales known for their importance in
317 remediation and symbiotic nitrogen fixation appeared susceptible to Cu-NPs (Shah et al. 2016). The NPs also have
318 significant effects on enzymatic activities (invertase, urease, catalase, and phosphatase, dehydrogenase), microbial
319 community structure, bacterial diversity nutrient cycling, changes in humic substances, and biological nitrogen
320 fixation. The CuO-NPs at 30-60 mg L⁻¹ affected the microbial enzymatic activity of activated sludge (Wang et al.
321 2017). Several other studies also report Cu-NPs effects on soil microbial community, enzymatic activities and
322 reduced C and N biomass (Ben-Moshe et al. 2013; Kumar et al. 2012; Xu et al. 2015). However, the effect of Cu-
323 based NPs on the soil microbial community has rarely been explored. While Cu-based NPs are known to exhibit
324 antimicrobial properties (Ingle et al. 2014), it is necessary to observe their impact on symbiotic microorganisms. It
325 can be assumed that NPs, besides influencing plant and microbes, could affect plants-microbe associations either
326 directly or indirectly. In this context, one of the classical examples is mycorrhizal symbiosis, which promotes plant
327 growth enhancing the plant nutrient acquisition through uptake of mineral nutrients. The formation of Cu-NPs at the
328 soil-root interface with the assistance of endomycorrhizal fungi was shown in *Phragmites australis*, and *Iris*
329 *pseudoacoru* and this mechanism helped to alleviate metal stress (Manceau et al. 2008). On the other hand, metallic
330 NPs were shown to inhibit mycorrhizal plant growth (Feng et al. 2013).

331 Furthermore, the CuO-NPs induced morphological and genetic alterations in leaf litter decomposing fungus
332 which could impact organic matter decomposition rate (Pradhan et al. 2011). A significant negative impact on
333 bacterial hydrolytic activity, oxidative potential, community composition and population size was also observed in

334 Bet-Dagan soil (Frenk et al. 2013). Cu-based NPs have also been reported to affect the growth and functionality of
335 green algae, cyanobacteria, and diatoms (Anyoogu et al. 2008). The most recent findings on Cu-based NPs action on
336 bacteria are summarized in Table 1.

337 The findings of recent studies dealing with the NPs action on bacteria are often controversial (Table 1).
338 Though, most studies show the increased toxicity of Cu-based NPs in comparison to ionic copper at similar dose
339 rates (VandeVoort and Arai 2018). Interesting results were also obtained when NPs interaction with pesticides was
340 studied. Parada et al. (2019) reported no major shift in microbial species composition; however, the degradation of
341 the pesticide was reduced. The possible explanation for this was given by Parra et al. (2019), wherein they showed a
342 decrease in spreading of pesticide-degradation genes bearing plasmids among the bacterial community. Therefore,
343 the current scenario demands the exploration of NPs toxicity mechanism on the soil microorganisms.

344 In addition, some studies report that Cu-based NPs can also have adverse effects on multicellular soil
345 organisms. For instance, the CuO-NPs affected growth and neuron morphology of a transgenic *Caenorhabditis*
346 *elegans* (Mashock et al. 2016), and disturbed immunity and reduced population density of a common earthworm
347 *Metaphire posthuma*, which is mostly distributed across the Indian subcontinent (Gautam et al. 2018).

348 Considering the presence of Cu-based NPs in the soil, it is imperative to study their influence on soil
349 biodiversity. The reviewed information indicates that NPs affected soil microbial community by decreasing their
350 abundance, enzymatic activities and soil microbial biomass. Therefore, the decrease in soil microbial biomass could
351 be a sensitive indicator for microbial changes in soils.

352

353 **5 Uptake and bioaccumulation of Cu-based NPs in plants**

354

355 The NPs are taken up by plant roots and transported to the aboveground plant tissues through the vascular
356 system, depending on the composition, shape, size of NPs, and anatomy of the plants (Rico et al. 2011). On the
357 other hand, some NPs remain adhered to the plant roots. It is well understood that NPs enter plant tissues either
358 via root tissues (root tips, rhizodermis, and lateral root junctions) or the aboveground organs and tissues
359 (cuticles, trichomes, stomata, stigma, and hydathodes) as well as through wounds and root junctions.
360 Interestingly, in the event of NPs-plant interaction, some metal-tolerant plants could limit the uptake of NPs into
361 the photosynthetic tissues by restricting the transport of metals across the root endodermis and storing them on
362 the root cortex, whereas, hyper-accumulating plants can take up excess amounts of NPs in the harvestable tissues
363 of plants (Manceau et al. 2008). It has been suggested that the plants can accumulate NPs in their original form
364 or as metal ions (Cota-Ruiz et al. 2018). However, the uptake and bioaccumulation vary with varying
365 physicochemical features of NPs (Ahmed et al. 2018b; Peng et al. 2015; Rico et al. 2011, 2015).

366 In a study, the translocation and biotransformation of CuO-NPs in rice plants were explored. It was
367 revealed that CuO-NPs get accumulated in epidermis and exodermis regions of the plants and get precipitated
368 with citrate or phosphate ligands or get bound to amino acids forming Cu-cysteine, Cu-citrate, and $\text{Cu}_3(\text{PO}_4)_2$
369 kind of products or get reduced to Cu(I) (Peng et al. 2015). Cu(I) is a highly redox active species capable of
370 producing hydroxyl radicals by Fenton-like reactions, and so its presence in even smaller quantities has
371 significant biological importance. Servin et al. (2017a) compared bioaccumulation of un-weathered and
372 weathered CuO-NPs, bulk and ions in lettuce plants after 70 days. In the case of CuO-bulk, weathered material
373 was found to decrease Cu accumulation in plant roots, whereas, weathering had a positive impact on
374 bioaccumulation of NPs. The authors further unearthed that in roots exposed to weathered NPs, the major
375 fraction of Cu, i.e., 94.2% was present in oxidized form as CuO, while the rest of the fraction i.e., 5.7% could
376 bind to sulfur in reduced form as Cu_2S . In contrast, roots exposed to un-weathered NPs showed negligible
377 biotransformation. As the ageing/weathering have a profound effect on the particle-size, particle-size
378 distribution, surface properties, composition, reactivity etc., it is an important aspect which needs to be
379 considered while assessing the environmental implication of Cu-based NPs. Similarly, the translocation and
380 biotransformation of NPs is a plant-specific phenomenon which requires adequate attention.

381 The nano-phytotoxicity studies on accumulation and uptake of NPs have generated important data for
382 understanding the fate of Cu-based NPs in plants (Ingle et al. 2014; Ma et al. 2010). Once NPs infiltrate the plant
383 system, they may traverse to different organs (leaves, stem, and fruits) or may get compartmentalized at different
384 locations *viz.* vacuoles, walls, stellar system, cytoplasmic matrix, lipid envelopes, and nucleus (Ahmed et al.
385 2018b; Rajput et al. 2017b, 2018a; Rastogi et al. 2017). The translocation efficiency varies greatly in different
386 plant species, for instance, alfalfa translocates 3-5% of Cu from root to shoot on exposure to $0\text{-}20\text{ mg L}^{-1}$ Cu-
387 NPs, whereas only 0.5-0.6% translocation was observed in lettuce (Hong et al. 2016). Before the plant uptake,
388 the dissolution of Cu-NPs increases the likelihood that Cu is internalized as Cu^{2+} ions or in the form of organic
389 complexes (Keller et al. 2017). A recent study revealed the adsorption and accumulation of Cu-based NPs in
390 tomato plants leads to the adsorption of nano-CuO on the roots (Ahmed et al. 2018b). Similarly, maize roots
391 showed 3.6 fold greater Cu content under CuO-NPs treatments (Wang et al. 2012). Also, the Cu content was 7
392 times higher in shoots of maize treated with 100 mg L^{-1} CuO-NPs. In this context, Zuverza-Mena et al. (2015)
393 also reported the translocation of Cu-based NPs in cilantro and their significant accumulation in shoots.
394 Differential accumulation profile of CuO-NPs has been reported in ryegrass and radish (Atha et al. 2012). Wheat
395 and bean seedlings grown on dual agar media have been adequately discussed pertaining to the bioavailability of
396 Cu-NPs and their relationship between accumulation and uptake (Woo-Mi et al. 2008). Cu-NPs were toxic to
397 both plants and also bioavailable. A Cu ion released from Cu-NPs has negligible effects in the studied

398 concentration range, and the apparent toxicity is clearly due to Cu-NPs. Bioaccumulation increased with
399 increasing concentration of Cu-NPs and agglomeration of particles was observed in the plant cells by using
400 transmission-electron microscopy-energy-dispersive spectroscopy (TEM-EDX). In shoots of wheat grown in the
401 sand matrix, the bioaccumulated Cu was detected as Cu(I)S complex and CuO (Dimkpa et al. 2012). The level of
402 Cu accumulation in wheat shoots under CuO-NPs exposure was almost equal to the concentrations quantitated in
403 bulk (Dimkpa et al. 2012).

404 In a very recent study, Keller and co-workers exposed leaf tissues of lettuce, collard green, and kale to
405 nano-CuO and detected CuO-NPs in leaf surfaces by use of single particle inductively coupled plasmon mass
406 spectroscopy (sp-ICP-MS) (Keller et al. 2018). Among all three vegetables, lettuce retained the highest amount
407 of CuO-NPs on leaf surface even after washing. For this retention, the varying degrees of leaf surface roughness
408 and hydrophilicity among the tested vegetables have been suggested to play an important role in holding CuO-
409 NPs (Keller et al. 2018). Overall the data from these studies indicate that certain fractions of CuO-NPs are taken
410 up by plants which may result in undesirable accumulation in edible plant tissues ultimately exposing humans
411 via the food chain.

412 The bioaccumulated nano-Cu or CuO is also subject to transportation and transformation in plants
413 (Ahmed et al. 2018c). For instance, the treatment of hydroponically cultured lettuce plant with CuO/Cu-based
414 NPs caused a greater accumulation of Cu than cupric ions (Trujillo-Reyes et al. 2014). Additionally, the xylem
415 and phloem based transport system to shoots and back to roots were proposed for CuO-NPs accumulation in root
416 cells, cytoplasm, intracellular space, and nuclei of xylem and cortical cells. However, the CuO-NPs was reduced
417 from Cu (II) → Cu (I) in due course of translocation (Wang et al. 2012). A similar transformation of CuO-NPs
418 has been reported with an elevation in the degree of saturation of fatty acids (Yuan et al. 2016). In another study,
419 when *Zea mays* were exposed to CuO-NPs, ionic, and bulk CuO, the Cu content in root and shoot of the plant
420 was found enhanced under CuO-NPs (Wang et al. 2012). A micro X-ray fluorescence (μ XRF) study revealed
421 that Cu-NPs may get accumulated in outer parts of the root (Servin et al. 2017a). The translocation of Cu-NPs
422 also varies depending upon the growth media. For instance, alfalfa, lettuce and cilantro exposed to CuO, Cu and
423 Cu(OH)₂ NPs based pesticide in soil showed >87-99% Cu accumulation mostly in roots with very little
424 transportation to shoots and negligible in leaves (Hong et al. 2015; Zuverza-Mena et al. 2015). In some recent
425 studies, Cu-NPs were also detected in leaves, stems, and fruits of cucumber and tomato when grown in soil
426 system (Zhao et al. 2016a). The uptake of CuO-NPs in tomato, alfalfa, cucumber, and radish seedlings was also
427 noticed in the range of 4-1748 $\mu\text{g g}^{-1}$ dry biomass when grown on semi-solid agar media (Ahmed et al. 2019). In
428 a comparative study between soil and hydroponically grown tomato plants, the organ wise distribution of CuO-
429 NPs in soil culture was found lesser than in hydroponic (Ahmed et al. 2018b). The Cu in soil grown root and

shoot of tomato plants was found lesser by 20% and 33% than in hydroponically grown plants (Ahmed et al. 2018b). This difference could be attributed to the NPs cluster formation due to the homo/hetero aggregation processes of the soil system. Besides root exposure, the atmospheric presence of Cu-based NPs also triggers their bio-uptake. For instance, during the foliar applications of Cu-NPs, most of the Cu remained in fruits or leaves with a little transport via phloem to roots. For example, *Lactuca sativa* exposed to Cu-based nano-pesticide accumulated 1350-2010 mg Cu kg⁻¹ dry biomass after 30 days (Zhao et al. 2016a, b). A small fraction (17-56 mg kg⁻¹) of Cu was also found in roots via phloem transport (Zhao et al. 2016a). In a study, the microscopic analysis showed the presence of dense material in root cells of *O. sativum* L. treated with CuO-NPs and confirmed the presence of Cu by bulk-X-ray absorption near edge structure (XANES), and interestingly the most dominant form of dense material was CuO (Peng et al. 2015).

Being very small in size, NPs have the potential to enter, translocate, and penetrate physiological barriers to travel within the plant tissues, and microscopic studies showed the accumulation of NPs in various parts of the plant (Ahmed et al. 2018a; Rajput et al. 2018a, d).

6 Toxicity of Cu-based-NPs in plant system

The long-term effects of Cu-based NPs accumulation in plant systems are still scarcely known. It has been suggested that the Cu-based NPs may cause morphological, physiological, genetic, and epigenetic changes which may alter plant growth and nutritional status. Plants as primary producers are very critical for the sustainability of an ecosystem and functions as an indispensable link for perpetual food supply and human nutrition. In the environment, plant roots make close associations with soil particles and virtually everything that enters in the soil system (Ahmed et al. 2017; Anjum et al. 2013). Variants of Cu-based NPs once released in the environment may eventually enter either intentionally or accidentally into the soil-plant system. Plants in soil environment can be the non-target organisms of Cu-based NPs. The critical toxicity level of Cu in many crop species varies between 20-30 µg g⁻¹ leaf dry biomass (Anjum et al. 2015; Yruela 2009). Thus, the potential toxicity assessment of Cu-based NPs to plants is relevant to a large extent. Several studies have reported the impact of different species of Cu-NPs in various culture media such as agar, hydroponic nutrient solution, sand, filter paper, soil, and soil-sand mixtures (Dimkpa et al. 2013; Kim et al. 2013; Moon et al. 2014; Musante and White 2012) (Table 2). The exact mechanism of plant defence under NPs toxicity is not fully understood. Generally, the phytotoxicity of NPs expressed in two steps: (1) chemical toxicity based on chemical composition, and (2) stress stimuli caused by the surface, size, or shape of the NPs. The antioxidant defence machinery of plants becomes activated against external/internal NPs stress stimuli. Underexposure with NPs

462 having enough physicochemical features to exert toxicity, plants trigger their antioxidant defence mechanism to
 463 prevent oxidative damage, as well as enhance their resistance towards NPs toxicity. For instance, cucumber
 464 plants grown hydroponically in the presence of CuO-NPs (50 nm) were found with augmented anti-oxidative
 465 enzymes *viz.* catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Kim et al. 2012). However,
 466 *C. sativus* when grown hydroponically in the presence of Cu NPs (10-30 nm) experienced significant phytotoxic
 467 effects which were not ameliorated by antioxidant enzymes adequately (Mosa et al. 2018). The NPs arbitrated
 468 phytotoxicity is predominantly related to their physicochemical properties. The Cu-based NPs cause
 469 phytotoxicity via the dissolution and release of higher concentration of ions such as Cu^{2+} or the production of
 470 excess reactive oxygen species (ROS) (Ahmed et al. 2019; Letelier et al. 2010). ROS can affect mitochondrial
 471 respiration, apoptosis, lipid peroxidation in the cell membrane, and induce a range of antioxidant responses
 472 (Dimkpa et al. 2012; Shaw and Hossain 2013). Recent studies of CuO-NPs phytotoxicity showed negative
 473 impacts on seed germination and overall plant growth of various crops such as *Lactuca sativa* (100-300 mg L⁻¹),
 474 *Medicago sativa* (0-20 mg L⁻¹), *Triticum aestivum* (200 mg L⁻¹), *Vigna radiata* (500 mg L⁻¹), *Zea mays* (2-100
 475 mg L⁻¹), *Cucumis sativus* (100-600 mg L⁻¹), *Oryza sativa* (0-1000 mg L⁻¹), *Brassica juncea* (0-1500 mg L⁻¹), and
 476 *Glycine max* (50-500 mg L⁻¹) (Rajput et al. 2017b).

477 The studies pertaining to toxicity assessment of Cu and Cu-based NPs, and understanding of its
 478 molecular mechanism warrant more systematic and in-depth investigations. The available data on the toxicity,
 479 chemistry, and Cu-NPs plant interactions suggesting adverse outcomes on plant growth are presented in Table 2.

480

481 **6.1 Effects on seed germination, morphometry and plant growth**

482

483 Seed germination commences a plant's physiological process, and therefore it is an important attribute when toxicity
 484 of a xenobiotic is examined. The Cu-based NPs have been found to inhibit seed germination in various crops (Table
 485 2). For instance, *Coriandrum sativum* cultivated in soil mixed with 20 and 80 mg kg⁻¹ of each Cu, CuO, and
 486 $\text{Cu}(\text{OH})_2$ NPs (Kocide and CuPRO) exhibited significant ($p \leq 0.05$) reduction in seed germination (Zuverza-Mena et
 487 al. 2015). In another study, the seed germination by CuO-NPs was reduced to almost 50%. Similarly, treatment with
 488 Cu-NPs at 80 mg kg⁻¹ reduces the shoot elongation by 11% (Zuverza-Mena et al. 2015). The CuO-NPs (~18.4 nm)
 489 at 0.02-2 mg ml⁻¹ also causes severe toxicity in tomato plants (Ahmed et al. 2018b). Furthermore, *Solanum*
 490 *lycopersicon* plants are grown in both soil and hydroponic media showed significant internalization of Cu in
 491 different plant organs with oxidative burst and reduction in plant height and weight (Ahmed et al. 2018b). Moreover,
 492 the Cu, CuO and core-shell Cu/CuO-NPs at different concentrations caused severe reduction in root length of
 493 *Hordeum vulgare* L. (Shaw et al. 2014), *H. sativum* distichum (Rajput et al. 2018a), *H. vulgare* (Qiu and Smolders

494 2017), *Z. mays*, *C. sativus* (Kim et al. 2013), *T. aestivum* (Gao et al. 2018; Woo-Mi et al. 2008), and *L. sativa* (Liu et
495 al. 2016; Trujillo-Reyes et al. 2014). The CuO-NPs (~ 40 nm) at 500 mg kg⁻¹ soil as fresh and after 28 days of
496 mixing of CuO-NPs with soil caused a significant decrease in maximal root length (Gao et al. 2018). In the same
497 study, it has been suggested that the exudates secreted from wheat roots in CuO-NPs amended soil enhanced the
498 dissolution of Cu ions in pore water, which played an important role in enhanced phytotoxicity (Gao et al. 2018).
499 Similarly, in a study by Qiu and Smolders (2017), CuO-NPs (~ 34 nm) at various concentrations ranging from 50-
500 1000 mg kg⁻¹ at two different pH (4.8 and 5.8) increases the toxicity of CuO-NPs affecting root elongation. The
501 CuO-NPs inhibited *C. sativus* seed germination when administered at 600 mg L⁻¹. At this rate, only 23.3%
502 germination was recorded over untreated of control (Moon et al. 2014). Some earlier studies also reported that CuO-
503 NPs reduced *C. pepo* biomass by 90% (Stampoulis et al. 2009), seedling growth of *Phaseolus radiatus* and *T.*
504 *aestivum* (Woo-Mi et al. 2008), shortened primary and lateral roots of the *B. juncea* L (Nair and Chung 2015a),
505 affected agronomical/physiological parameters in *Origanum vulgare* (Du et al. 2018), and decreased root growth in
506 *M. sativa* grown in hydroponic culture (Hong et al. 2015). In *Allium cepa*, 80 mg CuO-NPs L⁻¹ damaged the root cap
507 and meristematic zone and reduced the growth of the root tip (Deng et al. 2016).

508 Morphometric observations indicated a decline in root and shoot growth for Cu-based NPs treated plants.
509 Also, Cu-based NPs pose deleterious effects on plant germination (Deng et al. 2016; Moon et al. 2014; Nair and
510 Chung 2015a; Rajput et al. 2018a, b). The reduction in root and shoot growth could limit the surface area for water
511 uptake and photosynthesis respectively and consequently affects the plant performance.

512

513 **6.2 Effects on cellular ultrastructure**

514

515 Several studies on the ultrastructure of plants cells after Cu-based NPs exposure showed remarkable changes in
516 plant roots and leaves. In roots, violations of the integrity of the cell wall of the epidermis and endoderm,
517 vacuolization and disorganization of fragments in the endoplasmic reticulum, swelling of the mitochondria, and
518 destruction of the mitochondrial cristae have been observed with rare leucoplasts with disorganized and partially
519 destroyed thylakoid. In the chloroplasts of the leaf parenchyma, the size of starch grains and plastoglobules
520 increased significantly; the area of the thylakoids decreased, and inter-thylakoid space expanded (Rajput et al.
521 2018d). These changes can be indicative of lowering the photosynthetic processes with relation to CuO-NPs toxicity
522 (Rajput et al. 2015).

523 Plastoglobules are subcompartments of thylakoids that play an important role in lipid metabolic pathways
524 (Austin et al. 2006), the chloroplast to chromoplast transition and the formation of coloured carotenoid fibrils
525 (Vishnevetsky et al. 1999). Previous studies showed an increased number of plastoglobules due to biotic, abiotic and

526 CuO-NPs induced stress in *Landoltia punctata* (Lalau et al. 2015). The excess concentration of CuO-NPs severely
527 affected starch content, stomatal aperture, epidermis, endodermis, cell wall, mitochondria, nuclei and vascular
528 bundles of *H. sativum* (Rajput et al. 2018a).

529 The identified changes in the root and leaf cell ultrastructure, especially in the photosynthetic apparatus are
530 associated with altered plant growth and performance.

531

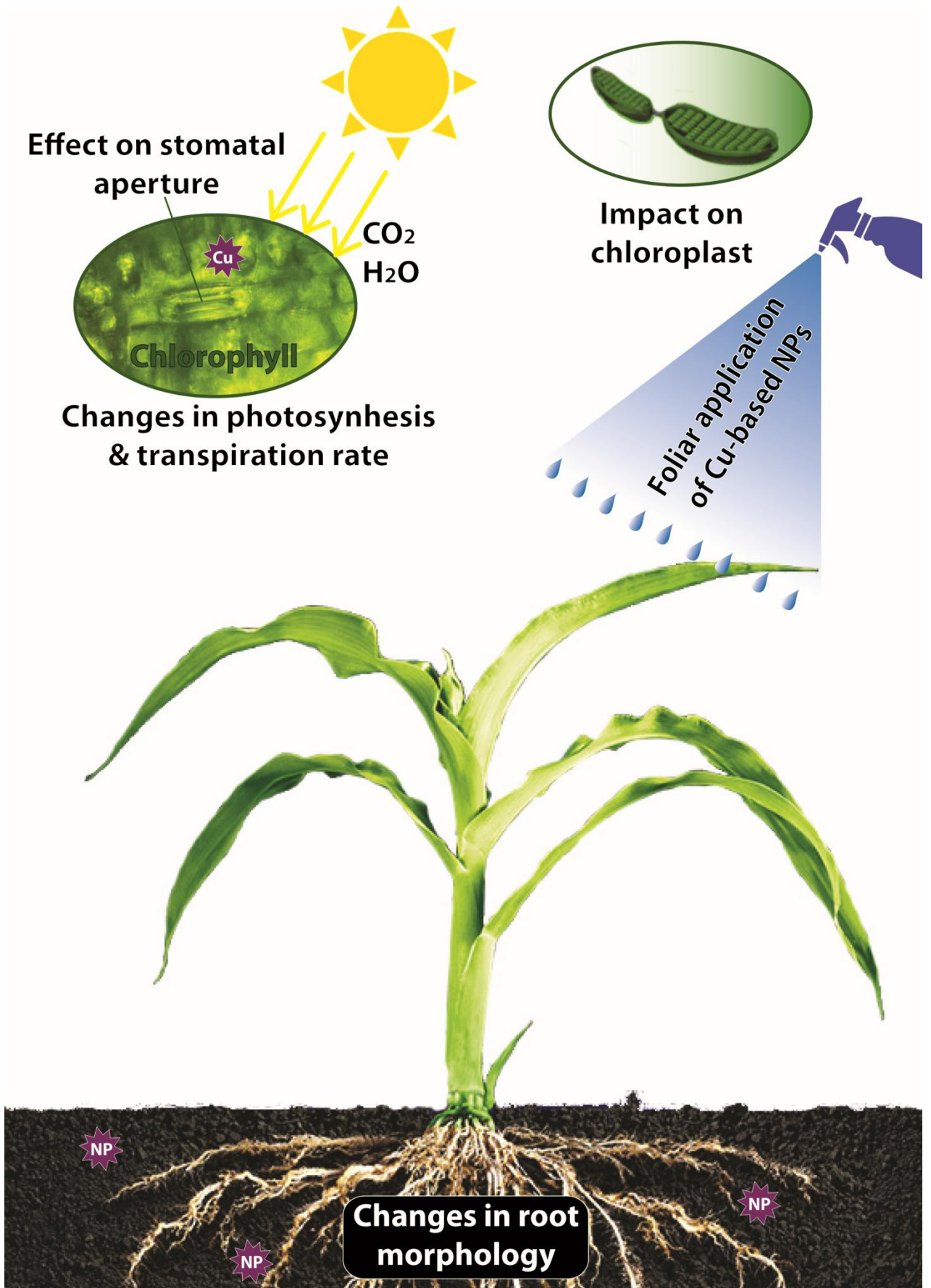
532 **6.3 Effects on plant physiology and photosynthetic systems**

533

534 Photosynthesis is a key process for the conversion of light energy into chemical energy, which is performed by
535 chloroplast, and other components of the photosynthetic machinery embedded in a highly dynamic matrix and
536 thylakoid membranes (Rottet et al. 2015). Cu-based NPs may also affect photosynthesis, and cause a decrease in
537 electron transport, thylakoid number per granum, photosynthetic rate, transpiration rate and stomatal conductance
538 (Da Costa and Sharma 2015; Perreault et al. 2014). Musante and White (2012) observed that both bulk Cu and Cu-
539 NPs reduced the transpiration rate by 60-70% in *C. pepo* relative to untreated controls. For the successful
540 photochemical phenomena, chloroplast ultrastructure, thylakoid, grana formation, and physiological activities of
541 photosynthetic machinery are important (Miller et al. 2017; Tighe-Neira et al. 2018). Thus, any structural and
542 ultrastructural alteration in chloroplast apparatus and functionality associated subcellular organelles such
543 plastoglobules starch grains may adversely impact the overall photosynthesis (Figure 2). Toxic effects of CuO-NPs
544 were further shown in experiments with *O. sativa*. The CuO-NPs decreased Fv/Fm up to a complete loss of
545 photosystem (PS) II photochemical quenching at a concentration of 1 mg L⁻¹ and declined the photosynthetic
546 pigment contents (Da Costa and Sharma 2015). It has further been reported that the CuO-NPs had a detrimental
547 impact on the structure and function of the photosynthetic apparatus especially on photosynthetic pigments,
548 chlorophyll, and grana (Tighe-Neira et al. 2018). Spring barley grown in hydroponic system showed accumulation
549 of CuO-NPs in leaf cells and disorganized chloroplast structure and thylakoid in the mesophyll cells (Rajput et al.
550 2018a).

551 Thus, the declining photosynthetic efficiency can be a good forecaster of NPs toxic effects on plants.

552



553

554 Fig 2. Schematic presentation of effects of Cu-based NPs on photosynthesis

555 **6.4 Effects on plant metabolism and nutrient content**

556

557 Several studies have demonstrated that Cu-based NPs also significantly affect the metabolism and nutrient content
558 of plants. For example, foliar application of Cu(OH)₂ nano pesticide (50-1000 nm) at 1050-2100 mg L⁻¹ alters
559 metabolite level of *L. sativa* leaves (Zhao et al. 2016b). Gas Chromatography-Time-of-Flight Mass Spectrometry
560 (GCTOF-MS) based analysis combined with Partial Least Squares-Discriminant Analysis (PLS-DA) multivariate
561 analysis shows disturbance in tricarboxylic acid (TCA) cycle and amino acid related pathways (Zhao et al. 2016b;
562 2017b). An increased level of potassium, putrescine, and spermidine in Cu(OH)₂ nano-pesticide treated plants has
563 been suggested to reduce the oxidative stress and enhance the tolerance (Zhao et al. 2016b). Similarly, in cucumber
564 grown with Cu-NPs (40 nm) in soil (200-800 mg kg⁻¹) and hydroponics (10 and 20 mg L⁻¹) exhibited perturbation in
565 iron, sodium, phosphorus, zinc, sulphur, and molybdenum uptake and alterations in cucumber fruit metabolite
566 profile (Zhao et al. 2016a). Additionally, TCA cycle and galactose metabolism also get compromised (Zhao et al.
567 2016b). CuO and Cu(OH)₂ nano pesticides also decrease the level of shoot phosphorus and iron in lettuce (Hong et
568 al. 2015). Moreover, CuO- NPs (<50 nm) at 500 mg kg⁻¹ soil has shown to reduce iron, manganese, zinc, and
569 calcium in common bean (Dimkpa et al. 2015). Moreover, micro- and macronutrients elemental composition in
570 cilantro has been found to be suppressed when grown with CuO-NPs (10¹-10² nm) and Cu-NPs (10²-10³ nm) at 0-80
571 mg kg⁻¹ soil (Zuverza-Mena et al. 2015). The Cu-based NPs have also been documented to bring down the
572 agronomically important characteristics of plants. The CuO-NPs (<50 nm) reduces carotenoids level in rice at 1 and
573 1.5 mM (Shaw and Hossain 2013). Similarly, the decrease in the firmness of cucumber fruits has been reported upon
574 treatment with CuO-NPs of <50 nm (Hong et al. 2016). Also, the grain yield of rice has been shown to reduce
575 significantly by CuO-NPs (~ 43 nm) at 500 and 1000 mg kg⁻¹ (Peng et al. 2017).

576 Summarizing these results, it can be concluded that Cu-based NPs at a certain concentration negatively
577 affected plant metabolism and nutrient content.

578

579 **6.5 Genotoxic and cytotoxic effects**

580

581 Genotoxicity is one of the most devastating effects exerted by NPs on plants. A variety of toxic effects have been
582 reported for NPs which may interact with biological systems via five main modes: (i) chemical effects as metal ions
583 in solution upon dissolution; (ii) mechanical effects owing to hard spheres and defined interfaces; (iii) catalytic
584 effects on surfaces; (iv) surface effects owing to binding of proteins to the surface, either by non-covalent or
585 covalent mechanisms or oxidative effects; and (v) changes in the chemical environment (pH). Metal and metal oxide
586 NPs have been shown to act as mediators of DNA damage in mammalian cells, organisms, and even in bacteria, but

587 the molecular mechanisms through which this occurs are poorly understood. For the first time, it was reported that
588 CuO-NPs induce DNA damage in crops and grassland plants (Atha et al. 2012). The Cu-NPs, up to 20 $\mu\text{g ml}^{-1}$
589 increased the mitotic index of actively dividing cells in *A. cepa* with a gradual decline in the mitotic index as the
590 concentration increased (Nagaonkar et al. 2015). Smaller sized NPs, increasing concentrations, and exposure
591 duration of NPs have been related to greater genotoxic responses, leading to mito-depressive effects in the cell
592 cycle. Micronuclei formation, disturbed chromosomes, chromosome fragments, stickiness, bridge, laggards'
593 chromosomes and decrease in mitotic index are the most obvious anomalies in plants exposure to silver, copper,
594 titanium dioxide, zinc, zinc oxide, selenium oxide, multi-wall carbon nanotube, tetramethylammonium hydroxide
595 and Bismuth (III) oxide NPs. The severity of abnormalities depending on the concentration, duration time and
596 particle size are different. Finally, if the DNA repair mechanisms are not enough to restore these alterations, it can
597 lead to loss of genetic material and mutation in DNA (Karami and Lima 2016). The plant DNA is also affected by
598 cellular oxidative stress generated by Cu-based NPs. Atha et al. (2012) reported oxidative-stress induced DNA
599 lesions in *R. sativus*, *Lolium perenne*, and *L. rigidum* by CuO-NPs (10-1000 mg L^{-1}) that include 2,6-diamino-4-
600 hydroxy-5-formamidopyrimidine, 8-OH-dG, the 2'-deoxynucleoside form of 8-OH-G, and 4,6-diamino-5-
601 formamidopyrimidine (Atha et al. 2012). Cu-based NPs exposure has been attributed to induce genotoxic effects and
602 affect the normal cell cycle. Chromosomal aberrations such as sticky and disturbed chromosomes in
603 metaphase/anaphase, c-metaphase, bridges, laggard chromosomes, disturbed telophase, and vacuolated nucleus
604 resulted after exposure to Cu/CuO-NPs in onion and black cumin (Deng et al. 2016; Kumbhakar et al. 2016;
605 Nagaonkar et al. 2015). These aberrations are very similar to those induced by ethyl methanesulphonate (EMS) and
606 gamma radiation. With the use of random amplified polymorphic DNA (RAPD), the genotoxicity of CuO-NPs (~50
607 nm) has been demonstrated in buckwheat (Lee et al. 2013). The authors demonstrated changes in DNA bands in
608 RAPAD profiles of buckwheat exposed by 2,000 and 4,000 of CuO NPs mg L^{-1} (Lee et al. 2013). The changes in the
609 genetic pattern induced by Cu-NPs toxicity could be attributed to changes in genomic DNA template stability due to
610 mutations homologous recombination, deletion of large DNA segments and might be due to the strong binding of
611 NPs with plant DNA (Ahmed et al. 2018b; Lee et al. 2013). The DNA isolated from young tomato leaves upon
612 interaction with various concentrations of CuO-NPs exhibited concentration-dependent fluorescence quenching of
613 acridine orange-DNA complex and ethidium bromide-DNA complex (Ahmed et al. 2018b). The CuO-NPs are able
614 to interact with plant DNA in both intercalative and non-intercalative mode with perceptible changes in other
615 macromolecules like amide I and II of proteins and carbohydrates (Ahmed et al. 2018b). The transfer of CuO-NPs to
616 progeny (harvested seeds) of *Arabidopsis thaliana* has been studied by XANES in the form of CuO (88.8%),
617 moreover, Cu in seeds has been detected as Cu-acetic acid (3.2%), $\text{Cu}_2(\text{OH})\text{PO}_4$ (2%), and Cu_2O (6%) (Wang et al.
618 2016). Recently, the change in the gene expression pattern of plants exposed to CuO-NPs has been reported. Wang

619 et al. (2016) documented differential expression of gene Fe-SOD and gene Aux/IAA in the regulation of *A. thaliana*
620 root growth when exposed to 20 and 50 mg L⁻¹ CuO-NPs. Similarly, altered gene expression has been observed by
621 surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF) in cucumber seeds after treatment with
622 nano-CuO at 600 mg L⁻¹ (Moon et al. 2014). In this study, among 34 differentially expressed proteins about 9
623 differed from those exposed to control and bulk-CuO treated plants. A protein (5977-m/z) has been found as the
624 most distinguished biomarker for the determination of CuO-NPs induced phytotoxicity (Moon et al. 2014).

625 Interaction of Cu-NPs with plant root exudates also influences the fate of Cu-NPs and magnitude of
626 toxicity. Huang et al. (2017) determined the thermodynamic parameters for the interaction of Cu-NPs (40 nm) with
627 a mixture of synthetic root exudates (SRE) and its components such as sugars, amino acids, organic acids, and
628 phenolic acids by nano isothermal titration calorimetry. The data revealed a strong binding constant ($K_d = 5.645 \times$
629 10^3 M^{-1}) for Cu-NPs SRE interaction, however, the binding of Cu²⁺ was found stronger but varied for individual
630 SRE components (Huang et al. 2017).

631 The DNA damage and chromosomal aberrations raise the concern about the safety associated with
632 applications of the NPs. However, the studies on the phytotoxicity of NPs are scarce, especially with regard to its
633 mechanisms, and on its potential uptake and subsequent fate within the food chain.

634

635 **6.6 Effects on plants ROS and anti-oxidative activities**

636

637 One of the widely reported toxicity mechanisms is the generation of NPs-induced ROS and consequent stimulation
638 of cellular antioxidant defence mechanisms in plants. The NPs could enhance ROS generation in plants, and cause
639 oxidative stress, protein oxidation, lipid peroxidation, DNA damage and finally cell death (Ahmed et al. 2018b;
640 Mosa et al. 2018). To avoid oxidative stress, plants activate a defence mechanism involving the anti-oxidative
641 enzymes (Rajput et al. 2015).

642 The ROS generation reportedly induces damage to cellular membranes resulting in respiratory loss and
643 lipid peroxidation leading to disruption of vital cellular functions (Gueraud et al. 2010; Maness et al. 1999). In the
644 presence of high concentrations, Cu can promote the generation of ROS by Fenton reaction ($\text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} +$
645 $\text{OH}^\bullet + \text{OH}^-$) due to its high redox-active nature (Halliwell and Gutteridge 1985). ROS interaction with protein
646 sulfhydryl (-SH) groups may cause enzyme inactivation which in all likelihood may lead to necrosis, chlorosis, and
647 growth inhibition (Das and Roychoudhury 2014; Xiong and Wang 2005; Yruela 2009). Among ROS, hydroxyl
648 radicals formed via Haber-Weiss reaction ($\text{H}_2\text{O}_2 + \text{O}_2^{\bullet-} \rightarrow \text{OH}^\bullet + \text{OH}^- + \text{O}_2$) are considered to be more toxic
649 (Letelier et al. 2010). To mitigate the ROS stress induced by Cu-NPs, plants elevate the activity of antioxidant
650 enzymes such as superoxide dismutase (SOD) (Wang et al. 2016), ascorbate peroxidase (APX) (Hong et al. 2015;

651 Shaw et al. 2014), glutathione reductase (GR) (Shaw et al. 2014), catalase (CAT) (Ahmed et al. 2018a,b; Trujillo-
652 Reyes et al. 2014), and peroxidase (POD) (Nair and Chung 2014). In addition to this, Cu-NPs arbitrated oxidative
653 stress can also be measured in terms of antioxidant levels and proline (Shaw and Hossain 2013; Zhao et al. 2016b).
654 The CuO-NPs exposure also increased the lipid peroxidation and triggered an imbalance in oxidative enzymes *viz.*
655 GSH, CAT and POD (Dimkpa et al. 2012). The enhanced lipid peroxidation also accompanies low GSH and
656 GSH/GSSG ratio (Shaw et al. 2014; Shaw and Hossain 2013) and high SOD activity that converts superoxide
657 radicals into hydrogen peroxide ($O_2^{\bullet} \rightarrow H_2O_2$) (Kim et al. 2012; Nekrasova et al. 2011). Besides, antioxidant
658 enzymes enhanced malondialdehyde (MDA) content also serves as an oxidative stress marker for Cu-based NPs. For
659 instance, the highest levels of MDA were observed in *C. sativus* shoots and roots treated with 100 and 200, and 50
660 and 100 mg L⁻¹ Cu-NPs grown in a hydroponic system, respectively. An increase in MDA levels is directly
661 proportional to the concentration of the Cu-NPs used for the treatment (Mosa et al. 2018). Similarly, the CuO-NPs
662 increased lipid peroxidation and ROS in *Pisum sativum* (Nair and Chung 2015b).

663 To better understand the toxic nature of Cu-based NPs and their targeted applications, the endpoints of
664 toxicity should be carefully scrutinized.

665

666 7. Toxicity on aquatic systems

667

668 The impact of Cu-based NPs on aquatic environment is an important issue due to extensive utilization of Cu-NPs,
669 releasing metal ions in aqueous solution, making them bioavailable and toxic (Bondarenko et al. 2013; Chang et al.
670 2012; Mukherjee and Acharya 2018). The probabilistic model predicts environmental concentrations of Cu-NPs
671 0.06 mg L⁻¹ in major Taiwanese rivers with 95% confidence interval (CI): 0.01–0.92) (Chio et al. 2012). This model
672 raised concern on Cu-based NPs adverse effects on aquatic organisms. In addition, several studies highlighted
673 toxicity of Cu-based NPs on aquatic organisms including gill injury and acute lethality in zebrafish and toxicity to
674 algal species (Aruoja et al. 2009; Griffitt et al. 2007; Griffitt et al. 2009), induction of oxidative stress in the liver,
675 gills and muscles of juvenile *Epinephelus coioides* (Wang et al. 2014) and in mussels (Gomes et al., 2014), damage
676 to gill filaments and gill pavement cells of freshwater fish (Song et al. 2015b), disruption of secondary lamellae of
677 gills, damage in the liver showing pyknotic nuclei (Gupta et al. 2016), affected proliferation, cell cycle progression
678 and cell death of amphibians (Thit et al. 2013). The summarized review on NPs toxicity on aquatic habitats suggests
679 lethal effects on *Pseudokirchneriella Subcapitata*, *Desmodesmus subspicatus*, *Xenopus laevis*, *Rana catesbeiana*,
680 *Mytilus edulis*, *Mytilus galloprovincialis*, *Crassostrea virginica*, *Daphnia magna*, *Thamnocephalus platyurus*, *Danio*
681 *rerio*, *Lytechinus pictus*, *Oncorhynchus mykiss* and *Cyprinus carpio* (Mukherjee and Acharya 2018). Pradhan et al.
682 (2015) found that CuO-NPs induce oxidative stress, damage to DNA and plasma membrane of aquatic fungi.

683 Similarly, Giannetto et al. (2018) found that CuO-NPs affected oxidative stress-related genes of *Arbacia lixula*
684 embryos. A short-term study on diatom showed that Cu-NPs inhibited the growth, photosynthesis and induced
685 oxidative stress on *Phacodactylum tricorutum* (Zhu et al. 2017). Three different Lemnaceae species (*Spirodela*
686 *polyrhiza*, *Lemna minor* and *Wolffia arrhiza*) commonly found in freshwater lakes exposed to Cu-NPs expressed
687 different sensitivities (Song et al. 2015a).

688 These data suggest that the toxicity of Cu-based NPs can be influenced by the species, exposure duration,
689 and dose.

690

691 **7.1 Toxicity on aquatic plants**

692

693 There are potentially many sources of NPs in the aquatic ecosystem such as geogenic sources, industrial sources
694 including medical and pharmaceutical, runoff from household's farms, leaching from landfills etc. Xenobiotic
695 substances could have a great impact on aquatic biota as well as constitute a serious danger for the aquatic
696 ecosystem (Moore 2006). One of the anthropogenic sources of Cu-based NPs in the aquatic system is polymer-
697 coating found in marine paints or fabric with antimicrobial and biocidal properties. This kind of material is used
698 for antifouling of boats and immersed structures, and CuO-NPs are frequently one of the ingredients (Almeida et
699 al. 2007). A study showed that CuO-NPs alone (0.004 g L^{-1}) is less toxic to green alga *Chlamydomonas*
700 *reinhardtii* than CuO-NPs coated with the polymer after 6 h of exposition (Melegari et al. 2013). Nonetheless,
701 CuO-NPs still decreased the activity of PS II and were found responsible for the generation of ROS. There were
702 observations for significantly higher intracellular Cu accumulation in the form of aggregate as compared to Cu-
703 free samples (Perreault et al. 2012). Similar results were observed in the plant *Lemna gibba* such as
704 morphological changes like abscission of the fronds from the colonies, decrease in frond size and whitening of
705 the fronds (Perreault et al. 2014). Both observations indicate that surface modification of NPs in order to enhance
706 their stabilization changes their mechanism of toxicity which seems to be an important issue for expanding
707 applications of Cu-based NPs in future. Aruoja et al. (2009) performed tests on the bioavailability of Cu-based
708 pollutants. The authors confirmed that Cu from CuO-NPs was 141-fold more bioavailable to aquatic flora in
709 comparison to that from bulk CuO. The greater toxicity of CuO-NPs was seen in algae *Pseudokirchneriella*
710 (Aruoja et al. 2009) and plant *Lemna minor* (Song et al. 2015a). That is consistent with the previous statement
711 that the Cu bioavailability rather than the total concentration is the primary toxicity (Campbell 1995). However,
712 Perreault et al. (2012) pose a hypothesis that during CuO-NPs solubilisation, a soluble form of copper, mostly
713 Cu^{2+} ions are released which can spread into the medium and become the main factor for CuO-NPs toxicity that
714 is similar to the danger posed by CuSO_4 . The *P. stratiotes* plants grown in the presence of Cu-NPs (1000 mg L^{-1}

715 ¹) for 14 days exhibited discolouration along with the visible signs of turgor loss in mesophilic cells.
716 Morphological changes in the root system were more prominent. In comparison to the control plant, blackening
717 of roots together with inhibition of new growth roots, and a decrease in plant weight, amino acids, and the
718 content of ascorbic acid reduced by 63% was observed in exposed plants (Olkhovych et al. 2016). The
719 morphological changes were also observed for plant *L. gibba* in the form of leaf reduction and detachment of
720 fronds from the plant. The symptoms were detected after 24 h CuO-NPs exposure with 1.0 mg L⁻¹ (Perreault et
721 al. 2014). The growth inhibition was observed at 6.4 mg L⁻¹ microalgae culture and for *L. minor* at 10 mg L⁻¹ in
722 comparison to Cu-free samples (Melegari et al. 2013, Song et al. 2016). The Cu-based NPs exposure on aquatic
723 flora is mostly reflected in photosystem dysfunction. The chlorophyll content of *L. minor* decreased with the
724 increase in concentration at 100 mg L⁻¹ CuO-NPs (Song et al. 2016). In the algal culture of *C. reinhardtii*, the
725 decrease of total chlorophyll and carotenoids was observed at 1000 mg L⁻¹ when exposure lasted for 72 h
726 (Aruoja et al. 2009). For microalgae, *Pseudokirchneriella* 6.4 mg L⁻¹ was sufficient to evoke abnormality in
727 photosynthetic system performance (Melegari et al. 2013). In the study of Perreault et al. (2014), lower
728 photosynthetic electron transport rate for *L. gibba* was observed. The Cu-NPs at a concentration higher than 1 mg
729 L⁻¹ clearly suppresses photosynthesis on *Elodea densa* (waterweed) while low concentration (<0.25 mg L⁻¹) has a
730 positive impact on photosynthesis effectiveness (Nekrasova et al. 2011). The main feature of Cu-based NPs is
731 that they have the ability to cross the plasma membrane that results in alteration of subcellular organelles. This
732 condition substantially may cause oxidative stress which is connected to increased enzymatic activity (i.e., POD,
733 CAT, and SOD) (Melegari et al. 2013). The production of ROS may be the result of conditions when plants are
734 subjected to harmful stress conditions. The chloroplasts and mitochondria of plant cells are important in
735 intracellular generators of ROS. Internal O₂ concentration is high during photosynthesis, and chloroplasts are
736 particularly prone to generate ROS; therefore, these cytotoxic ROS can remarkably disrupt normal metabolism
737 through oxidative damage of lipids, nucleic acids, and proteins.

738 In general Cu-based pollutants induce various responses within the photosynthetic organism. The
739 changes seem to be the most prominent for the CuO-NPs and Cu-NPs following by CuSO₄ and bulk CuO. The
740 Cu-NPs toxicity heavily depends on dosage and further surface modification.

741

742 ***7.2 Toxicity on aquatic animals***

743

744 There is currently a significant gap in our knowledge about CuO-NPs toxicity to aquatic animals. In general, the
745 Cu(O) NPs toxicity may be a potential environmental concern for crustaceans, as LC50 values are within an order of
746 magnitude of predicted wastewater concentrations, while chronic and developmental toxicity are a more relevant

747 concern for fishes (Braz-Mota et al. 2018). A few studies have noted bioactivity in these animals at high
748 concentrations ($20 \mu\text{g L}^{-1}$). The release of manufactured Cu-based NPs into the aquatic environment is rather rarely
749 known (Moore 2006). Nevertheless, it was proven that NPs association with naturally occurring colloids may affect
750 their bioavailability and uptake into cells and organisms. Uptake by endocytic routes was previously identified as
751 probable major mechanisms of entry into cells; potentially leading to various types of toxic cell injury (Moore
752 2006). Griffitt et al. (2009) demonstrated that the effects of Cu-NPs were not solely due to the release of soluble
753 metals into the water column. These studies highlight the need for further studies focused on understanding the
754 mechanisms of NPs toxicity to aquatic organisms as dissolution and the presence of a generic NPs response are not
755 sufficient to explain the observed effects.

756 Sedimentation following hetero-aggregation with organic matter and free anions poses a threat due to
757 benthic, sediment-dwelling and filter feeding organisms. In marine systems, NPs can be absorbed by
758 microorganisms and transferred to the next trophic levels by consumption. Filter feeders, especially bivalves,
759 accumulate CuO-NPs through trapping them in mucus prior to ingestion. Benthic fauna may directly ingest sediment
760 CuO-NPs. In fish, uptake is principally via the gut following drinking, whilst CuO-NPs caught in gill mucus may
761 affect respiratory processes and ion transport. Currently, environmentally realistic CuO-NPs concentrations are
762 unlikely to cause significant adverse acute health problems, however, sub-lethal effects e.g. oxidative stress have
763 been noted in many organisms, often deriving from the dissolution of Cu^{2+} , and this could result in chronic health
764 impacts (Baker et al. 2014).

765 The effect of waterborne Cu-NPs and copper sulphate on rainbow trout (*Oncorhynchus mykiss*) in the
766 context of physiology and accumulation was also evaluated by Shaw et al. (2012). Overall, these data showed that
767 Cu-NPs have similar types of toxic effects to CuSO_4 , which can occur at lower tissue Cu concentrations than
768 expected for the dissolved metal. It was also proved that CuO-NPs can induce toxicity to the freshwater shredder
769 (*Allogamus ligonifer*) (Pradhan et al. 2012).

770 Abdel-Khalek et al. (2015) compared the toxicity of CuO-NPs to Nile Tilapia (*Oreochromis niloticus*) with
771 its bulk counterpart and reported that the $\text{LC}_{50/96 \text{ h}}$ of CuO bulk particles (BPs) was higher than that of NPs
772 indicating that CuO-NPs are more toxic. The CuO-NPs could exert more toxic effects despite the fact that they are
773 smaller in size than the CuO-BPs, and they can form aggregates in suspensions. The authors demonstrated CuO
774 (BPs & NPs) induced biochemical alterations and oxidative stress in *O. niloticus*, which suggest ecological
775 implications of CuO-NPs released in aquatic ecosystems. The study conducted by Braz-Mota et al. (2018) aimed to
776 understand the effects of CuO-NPs and Cu on two ornamental Amazon fish species: dwarf cichlid (*Apistogramma*
777 *agassizii*) and cardinal tetra (*Paracheirodon axelrodi*). For fish exposed to 50% of the LC_{50} for CuO-NPs, aerobic
778 metabolic rate (MO_2), gill osmoregulatory physiology and mitochondrial function, oxidative stress markers, and

779 morphological damage were evaluated. The results revealed species specificity in metabolic stress responses. An
780 increase of MO_2 was noted in cardinal tetra exposed to Cu, but not CuO-NPs, whereas MO_2 in dwarf cichlid showed
781 little change with either treatment. In contrast, mitochondria from dwarf cichlid exhibited increased proton leak and
782 a resulting decrease in respiratory control ratios in response to CuO-NPs and Cu exposure. This uncoupling was
783 directly related to an increase in ROS levels. The authors revealed different metabolic responses between these two
784 species in response to CuO-NPs and Cu, which are probably caused by the differences between species natural
785 histories, indicating that different mechanisms of toxic action of the contaminants are associated to differential
786 osmoregulatory strategies among species.

787 Gupta et al. (2016) described the effect of Cu-NPs exposure in the physiology of the common carp
788 (*Cyprinus carpio*) using biochemical, histological and proteomic approaches. The results indicated that the activity
789 of oxidative stress enzymes catalase, superoxide dismutase, and glutathione-S-transferase were significantly
790 increased in the kidney, liver and gills of the treated groups when compared to control. Histological analysis
791 revealed that after exposure, disruption of the secondary lamellae of gills, liver damage with pyknotic nuclei and
792 structural disarray of the kidney occurred. Proteomic analysis of the liver showed down-regulation of several
793 proteins including the ferritin heavy chain, Rho guanine nucleotide exchange factor 17-like, cytoglobin-1, regulation
794 of diphosphomevalonate decarboxylase and selenide & water dikinase-1.

795 The effect of Cu-NPs on the development of zebrafish embryos was depicted by Sun et al. (2016). The
796 exposure to CuO-NPs at concentrations of 12.5 mg L^{-1} or higher leads to abnormal phenotypes and induces an
797 inflammatory response in a dose-dependent pattern. Moreover, exposure to CuO-NPs at high doses results in an
798 underdeveloped liver and a delay in retinal neurodifferentiation accompanied by reduced locomotor ability. The
799 authors demonstrated that short-term exposure to CuO-NPs at high doses shows hepatotoxicity and neurotoxicity.
800 On the other hand, cellular and molecular responses of adult zebrafish after exposure to CuO-NPs or ionic Cu were
801 tested by Vicario-Pares et al. (2018). Another study performed by Bai et al. (2010) was undertaken to test the
802 toxicity of nano-Cu suspension to zebrafish embryos. It was found that nano-Cu retarded the hatching of zebrafish
803 embryos and caused morphological malformation of the larvae. The authors claimed that high concentrations (>0.1
804 mg L^{-1}) of nano-Cu can kill the gastrula-stage zebrafish embryos. Denluck et al. (2018) investigated the role of the
805 chorion in nanomaterial toxicity. The authors found that the presence of the chorion inhibited Cu-NPs toxicity:
806 while dechorionated embryonic zebrafish exposed to Cu-NPs had an LC_{50} of $2.5 \pm 0.3 \text{ mg L}^{-1}$, a chorion-intact had
807 LC_{50} of $13.7 \pm 0.8 \text{ mg L}^{-1}$. In summary, embryo sensitivity increased by at least one order of magnitude when
808 chorions were removed.

809 The toxicity of Cu-based NPs in aquatic environment appears to be one of the most important issues for
810 assessing whole ecosystem safety. With no doubts, zebrafish embryos are excellent models for the study of
811 nanomaterial-biological interactions and toxicity.

812

813 **8 Techniques used to detect the presence of Cu in plant tissues treated with Cu-based NPs**

814

815 It has already been mentioned that new developments in nanotechnology industry increase the amount of such
816 engineered nanomaterials in the environment, particularly in soils and aquatic ecosystems. This could lead to
817 unpredicted consequences in the nearest future as plants play a vital role in the ecosystem and worldwide food
818 supply. That is why NPs detection in environmental samples is of importance (Chaudhry et al. 2008; Mukherjee et
819 al. 2016). However, not all methods are applicable to this problem due to low concentrations of NPs in
820 environmental samples and experimental complications in sample preparation. Still, there are several modern
821 techniques which are being widely applied to detect the presence, visualise the distribution and analyse chemical
822 properties of NPs in plant tissues or in the soil. The available detection methods could be classified into three broad
823 sections: spectroscopy, diffraction and imaging. However, the most comprehensive results could be obtained using
824 the combination of all three methods. Besides, one of the most sensitive techniques is Atomic Absorption
825 Spectroscopy (AAS). However, this method is destructive and requires special sample preparation procedures.

826 Different types and combinations of electron microscopy techniques offer environmental scientists a wide
827 range of capabilities. Scanning Electron Microscopy (SEM) gives a possibility to find and locate metal NPs which
828 usually have higher electron density. SEM microscopes are often equipped with EDX that extend analytical
829 capabilities to qualitative determination of elements present in the sample and quantitative determination of element
830 concentration, thus opening a possibility to study the chemical composition of NPs. High-Resolution TEM reveal
831 the shape and morphology of tiny NPs of several nanometers in diameter. Selected Area Electron Diffraction
832 (SAED) and images acquired in bright and dark-field modes could be used to study NPs phase composition and
833 distribution in the samples. Microscopes equipped with Electron Energy Loss Spectra (EELS) cameras are capable
834 of revealing the oxidation state of 3d transition metals at nanoscale resolution (Tan et al. 2012). Moreover, these
835 electron-based methods could be combined in one microscope that provides a great possibility to study the presence,
836 distribution, chemical composition, morphology, shape and size distribution of NPs in soils and plants. However, the
837 shortcomings of the method are the limitations on the size of the sample, special sample preparation procedures and
838 the requirement of ultra-high vacuum.

839 Furthermore, X-Ray Fluorescence (XRF) is one of the powerful tools to estimate the relative quantity of
840 elements present in the sample semi-quantitatively (mass %). Often laboratory equipment has a focused X-ray beam

841 up to 20-50 micrometres (μ -XRF) that gives a possibility to obtain element concentration maps of the samples with
842 appropriate resolution. The latter could be used to detect and locate NPs aggregation in plants. There is also a
843 particular interest in portable XRF devices (pXRF) (McLaren et al. 2012) for agronomic and environmental science
844 applications as it opens possibilities to conduct in field studies. Such equipment could be used to relate plant
845 conditions to elemental nutrient deficiencies in the soil (Towett et al. 2016). However, such devices are limited to
846 spectroscopic data and low sensitivity. On the contrary, sub-micron resolution and high sensitivity of synchrotron-
847 based micro- and nano- X-ray techniques open new possibilities to investigate the interactions between plants and
848 engineered nanomaterials. Synchrotron-based techniques require minimal sample preparation, are non-destructive,
849 offer the best balance between sensitivity, chemical specificity, and spatial resolution (Castillo-Michel et al. 2017).
850 These techniques are particularly adapted to investigate localization and speciation of NPs in plants: μ -XRF and
851 synchrotron X-ray fluorescence mapping (SR-XFM) offers multi-elemental detection with resolution down to the
852 tens of nm, in combination with spatially resolved X-ray absorption spectroscopy (μ -XAS or μ -XANES) speciation.
853 Moreover, such synchrotron-based techniques could be combined with μ -XRD (micro X-Ray Diffraction) and μ -
854 FTIR (micro Fourier-Transform Infrared Spectroscopy) techniques in one beamline (Cotte et al. 2017).

855 One of the most promising methods to detect the presence of NPs at environmentally relevant concentration
856 is sp-ICP-MS (Laborda et al. 2014; Laborda et al. 2013). It gives a possibility to obtain qualitative information about
857 the presence of particulate and/or dissolved forms, quantitative information as particle number as well as mass
858 concentrations, and characterization information about the mass of element/s per particle and particle size (Laborda
859 et al. 2016).

860 TEM remains one of the main tools to analyse Cu-based NPs distribution (Lee et al. 2008; Nhan Le et al.
861 2016) and composition in plants (Trujillo-Reyes et al. 2014; Wang et al. 2011). The XRF technique was applied to
862 reveal the elemental composition of *C. sativus* shoot and root samples treated with Cu-NPs (Mosa et al. 2018). The
863 microscopic analysis showed the presence of dense material in root cells of *O. sativum* L. treated with CuO-NPs and
864 confirmed the presence of Cu by bulk-XANES, and the most dominant form of Cu was from CuO-NPs (Peng et al.
865 2015). A combination of μ -XRF and μ -XANES was used to study bioaccumulation un-weathered (U) and weathered
866 (W) CuO-NPs, bulk and ionic form by lettuce (Servin et al. 2017b). The μ -XRF analysis of W-NP-exposed roots
867 showed a homogenous distribution of Cu in the tissues, while μ -XANES analysis of W-NP-exposed roots showed
868 near complete transformation of CuO to Cu (I)-sulfur and oxide complexes in the tissues. Duran et al. (2017)
869 showed that CuO-NPs did not affect seed germination of *Phaseolus vulgaris* L., but seedling weight gain was
870 promoted by 100 mg Cu L⁻¹ and inhibited by 1000 mg Cu L⁻¹ of 25 nm CuO and CuSO₄. The μ -XRF analysis
871 showed that most of the Cu taken up remained in the seed coat with Cu hotspots in the hilum. Moreover, μ -XANES
872 unravelled that most of Cu remained in its pristine form. Zhao et al. (2017a) showed significant growth inhibition on

873 both roots and shoots of *E. crassipes* after 8-day exposure of CuO-NPs (50 mg L⁻¹) which was much higher than that
874 of the bulk CuO particles and dissolved Cu²⁺ ions of the same Cu concentration. The XANES was used to reveal the
875 presence of CuO-NPs as well as Cu₂S and other Cu species in roots, submerged leaves, and emerged leaves of plants
876 providing solid evidence of the transformation of CuO-NPs. Electron microscopy remains one of the most widely
877 used tools to study distribution, morphology and composition of metal NPs in plants. The possibilities that such
878 synchrotron radiation techniques as μ -XRF and μ -XANES open to environmental scientists could significantly
879 change the situation in the sense of revealing precise information on its structure. Moreover, an sp-ICP-MS becomes
880 one of the most promising technique to obtain the presence and size distribution of NPs at environmentally relevant
881 concentrations.

882

883 **9 Conclusion and future outlook**

884

885 The literature unequivocally suggests that the higher concentrations of Cu-based NPs are detrimental to beneficial
886 soil microorganisms, food crops, aquatic animals and plants. The toxicity of Cu-based NPs is influenced by their
887 composition, capping/coating material, size, and interactions with environmental components such as abiotic factors
888 (e.g. pH) and microbial/plant secretions, and naturally occurring organic matter etc. Furthermore, the phytotoxicity
889 may vary with the varying physiology/anatomy of plant species. Cu-based NPs are either taken up by organisms
890 (internal efficiency) or adsorbed on external structures (external efficiency). The adherence and bioaccumulation
891 may also be changed by physicochemical properties of Cu-based NPs, plant genotypes, and
892 physical/chemical/biological transformation. The available studies considered in this review showed the inadequate
893 characterization of Cu-based NPs, which could be the major obstacle in properly assessing its toxicity. Moreover,
894 the disposal/discharge of Cu-based NPs into the environment is not regulated appropriately. After reviewing those
895 studies, many questions still persist unanswered when the behaviour and fate of Cu-based NPs in biological systems
896 are taken into consideration. For instance, most of the studies on Cu-based NPs and plants interactions were
897 performed on agar or in hydroponic media which do not reflect the actual interaction in the more realistic
898 environment such as the soil system. The fate of Cu-based NPs, their toxicity and accumulation in the soil can vary
899 significantly in different soil types due to the difference in pH, organic matter content and composition, etc.
900 Therefore, understanding the connection between association and dissociation/dissolution of adequately
901 characterized Cu-based NPs in a range of environmental media and the physiology/anatomy of affected organisms is
902 most urgently needed to further our knowledge regarding the potential toxicity exerted by Cu-based NPs. After all,
903 we conclude that Cu-based NPs comprised of Cu-NPs, CuO-NPs, and nano-Cu based products used in agricultural
904 practices have a great potential to negatively impact soil and aquatic micro/macro biota. The current scenario also

905 emphasizes the regulated and safe dumping of waste containing Cu-based NPs into agro-ecosystems. In the future,
906 the concentration of Cu-based NPs in edible parts of food crops must be measured carefully before supplying the
907 products to consumers.

908 It is also crucial to develop a unified methodology for testing the NPs toxicity in natural environments.
909 With the help of this methodology, joint research should be conducted to determine the toxicity of the same NPs
910 under different climatic conditions and soil types. Such international research could help to develop the permissible
911 levels of Cu-based NPs application and determine the threshold levels of their contents in different soils. The
912 kinetics of NPs dissolution and migration to the groundwater should be specifically considered to avoid their
913 accumulation above the safe levels. Sustainable use of Cu-based NPs could help to utilize the beneficial effects of
914 their application (i.e. in the form of nanopesticides) without posing a threat to the living organisms.

915 The increased application of Cu-based NPs clearly indicates their negative impact on ecosystems. It is,
916 therefore, imperative to explore Cu-based NPs toxicity and behaviour in water, living organisms (biota), soil and
917 sediments individually, and their toxicity in a combination of other metallic NPs. Past and future research must
918 be placed in the context of current risk assessments associated with Cu-based NPs, their use, distribution, and
919 release in the environment.

920

921 **Acknowledgements**

922 This research was supported by the Ministry of Education and Science of Russia (project no. 5.948.2017/PP).

923 We are grateful to Prof. Dr. Lok R. Pokhrel, Department of Public Health, East Carolina University, USA, for
924 help in language editing.

925

926 **Conflict of interest**

927 The authors declare that they have no conflict of interest.

928

929 **References:**

- 930 Abdel-Khalek AA, Kadry MAM, Badran SR, Marie MAS (2015) Comparative toxicity of copper oxide bulk and
931 nano particles in Nile Tilapia; *Oreochromis niloticus*: biochemical and oxidative stress. J Basic Appl Zool
932 72:43-57
- 933 Adeleye AS, Conway JR, Perez T, Rutten P, Keller AA (2014) Influence of extracellular polymeric substances on
934 the long-term fate, dissolution, and speciation of copper-based nanoparticles. Environ Sci Technol
935 48:12561-12568
- 936 AdeleyeAS, Oranu EA, Tao M, Keller AA (2016) Release and detection of nanosized copper from a commercial
937 antifouling paint. Water Res 102:374-382
- 938 Adhikari T, Dube G, Kundu S, Patra AK (2018) Impact of copper oxide nanoparticles on growth of different
939 bacterial species. In Energy and Environment (pp. 47-55). Springer, Singapore
- 940 Ahmed B, Rizvi A, Zaidi A, Khan MS, Musarrat J (2019) Understanding the phyto-interaction of heavy metal oxide
941 bulk and nanoparticles: evaluation of seed germination, growth, bioaccumulation, and metallothionein
942 production. RSC Adv 9:4210-4225

- 943 Ahmed B, Dwivedi S, Abdin MZ, Azam A, Al-Shaeri M, Khan MS, Saquib Q, Al-Khedhairi AA, Musarrat J (2017)
944 Mitochondrial and chromosomal damage induced by oxidative stress in Zn⁽²⁺⁾ ions, ZnO-bulk and ZnO-
945 NPs treated *Allium cepa* roots. *Sci Rep* 7:40685
- 946 Ahmed B, Hashmi A, Khan MS, Musarrat J (2018a) ROS mediated destruction of cell membrane, growth and
947 biofilms of human bacterial pathogens by stable metallic AgNPs functionalized from bell pepper extract
948 and quercetin. *Adv Powder Technol* 29:1601-1616
- 949 Ahmed B, Khan MS, Musarrat J (2018b) Toxicity assessment of metal oxide nano-pollutants on tomato (*Solanum*
950 *lycopersicon*): A study on growth dynamics and plant cell death. *Environ Pollut* 240:802-816
- 951 Ahmed B, Khan MS, Saquib Q, Al-Shaeri M, Musarrat J (2018c) Interplay Between Engineered Nanomaterials
952 (ENMs) and Edible Plants: A Current Perspective. In "Phytotoxicity of Nanoparticles" (Faisal M, Saquib
953 Q, Alatar AA, Al-Khedhairi A A, eds.), pp. 63-102. Springer International Publishing, Cham
- 954 Ali K, Ahmed B, Dwivedi S, Saquib Q, Al-Khedhairi AA, Musarrat J (2015) Microwave accelerated green
955 synthesis of stable silver nanoparticles with *Eucalyptus globulus* leaf extract and their antibacterial and
956 antibiofilm activity on clinical isolates. *PLoS One* 10:e0131178
- 957 Almeida E, Diamantino TC, de Sousa O (2007) Marine paints: The particular case of antifouling paints. *Prog Org*
958 *Coat* 59:2-20
- 959 Anjum NA, Adam V, Kizek R, Duarte AC, Pereira E, Iqbal M, Lukatkin AS, Ahmad I (2015) Nanoscale copper in
960 the soil-plant system - toxicity and underlying potential mechanisms. *Environ Res* 138:306-25
- 961 Anjum NA, Gill SS, Duarte AC, Pereira E, Ahmad I (2013) Silver nanoparticles in soil-plant systems. *J Nanopart*
962 *Res* 15
- 963 Anyaogu KC, Fedorov AV, Neckers DC (2008) Synthesis, characterization, and antifouling potential of
964 functionalized copper nanoparticles. *Langmuir* 24:4340-4346
- 965 Arruda SC, Silva AL, Galazzi RM, Azevedo RA, Arruda MA (2015) Nanoparticles applied to plant science: a
966 review. *Talanta* 131:693-705
- 967 Aruoja V, Dubourguier HC, Kasemets K, Kahru A (2009) Toxicity of nanoparticles of CuO, ZnO and TiO₂ to
968 microalgae *Pseudokirchneriella subcapitata*. *Sci Total Environ* 407:1461-148
- 969 Atha DH, Wang H, Petersen EJ, Cleveland D, Holbrook RD, Jaruga P, Dizdaroglu M, Xing B, Nelson BC (2012)
970 Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. *Environ Sci Technol*
971 46:1819-1827.
- 972 Austin JR, Frost E, Vidi PA, Kessler F, Staehelin LA (2006) Plastoglobules are lipoprotein subcompartments of the
973 chloroplast that are permanently coupled to thylakoid membranes and contain biosynthetic enzymes. *Plant*
974 *Cell* 18:1693-1703
- 975 Bai W, Tian W, Zhang Z, He X, Ma Y, Liu N, Chai Z (2010) Effects of Copper Nanoparticles on the Development
976 of Zebrafish Embryos. *J Nanosci Nanotechnol* 10:8670-8676
- 977 Baker TJ, Tyler CR, Galloway TS (2014) Impacts of metal and metal oxide nanoparticles on marine organisms.
978 *Environ Pollut* 186:257-271
- 979 Ben-Moshe T, Frenk S, Dror I, Minz D, Berkowitz B (2013) Effects of metal oxide nanoparticles on soil properties.
980 *Chemosphere* 90:640-646
- 981 Ben-Sasson M, Lu X, Nejati S, Jaramillo H, Elimelech M (2016) In situ surface functionalization of reverse osmosis
982 membranes with biocidal copper nanoparticles. *Desalination* 388:1-8
- 983 Blosi M, Albonetti S, Dondi M, Martelli C, Baldi G (2010) Microwave-assisted polyol synthesis of Cu
984 nanoparticles. *J Nanopart Res* 13:127-138
- 985 Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A (2013) Toxicity of Ag, CuO and ZnO
986 nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical
987 review. *Arch Toxicol* 87:1181-1200
- 988 Braz-Mota S, Campos DF, MacCormack TJ, Duarte RM, Val AL, Almeida-Val VMF (2018) Mechanisms of toxic
989 action of copper and copper nanoparticles in two Amazon fish species: Dwarf cichlid (*Apistogramma*
990 *agassizii*) and cardinal tetra (*Paracheirodon axelrodi*). *Sci Total Environ* 630:1168-1180
- 991 Buffet PE, Richard M, Caupos F, Vergnoux A, Perrein-Ettajani H, Luna-Acosta A, Akcha F, Amiard JC, Amiard-
992 Triquet C, Guibolini M, Risso-De Faverney C, Thomas-Guyon H, Reip P, Dybowska A, Berhanu D,
993 Valsami-Jones E, Mouneyrac C (2013) A mesocosm study of fate and effects of CuO nanoparticles on
994 endobenthic species (*Scrobicularia plana*, *Hediste diversicolor*). *Environ Sci Technol* 47:1620-8
- 995 Bundschuh M, Filser J, Luderwald S, McKee MS, Metreveli G, Schaumann GE, Schulz R, Wagner S (2018)
996 Nanoparticles in the environment: where do we come from, where do we go to? *Environ Sci Eur* 30:6
- 997 Castillo-Michel HA, Larue C, Pradas del Real AE, Cotte M, Sarret G (2017) Practical review on the use of
998 synchrotron based micro- and nano- X-ray fluorescence mapping and X-ray absorption spectroscopy to
999 investigate the interactions between plants and engineered nanomaterials. *Plant Physiol Biochem* 110:13-32
- 1000 Campbell PGC (1995) Interactions between trace metals and aquatic organisms: a critique of the free-ion activity
1001 model. John Wiley and Sons, New York, NY
- 1002 Chang YN, Zhang M, Xia L, Zhang J, Xing G (2012) The toxic effects and mechanisms of CuO and ZnO
1003 nanoparticles. *Materials* 5:2850-2871

- 1004 Chaudhry Q, Scotter M, Blackburn J, Ross B, Boxall A, Castle L, Aitken R, Watkins R (2008) Applications and
1005 implications of nanotechnologies for the food sector. *Food Addit Contam Part A Chem Anal Control Expo*
1006 *Risk Assess* 25:241-258
- 1007 Chen Z, Gao SH, Jin M, Sun S, Lu J, Yang P, ... Guo J (2019) Physiological and transcriptomic analyses reveal CuO
1008 nanoparticle inhibition of anabolic and catabolic activities of sulfate-reducing bacterium. *Environ Int*
1009 125:65-74
- 1010 Chio CP, Chen WY, Chou WC, Hsieh NH, Ling MP, Liao CM (2012) Assessing the potential risks to zebrafish
1011 posed by environmentally relevant copper and silver nanoparticles. *Sci Total Environ* 420:111-8
- 1012 Colman BP, Arnaout CL, Anciaux S, Gunsch CK, Hochella MF, Jr Kim B, Lowry GV, McGill BM, Reinsch BC,
1013 Richardson CJ, Unrine JM, Wright JP, Yin L, Bernhardt ES (2013) Low concentrations of silver
1014 nanoparticles in biosolids cause adverse ecosystem responses under realistic field scenario. *PLoS One* 8:
1015 e57189
- 1016 Concha-Guerrero SI, Brito EMS, Piñón-Castillo HA, Tarango-Rivero SH, Caretta CA, Luna-Velasco A, Duran R,
1017 Orrantia-Borunda E (2014) Effect of CuO nanoparticles over isolated bacterial strains from agricultural
1018 soil. *J Nanomater* 1-13
- 1019 Conway JR, Beaulieu AL, Beaulieu NL, Mazer SJ, Keller AA (2015) Environmental stresses increase
1020 photosynthetic disruption by metal oxide nanomaterials in a soil-grown plant. *ACS Nano* 9:11737-11749
- 1021 Cornelis G, Hund-Rinke K, Kuhlbusch T, van den Brink N, Nickel C (2014) Fate and bioavailability of engineered
1022 nanoparticles in soils: a review. *Crit Rev Environ Sci Technol* 44:2720-2764
- 1023 Cota-Ruiz K, Delgado-Rios M, Martínez-Martínez A, Núñez-Gastelum JA, Peralta-Videa JR, Gardea-Torresdey JL
1024 (2018) Current findings on terrestrial plants-engineered nanomaterial interactions: Are plants capable of
1025 phytoremediating nanomaterials from soil? *Curr Opin Environ Sci Health* 6:9-15
- 1026 Cotte M et al. (2017) The ID21 X-ray and infrared microscopy beamline at the ESRF: status and recent applications
1027 to artistic materials. *J Anal At Spectrom* 32:477-493
- 1028 Da Costa MVJ, Sharma PK (2015) Effect of copper oxide nanoparticles on growth, morphology, photosynthesis,
1029 and antioxidant response in *Oryza sativa*. *Photosynthetica* 54:110-119
- 1030 Das K, Roychoudhury A (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers
1031 during environmental stress in plants. *Front Environ Sci* 2
- 1032 del Real AEP, Castillo-Michel H, Kaegi R, Larue C, de Nolf W, Reyes-Herrera J, ... Sarret G (2018) Searching for
1033 relevant criteria to distinguish natural vs. anthropogenic TiO₂ nanoparticles in soils. *Environ Sci: Nano*
1034 5(12):2853-2863
- 1035 Deng F, Wang S, Xin H (2016) Toxicity of CuO nanoparticles to structure and metabolic activity of *Allium cepa*
1036 root tips. *Bull Environ Contam Toxicol* 97:702-708
- 1037 Denluck L, Wu F, Crandon LE, Harper BJ, Harper SL (2018) Reactive oxygen species generation is likely a driver of
1038 copper based nanomaterial toxicity. *Environ Sci: Nano* 6:1473-1481
- 1039 Dietz KJ, Herth S (2011) Plant nanotoxicology. *Trends Plant Sci* 16:582-9
- 1040 Dimkpa CO, Latta DE, McLean JE, Britt DW, Boyanov MI, Anderson AJ (2013) Fate of CuO and ZnO nano- and
1041 microparticles in the plant environment. *Environ Sci Technol* 47:4734-42
- 1042 Dimkpa CO, McLean JE, Britt DW, Anderson AJ (2015) Nano-CuO and interaction with nano-ZnO or soil
1043 bacterium provide evidence for the interference of nanoparticles in metal nutrition of plants. *Ecotoxicology*
1044 24:119-129
- 1045 Dimkpa CO, McLean JE, Latta DE, Manangón E, Britt DW, Johnson WP, Boyanov MI, Anderson A J (2012) CuO
1046 and ZnO nanoparticles: phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown
1047 wheat. *J Nanopart Res* 14
- 1048 Du W, Tan W, Yin Y, Ji R, Peralta-Videa JR, Guo H, Gardea-Torresdey JL (2018) Differential effects of copper
1049 nanoparticles/microparticles in agronomic and physiological parameters of oregano (*Origanum vulgare*).
1050 *Sci Total Environ* 618:306-312
- 1051 Duan X, Xu M, Zhou Y, Yan Z, Du Y, Zhang L, Zhang C, Bai L, Nie J, Chen G, Li F (2016) Effects of soil
1052 properties on copper toxicity to earthworm *Eisenia fetida* in 15 Chinese soils. *Chemosphere* 145:185-192
- 1053 Duran NM, Savassa SM, Lima RG, de Almeida E, Linhares FS, van Gestel CAM, Pereira de Carvalho HW (2017)
1054 X-ray spectroscopy uncovering the effects of Cu based nanoparticle concentration and structure on
1055 *Phaseolus vulgaris* germination and seedling development. *J Agric Food Chem* 65:7874-7884
- 1056 Feng Y, Cui X, He S, Dong G, Chen M, Wang J, Lin X (2013) The role of metal nanoparticles in influencing
1057 arbuscular mycorrhizal fungi effects on plant growth. *Environ Sci Technol* 47:9496-9504
- 1058 Frenk S, Ben-Moshe T, Dror I, Berkowitz B, Minz D (2013) Effect of metal oxide nanoparticles on microbial
1059 community structure and function in two different soil types. *Plos One* 8:e84441
- 1060 Gao X, Avellan A, Loughton S, Vaidya R, Rodrigues SM, Casman E A, Lowry GV (2018) CuO nanoparticle
1061 dissolution and toxicity to wheat (*Triticum aestivum*) in Rhizosphere soil. *Environ Sci Technol* 52:2888-
1062 2897
- 1063 Garner KL, Keller AA (2014) Emerging patterns for engineered nanomaterials in the environment: a review of fate
1064 and toxicity studies. *J Nanopart Res* 16
- 1065 Gautam A, Ray A, Mukherjee S, Das S, Pal K, Das S, Karmakar P, Ray M, Ray S (2018) Immunotoxicity of copper
1066 nanoparticle and copper sulfate in a common Indian earthworm. *Ecotoxicol Environ Saf* 148:620-631

- 1067 Ge Y, Schimel JP, Holden PA (2011) Evidence for negative effects of TiO₂ and ZnO nanoparticles on soil bacterial
1068 communities. *Environ Sci Technol* 45:1659-64
- 1069 Giannetto A, Cappello T, Oliva S, Parrino V, De Marco G, Fasulo S, Mauceri A, Maisano M (2018) Copper oxide
1070 nanoparticles induce the transcriptional modulation of oxidative stress-related genes in *Arbacia lixula*
1071 embryos. *Aquat Toxicol* 201:187-197
- 1072 Gogos A, Thalmann B, Voegelin A, Kaegi R (2017) Sulfidation kinetics of copper oxide nanoparticles. *Environ Sci:*
1073 *Nano* 4:1733-1741
- 1074 Gomes T, Chora S, Pereira CG, Cardoso C, Bebianno MJ (2014) Proteomic response of mussels *Mytilus*
1075 *galloprovincialis* exposed to CuO NPs and Cu(2)(+): an exploratory biomarker discovery. *Aquat Toxicol*
1076 155:327-36
- 1077 Griffitt RJ, Weil R, Hyndman KA, Denslow ND, Powers K, Taylor D, Barber DS (2007) Exposure to copper
1078 nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). *Environ Sci Technol*
1079 41:8178-8186
- 1080 Griffitt RJ, Luo J, Gao J, Bonzongo JC, Barber DS (2009) Effects of particle composition and species on toxicity of
1081 metallic nanomaterials in aquatic organisms. *Environmental Toxicology and Chemistry* 27:1972-1978
- 1082 Gueraud F, Atalay M, Bresgen N, Cipak A, Eckl PM, Huc L, Jouanin I, Siems W, Uchida K (2010) Chemistry and
1083 biochemistry of lipid peroxidation products. *Free Radic Res* 44:1098-124
- 1084 Gupta YR, Sellegounder D, Kannan M, Deepa S, Senthilkumaran B, Basavaraju Y (2016) Effect of copper
1085 nanoparticles exposure in the physiology of the common carp (*Cyprinus carpio*): biochemical, histological
1086 and proteomic approaches. *Aquac Fish* 1:15-23
- 1087 Halliwell B, Gutteridge JMC (1985) Free radicals in biology and medicine. *Free Radic Biol Med* 1:331-332
- 1088 Handy RD, Al-Bairuty G, Al-Jubory A, Ramsden CS, Boyle D, Shaw BJ, Henry TB (2011) Effects of manufactured
1089 nanomaterials on fishes: a target organ and body systems physiology approach. *J Fish Biol* 79:821-53
- 1090 Hanna SK, Miller RJ, Zhou D, Keller AA, Lenihan HS (2013) Accumulation and toxicity of metal oxide
1091 nanoparticles in a soft-sediment estuarine amphipod. *Aquat Toxicol* 142-143:441-6
- 1092 Haris Z, Ahmad I (2017) Impact of metal oxide nanoparticles on beneficial soil microorganisms and their secondary
1093 metabolites. *Int J Sci Res* 3
- 1094 He S, Feng Y, Ni J, Sun Y, Xue L, Feng Y, Yu Y, Lin X, Yang L (2016) Different responses of soil microbial
1095 metabolic activity to silver and iron oxide nanoparticles. *Chemosphere* 147:195-202
- 1096 Hong J, Rico CM, Zhao L, Adeleye AS, Keller AA, Peralta-Videa JR, Gardea-Torresdey JL (2015) Toxic effects of
1097 copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*).
1098 *Environ Sci Process Impacts* 17:177-185
- 1099 Hong J, Wang L, Sun Y, Zhao L, Niu G, Tan W, Rico CM, Peralta-Videa JR, Gardea-Torresdey JL (2016) Foliar
1100 applied nanoscale and microscale CeO₂ and CuO alter cucumber (*Cucumis sativus*) fruit quality. *Sci Total*
1101 *Environ* 563-564:904-911
- 1102 Hu W, Culloty S, Darmody G, Lynch S, Davenport J, Ramirez-Garcia S, Dawson KA, Lynch I, Blasco J, Sheehan D
1103 (2014) Toxicity of copper oxide nanoparticles in the blue mussel, *Mytilus edulis*: a redox proteomic
1104 investigation. *Chemosphere* 108:289-299
- 1105 Huang Y, Zhao L, Keller AA (2017) Interactions, transformations, and bioavailability of nano-copper exposed to
1106 root exudates. *Environ Sci Technol* 51:9774-9783
- 1107 Ingle AP, Duran N, Rai M (2014) Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles:
1108 a review. *Appl Microbiol Biotechnol* 98:1001-1009
- 1109 Jarvi S, Gollan PJ, Aro EM (2013) Understanding the roles of the thylakoid lumen in photosynthesis regulation.
1110 *Front Plant Sci* 4:434
- 1111 Karami MS, De Lima R (2016) Nanoparticles cyto and genotoxicity in plants: mechanisms and abnormalities.
1112 *Environ Nanotechnol Monit Manage* 6:184-193
- 1113 Kasemets K, Ivask A, Dubourguier HC, Kahru A (2009) Toxicity of nanoparticles of ZnO, CuO and TiO₂ to yeast
1114 *Saccharomyces cerevisiae*. *Toxicol In Vitro* 23:1116-1122
- 1115 Katsumiti A, Thorley AJ, Arostegui I, Reip P, Valsami-Jones E, Tetley TD, Cajaraville MP (2018) Cytotoxicity and
1116 cellular mechanisms of toxicity of CuO NPs in mussel cells in vitro and comparative sensitivity with
1117 human cells. *Toxicol In Vitro* 48:146-158
- 1118 Keller AA, Adeleye AS, Conway JR, Garner KL, Zhao L, Cherr GN, Hong J, Gardea-Torresdey JL, Godwin HA,
1119 Hanna S, Ji Z, Kaweeteerawat C, Lin S, Lenihan HS, Miller RJ, Nel AE, Peralta-Videa JR, Walker SL,
1120 Taylor AA, Torres-Duarte C, Zink JI, Zuverza-Mena N (2017) Comparative environmental fate and
1121 toxicity of copper nanomaterials. *NanoImpact* 7:28-40
- 1122 Keller AA, Huang Y, Nelson J (2018) Detection of nanoparticles in edible plant tissues exposed to nano copper
1123 using single particle ICP-MS. *J Nanopart Res* 20:101
- 1124 Keller AA, Lazareva A (2013) Predicted releases of engineered nanomaterials: from global to regional to local.
1125 *Environ Sci Technol Lett* 1:65-70
- 1126 Keller AA, McFerran S, Lazareva A, Suh S (2013) Global life cycle releases of engineered nanomaterials. *J*
1127 *Nanopart Res* 15
- 1128 Kessler F, Vidi PA (2007) Plastoglobule lipid bodies: their functions in chloroplasts and their potential for
1129 applications. *Adv Biochem Eng Biotechnol* 107:153-72

- 1130 Kim S, Lee S, Lee I (2012) Alteration of phytotoxicity and oxidant stress potential by metal oxide nanoparticles in
1131 *Cucumis sativus*. *Water Air Soil Pollut* 223:2799-2806
- 1132 Kim S, Sin H, Lee S, Lee I (2013) Influence of metal oxide particles on soil enzyme activity and bioaccumulation of
1133 two plants. *J Microbiol Biotechnol* 23:1279-1286
- 1134 Kovacec E, Regvar M, van Elteren JT, Arcon I, Papp T, Makovec D, Vogel-Mikus K (2017) Biotransformation of
1135 copper oxide nanoparticles by the pathogenic fungus *Botrytis cinerea*. *Chemosphere* 180:178-185
- 1136 Kumar N, Shah V, Walker VK (2012) Influence of a nanoparticle mixture on an arctic soil community. *Environ*
1137 *Toxicol Chem* 31:131-135
- 1138 Kumbhakar DV, Datta AK, Mandal A, Das D, Gupta S, Ghosh B, Halder S, Dey S (2016) Effectivity of copper and
1139 cadmium sulphide nanoparticles in mitotic and meiotic cells of *Nigella sativa* L. (black cumin) – can
1140 nanoparticles act as mutagenic agents? *J Exp Nanosci* 11:823-839
- 1141 Laborda F, Bolea E, Jimenez-Lamana J (2014) Single particle inductively coupled plasma mass spectrometry: a
1142 powerful tool for nanoanalysis. *Anal Chem* 86:2270-2278
- 1143 Laborda F, Bolea E, Jimenez-Lamana J (2016) Single particle inductively coupled plasma mass spectrometry for the
1144 analysis of inorganic engineered nanoparticles in environmental samples. *Trends Environ Anal Chem* 9:15-
1145 23
- 1146 Laborda F, Jimenez-Lamana J, Bolea E, Castillo JR (2013) Critical considerations for the determination of
1147 nanoparticle number concentrations, size and number size distributions by single particle ICP-MS. *J Anal*
1148 *At Spectrom* 28:1220-1232
- 1149 Lalau CM, Mohedano Rde A, Schmidt EC, Bouzon ZL, Ouriques LC, dos Santos RW, da Costa CH, Vicentini DS,
1150 Matias WG (2015) Toxicological effects of copper oxide nanoparticles on the growth rate, photosynthetic
1151 pigment content, and cell morphology of the duckweed *Landoltia punctata*. *Protoplasma* 252:221-229
- 1152 Laratte B, Guillaume B (2014) Epistemic and methodological challenges of dynamic environmental assessment: a
1153 case-study with energy production from solar cells. *Key Eng Mater* 572:535-538
- 1154 Laratte B, Guillaume B, Kim J, Birregah B (2014) Modeling cumulative effects in life cycle assessment: the case of
1155 fertilizer in wheat production contributing to the global warming potential. *Sci Total Environ* 481:588-595
- 1156 Lee S, Chung H, Kim S, Lee I (2013) The genotoxic effect of ZnO and CuO nanoparticles on early growth of
1157 buckwheat, *Fagopyrum esculentum*. *Water Air Soil Pollut* 224
- 1158 Lee WM, An YJ, Yoon H, Kweon HS (2008) Toxicity and bioavailability of copper nanoparticles to the terrestrial
1159 plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): plant agar test for water-insoluble
1160 nanoparticles. *Environ Toxicol Chem* 27:1915-1921
- 1161 Letelier ME, Sanchez-Jofre S, Peredo-Silva L, Cortes-Troncoso J, Aracena-Parks P (2010) Mechanisms underlying
1162 iron and copper ions toxicity in biological systems: pro-oxidant activity and protein-binding effects. *Chem*
1163 *Biol Interact* 188:220-227
- 1164 Liu J, Dhungana B, Cobb GP (2018) Copper oxide nanoparticles and arsenic interact to alter seedling growth of rice
1165 (*Oryza sativa japonica*). *Chemosphere* 206:330-337
- 1166 Liu Q, Zhou D, Yamamoto Y, Ichino R, Okido M (2012) Preparation of Cu nanoparticles with NaBH₄ by aqueous
1167 reduction method. *Trans Nonferrous Met Soc China* 22:117-123
- 1168 Liu Y, Baas J, Peijnenburg WJ, Vijver MG (2016) Evaluating the combined toxicity of Cu and ZnO nanoparticles:
1169 utility of the concept of additivity and a nested experimental design. *Environ Sci Technol* 50:5328-5337
- 1170 Lofts S, Criel P, Janssen CR, Lock K, McGrath SP, Oorts K, Rooney CP, Smolders E, Spurgeon DJ, Svendsen C,
1171 Van Eeckhout H, Zhao FZ (2013) Modelling the effects of copper on soil organisms and processes using
1172 the free ion approach: towards a multi-species toxicity model. *Environ Pollut* 178:244-253
- 1173 Lowry GV, Gregory KB, Apte SC, Lead JR (2012) Transformations of nanomaterials in the environment. *Environ*
1174 *Sci Technol* 46:6893-6899
- 1175 Ma R, Stegemeier J, Levard C, Dale JG, Noack CW, Yang T, Brown GE, Lowry GV (2014) Sulfidation of copper
1176 oxide nanoparticles and properties of resulting copper sulfide. *Environ Sci Nano* 1:347-357
- 1177 Ma X, Geisler-Lee J, Deng Y, Kolmakov A (2010) Interactions between engineered nanoparticles (ENPs) and
1178 plants: phytotoxicity, uptake and accumulation. *Sci Total Environ* 408:3053-3061
- 1179 McLaren TI, Guppy CN, Tighe MK (2012) A rapid and nondestructive plant nutrient analysis using portable X-ray
1180 fluorescence. *Soil Sci Soc Am J* 76:1446-1453
- 1181 Manceau A, Nagy KL, Marcus MA, Lanson M, Geoffroy N, Jacquet T, Kirpichtchikova T (2008) Formation of
1182 metallic copper nanoparticles at the soil-root interface. *Environ Sc Technol* 42:1766-1772
- 1183 Maness PC, Smolinski S, Blake DM, Huang Z, Wolfrum EJ, Jacoby WA (1999) Bactericidal activity of
1184 photocatalytic TiO₂ reaction: toward an understanding of its killing mechanism. *Appl Environ Microbiol*
1185 65:4094-4098
- 1186 Manusadzianas L, Caillet C, Fachetti L, Gylte B, Grigutyte R, Jurkoniene S, Karitonas R, Sadauskas K, Thomas F,
1187 Vitkus R, Ferard JF (2012) Toxicity of copper oxide nanoparticle suspensions to aquatic biota. *Environ*
1188 *Toxicol Chem* 31:108-114
- 1189 Mashock MJ, Zanon T, Kappell AD, Petrella LN, Andersen EC, Hristova KR (2016) Copper oxide nanoparticles
1190 impact several toxicological endpoints and cause neurodegeneration in *Caenorhabditis elegans*. *PLoS One*
1191 11:e0167613

- 1192 Melegari SP, Perreault F, Costa RH, Popovic R, Matias WG (2013) Evaluation of toxicity and oxidative stress
1193 induced by copper oxide nanoparticles in the green alga *Chlamydomonas reinhardtii*. *Aquat Toxicol* 142-
1194 143:431-440
- 1195 Miao L, Wang C, Hou J, Wang P, Ao Y, Li Y, Lv B, Yang Y, You G, Xu Y (2015) Enhanced stability and
1196 dissolution of CuO nanoparticles by extracellular polymeric substances in aqueous environment. *J*
1197 *Nanopart Res* 17
- 1198 Miller RJ, Muller EB, Cole B, Martin T, Nisbet R, Bielmyer-Fraser GK, Jarvis TA, Keller AA, Cherr G, Lenihan
1199 HS (2017) Photosynthetic efficiency predicts toxic effects of metal nanomaterials in phytoplankton. *Aquat*
1200 *Toxicol* 183:85-93
- 1201 Montes M, Pierce CG, Lopez-Ribot JL, Bhalla AS, Guo RY (2016) Properties of silver and copper nanoparticle
1202 containing aqueous suspensions and evaluation of their In vitro activity against *Candida albicans* and
1203 *Staphylococcus aureus* biofilms. *J Nano Res* 37:109-121
- 1204 Moon YS, Park ES, Kim TO, Lee HS, Lee SE (2014) SELDI-TOF MS-based discovery of a biomarker in *Cucumis*
1205 *sativus* seeds exposed to CuO nanoparticles. *Environ Toxicol Pharmacol* 38:922-931
- 1206 Moore MN (2006) Do nanoparticles present ecotoxicological risks for the health of the aquatic environment?
1207 *Environ Inter* 32:967-976
- 1208 Mosa KA, El-Naggar M, Ramamoorthy K, Alawadhi H, Elnaggar A, Wartanian S, Ibrahim E, Hani H (2018)
1209 Copper nanoparticles induced genotoxicity, oxidative stress, and changes in superoxide dismutase (SOD)
1210 gene expression in cucumber (*Cucumis sativus*) plants. *Front Plant Sci* 9
- 1211 Mudunkotuwa IA, Pettibone JM, Grassian VH (2012) Environmental implications of nanoparticle aging in the
1212 processing and fate of copper-based nanomaterials. *Environ Sci Technol* 46:7001-7010
- 1213 Mukherjee A, Peralta-Videa JR, Gardea-Torresdey J (2016) Effects and Uptake of Nanoparticles in Plants. In
1214 "Engineered Nanoparticles and the Environment: Biophysicochemical Processes and Toxicity" (C.D.V.
1215 Baoshan Xing, Nicola Senesi, ed.), pp. 386-408. John Wiley & Sons
- 1216 Mukherjee K, Acharya K (2018) Toxicological effect of metal oxide nanoparticles on soil and aquatic habitats. *Arch*
1217 *Environ Contam Toxicol* 75:175-186
- 1218 Musante C, White JC (2012) Toxicity of silver and copper to Cucurbita pepo: differential effects of nano and bulk-
1219 size particles. *Environ Toxicol* 27:510-517
- 1220 Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. *Environ*
1221 *Chem Letters* 8:199-216
- 1222 Nagaonkar D, Shende S, Rai M (2015) Biosynthesis of copper nanoparticles and its effect on actively dividing cells
1223 of mitosis in *Allium cepa*. *Biotechnol Prog* 31:557-565
- 1224 Nair PM, Chung IM (2014) A mechanistic study on the toxic effect of copper oxide nanoparticles in soybean
1225 (*Glycine max* L.) root development and lignification of root cells. *Biol Trace Elem Res* 162:342-352
- 1226 Nair PM, Chung IM (2015a) Study on the correlation between copper oxide nanoparticles induced growth
1227 suppression and enhanced lignification in Indian mustard (*Brassica juncea* L.). *Ecotoxicol Environ Saf*
1228 113:302-313
- 1229 Nair PMG, Chung IM (2015b) The responses of germinating seedlings of green peas to copper oxide nanoparticles.
1230 *Biologia Plantarum* 59:591-595
- 1231 Nekrasova GF, Ushakova OS, Ermakov AE, Uimin MA, Byzov IV (2011) Effects of copper(II) ions and copper
1232 oxide nanoparticles on *Elodea densa* Planch. *Russ J Ecol* 42:458-463
- 1233 Nhan Le V, Ma C, Shang J, Rui Y, Liu S, Xing B (2016) Effects of CuO nanoparticles on insecticidal activity and
1234 phytotoxicity in conventional and transgenic cotton. *Chemosphere* 144:661-670
- 1235 Ojha NK, Zyryanov GV, Majee A, Charushin VN, Chupakhin ON, Santra S (2017) Copper nanoparticles as
1236 inexpensive and efficient catalyst: A valuable contribution in organic synthesis. *Corros Rev Corrosion*
1237 *Reviews* 353:1-57
- 1238 Olkhovych O, Volkogon M, Taran N, Batsmanova L, Kravchenko I (2016) The effect of copper and zinc
1239 nanoparticles on the growth parameters, contents of ascorbic acid, and qualitative composition of amino
1240 acids and acylcarnitines in *Pistia stratiotes* L. (Araceae). *Nanoscale Res Lett* 11:218
- 1241 Owens JW (2006) Life-cycle assessment in relation to risk assessment: an evolving perspective. *Risk Anal* 17:359-
1242 365
- 1243 Parada J, Rubilar O, Diez MC, Cea M, da Silva ASA, Rodríguez-Rodríguez CE, Tortella GR (2019) Combined
1244 pollution of copper nanoparticles and atrazine in soil: Effects on dissipation of the pesticide and on
1245 microbiological community profiles. *J Hazard Mater* 361:228-236
- 1246 Parra B, Tortella GR, Cuzzo S, Martínez M (2019) Negative effect of copper nanoparticles on the conjugation
1247 frequency of conjugative catabolic plasmids. *Ecotoxicol Environ Saf* 169:662-668
- 1248 Peng C, Duan D, Xu C, Chen Y, Sun L, Zhang H, Yuan X, Zheng L, Yang Y, Yang J, Zhen X, Chen Y, Shi J (2015)
1249 Translocation and biotransformation of CuO nanoparticles in rice (*Oryza sativa* L.) plants. *Environ Pollut*
1250 197:99-107
- 1251 Peng C, Xu C, Liu Q, Sun L, Luo Y, Shi J (2017) Fate and transformation of CuO nanoparticles in the soil-rice
1252 system during the life cycle of rice plants. *Environ Sci Technol* 51:4907-4917

- 1253 Perreault F, Oukarroum A, Melegari SP, Matias WG, Popovic R (2012) Polymer coating of copper oxide
1254 nanoparticles increases nanoparticles uptake and toxicity in the green alga *Chlamydomonas reinhardtii*.
1255 *Chemosphere* 87:1388-1394
- 1256 Perreault F, Popovic R, Dewez D (2014) Different toxicity mechanisms between bare and polymer-coated copper
1257 oxide nanoparticles in *Lemna gibba*. *Environ Pollut* 185:219-227
- 1258 Philippe A, Schaumann GE (2014) Interactions of dissolved organic matter with natural and engineered inorganic
1259 colloids: a review. *Environ Sci Technol* 48:8946-8962
- 1260 Ponmurugan P, Manjugarunambika K, Elango V, Gnanamangai BM (2016) Antifungal activity of biosynthesised
1261 copper nanoparticles evaluated against red root-rot disease in tea plants. *J Exp Nanosci* 11:1019-1031
- 1262 Pradhan A, Seena S, Pascoal C, Cassio F (2011) Can metal nanoparticles be a threat to microbial decomposers of
1263 plant litter in streams? *Microb Ecol* 62:58-68
- 1264 Pradhan A, Seena S, Pascoal C, Cassio F (2012) Copper oxide nanoparticles can induce toxicity to the freshwater
1265 shredder *Allogamus ligonifer*. *Chemosphere* 89:1142-1150
- 1266 Pradhan A, Seena S, Schlosser D, Gerth K, Helm S, Dobritzsch M, Krauss GJ, Dobritzsch D, Pascoal C, Cassio F
1267 (2015) Fungi from metal-polluted streams may have high ability to cope with the oxidative stress induced
1268 by copper oxide nanoparticles. *Environ Toxicol Chem* 34:923-930
- 1269 Pullagurala VLR, Rawat S, Adisa IO, Hernandez-Viezcas JA, Peralta-Videa JR, Gardea-Torresdey JL (2018) Plant
1270 uptake and translocation of contaminants of emerging concern in soil. *Sci Total Environ* 636:1585-1596
- 1271 Qiu H, Smolders, E (2017) Nanospecific phytotoxicity of CuO nanoparticles in soils disappeared when
1272 bioavailability factors were considered. *Environ Sci Technol* 51:11976-11985
- 1273 Rajput V, Minkina T, Fedorenko A, Sushkova S, Mandzhieva S, Lysenko V, Duplii N, Fedorenko G, Dvadenko K,
1274 Ghazaryan K (2018a) Toxicity of copper oxide nanoparticles on spring barley (*Hordeum sativum*
1275 *distichum*). *Sci Total Environ* 645:1103-1113
- 1276 Rajput VD, Chen Y, Ayup M (2015) Effects of high salinity on physiological and anatomical indices in the early
1277 stages of *Populus euphratica* growth. *Russ J Plant Physiol* 62:229-236
- 1278 Rajput VD, Minkina T, Fedorenko A, Tsitsuashvili V, Mandzhieva S, Sushkova S, Azarov A (2018b) Metal oxide
1279 nanoparticles: Applications and effects on soil ecosystems. In "Soil Contamination: Sources, Assessment
1280 and Remediation", pp. 81-106
- 1281 Rajput VD, Minkina T, Sushkova S, Tsitsuashvili V, Mandzhieva S, Gorovtsov A, Nevidomskaya D, Gromakova
1282 N (2017a) Effect of nanoparticles on crops and soil microbial communities. *J Soils Sediments* 18:2179-
1283 2187
- 1284 Rajput VD, Minkina T, Sushkova S, Mandzhieva S, Tsitsuashvili V, Chaplgin V, Fedorenko A (2017b) Effects of
1285 copper nanoparticles (CuO NPs) on crop plants: a mini review. *BioNanoSci* 8:36-42
- 1286 Rajput VD, Minkina TM, Behal A, Sushkova SN, Mandzhieva S, Singh R, Gorovtsov A, Tsitsuashvili VS, Purvis
1287 WO, Ghazaryan KA, Movsesyan HS (2018c) Effects of zinc-oxide nanoparticles on soil, plants, animals
1288 and soil organisms: a review. *Environ Nanotechnol Monit* 9:76-84
- 1289 Rajput VD, Minkina T, Fedorenko A, Mandzhieva S, Sushkova S, Lysenko V, Duplii N, Azarov A, Chokheli V
1290 (2018d) Destructive effect of copper oxide nanoparticles on ultrastructure of chloroplast, plastoglobules
1291 and starch grains in spring barley (*Hordeum sativum distichum*). *Int J Agric Biol*
1292 <https://doi.org/10.17957/IJAB/15.0877>
- 1293 Raliya R, Franke C, Chavalmane S, Nair R, Reed N, Biswas P (2016) Quantitative understanding of nanoparticle
1294 uptake in watermelon plants. *Front Plant Sci* 7:1288
- 1295 Rastogi A, Zivcak M, Sytar O, Kalaji HM, He X, Mbarki S, Brestic M (2017) Impact of metal and metal oxide
1296 nanoparticles on plant: a critical review. *Front Chem* 5:78
- 1297 Ray D, Pramanik S, Prasad MR, Chaudhuri S, De, S (2015) Sugar-mediated 'green' synthesis of copper
1298 nanoparticles with high antifungal activity. *Mater Res Express* 2:105002
- 1299 Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresdey JL (2011) Interaction of
1300 nanoparticles with edible plants and their possible implications in the food chain. *J Agric Food Chem*
1301 59:3485-3498
- 1302 Rico CM, Peralta-Videa JR, Gardea-Torresdey JL (2015) Differential effects of cerium oxide nanoparticles on rice,
1303 wheat, and barley roots: a fourier transform infrared (FT-IR) microspectroscopy study. *Appl Spectrosc*
1304 69:287-295
- 1305 Rottet S, Besagni C, Kessler F (2015) The role of plastoglobules in thylakoid lipid remodeling during plant
1306 development. *Biochim Biophys Acta* 1847:889-899
- 1307 Rui M, Ma C, White JC, Hao Y, Wang Y, Tang X, Yang J, Jiang F, Ali A, Rui Y, Cao W, Chen G, Xing B (2018)
1308 Metal oxide nanoparticles alter peanut (*Arachis hypogaea* L.) physiological response and reduce nutritional
1309 quality: a life cycle study. *Environ Sci-Nano* 5:2088-2102
- 1310 Ruiz P, Katsumiti A, Nieto JA, Bori J, Jimeno-Romero A, Reip P, Arostegui I, Orbea A, Cajaraville MP (2015)
1311 Short-term effects on antioxidant enzymes and long-term genotoxic and carcinogenic potential of CuO
1312 nanoparticles compared to bulk CuO and ionic copper in mussels *Mytilus galloprovincialis*. *Mar Environ*
1313 *Res* 111:107-120

- 1314 Saleem S, Ahmed B, Khan MS, Al-Shaeri M, Musarrat J (2017) Inhibition of growth and biofilm formation of
1315 clinical bacterial isolates by NiO nanoparticles synthesized from *Eucalyptus globulus* plants. *Microb*
1316 *Pathog* 111:375-387
- 1317 Sedighi A, Montazer M (2016) Tunable shaped N-doped CuO nanoparticles on cotton fabric through processing
1318 conditions: synthesis, antibacterial behavior and mechanical properties. *Cellulose* 23:2229-2243
- 1319 Servin AD, De la Torre-Roche R, Castillo-Michel H, Pagano L, Hawthorne J, Musante C, Pignatello J, Uchimiya M,
1320 White JC (2017a) Exposure of agricultural crops to nanoparticle CeO₂ in biochar-amended soil. *Plant*
1321 *Physiol Biochem* 110:147-157
- 1322 Servin AD, Pagano L, Castillo-Michel H, De la Torre-Roche R, Hawthorne J, Hernandez-Viezcás JA, Loredó-
1323 Portales R, Majumdar S, Gardea-Torresday J, Dhankher OP, White JC (2017b) Weathering in soil
1324 increases nanoparticle CuO bioaccumulation within a terrestrial food chain. *Nanotoxicology* 11:98-111
- 1325 Shah V, Luxton TP, Walker VK, Brumfield T, Yost J, Shah S, Wilkinson JE, Kambhampati M (2016) Fate and
1326 impact of zero-valent copper nanoparticles on geographically-distinct soils. *Sci Total Environ* 573:661-670
- 1327 Shahid M, Saghir KM (2017) Assessment of glyphosate and quizalofop mediated toxicity to greengram (*Vigna*
1328 *radiata* (L.) Wilczek), stress abatement and growth promotion by herbicide tolerant bradyrhizobium and
1329 pseudomonas species. *Int J Curr Microbiol Appl Sci* 6:3001-3016
- 1330 Shaw AK, Ghosh S, Kalaji HM, Bosa K, Brestic M, Zivcak M, Hossain Z (2014) Nano-CuO stress induced
1331 modulation of antioxidative defense and photosynthetic performance of Syrian barley (*Hordeum vulgare*
1332 L.). *Environ Exp Bot* 102:37-47
- 1333 Shaw AK, Hossain Z (2013) Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings. *Chemosphere* 93:906-
1334 915
- 1335 Shaw BJ, Al-Bairuty G, Handy RD (2012) Effects of waterborne copper nanoparticles and copper sulphate on
1336 rainbow trout, (*Oncorhynchus mykiss*): physiology and accumulation. *Aquat Toxicol* 116-117:90-101
- 1337 Shi J, Ye J, Fang H, Zhang S, Xu C (2018) Effects of copper oxide nanoparticles on paddy soil properties and
1338 components. *Nanomaterials* 8(10):839
- 1339 Simonin M, Richaume A (2015) Impact of engineered nanoparticles on the activity, abundance, and diversity of soil
1340 microbial communities: a review. *Environ Sci Pollut Res Int* 22:13710-13723
- 1341 Simonin M, Cantarel AA, Crouzet A, Gervais J, Martins JM, Richaume A (2018) Negative Effects of copper oxide
1342 nanoparticles on carbon and nitrogen cycle microbial activities in contrasting agricultural soils and in
1343 presence of plants. *Front microbiol* 9:3102
- 1344 Slotte M, Zevenhoven R (2017) Energy requirements and life cycle assessment of production and product
1345 integration of silver, copper and zinc nanoparticles. *J Clean Prod* 148:948-957
- 1346 Song G, Hou W, Gao Y, Wang Y, Lin L, Zhang Z, Niu Q, Ma R, Mu L, Wang H (2016) Effects of CuO
1347 nanoparticles on *Lemna minor*. *Bot Stud* 57:3
- 1348 Song L, Vijver MG, Peijnenburg WJ (2015a) Comparative toxicity of copper nanoparticles across three Lemnaceae
1349 species. *Sci Total Environ* 518-519:217-224
- 1350 Song L, Vijver MG, Peijnenburg WJ, Galloway TS, Tyler CR (2015b) A comparative analysis on the in vivo
1351 toxicity of copper nanoparticles in three species of freshwater fish. *Chemosphere* 139:181-189
- 1352 Stampoulis D, Sinha SK, White JC (2009) Assay-dependent phytotoxicity of nanoparticles to plants. *Environ Sci*
1353 *Technol* 43:9473-9479
- 1354 Sun L, Yang J, Fang H, Xu C, Peng C, Huang H, Lu L, Duan D, Zhang X, Shi J (2017) Mechanism study of sulfur
1355 fertilization mediating copper translocation and biotransformation in rice (*Oryza sativa* L.) plants. *Environ*
1356 *Pollut* 226:426-434
- 1357 Sun Y, Zhang G, He Z, Wang Y, Cui J, Li Y (2016) Effects of copper oxide nanoparticles on developing zebrafish
1358 embryos and larvae. *Int J Nanomedicine* 11:905-918
- 1359 Tan HY, Verbeeck J, Abakumov A, Van Tendeloo G (2012) Oxidation state and chemical shift investigation in
1360 transition metal oxides by EELS. *Ultramicroscopy* 116:24-33
- 1361 Thit A, Selck H, Bjerregaard HF (2013) Toxicity of CuO nanoparticles and Cu ions to tight epithelial cells from
1362 *Xenopus laevis* (A6): effects on proliferation, cell cycle progression and cell death. *Toxicol In Vitro*
1363 27:1596-1601
- 1364 Tighe-Neira R, Carmora E, Recio G, Nunes-Nesi A, Reyes-Diaz M, Alberdi M, Rengel Z, Inostroza-Blancheteau C
1365 (2018) Metallic nanoparticles influence the structure and function of the photosynthetic apparatus in plants.
1366 *Plant Physiol Biochem* 130:408-417
- 1367 Torres-Duarte C, Adeleye AS, Pokhrel S, Madler L, Keller AA, Cherr GN (2016) Developmental effects of two
1368 different copper oxide nanomaterials in sea urchin (*Lytechinus pictus*) embryos. *Nanotoxicology* 10:671-
1369 679
- 1370 Trujillo-Reyes J, Majumdar S, Botez CE, Peralta-Videa JR, Gardea-Torresday JL (2014) Exposure studies of core-
1371 shell Fe/Fe(3)O(4) and Cu/CuO NPs to lettuce (*Lactuca sativa*) plants: are they a potential physiological
1372 and nutritional hazard? *J Hazard Mater* 267:255-263
- 1373 Towett EK, Shepherd KD, Drake BL (2016) Plant elemental composition and portable X-ray fluorescence (pXRF)
1374 spectroscopy: quantification under different analytical parameters X-ray. *Spectrometry* 45:117-124
- 1375 Van NL, Ma C, Shang J, Rui Y, Liu S, Xing B (2016) Effects of CuO nanoparticles on insecticidal activity and
1376 phytotoxicity in conventional and transgenic cotton. *Chemosphere* 144:661-670

- 1377 Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF, JrRejeski D, Hull MS (2015) Nanotechnology in
1378 the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein J Nanotechnol*
1379 6:1769-1780
- 1380 VandeVoort A, Arai Y (2018) Macroscopic observation of soil nitrification kinetics impacted by copper
1381 nanoparticles: implications for micronutrient nanofertilizer. *Nanomaterials* 8(11):927
- 1382 Vicario-Pares U, Lacave JM, Reip P, Cajaraville MP, Orbea A (2018) Cellular and molecular responses of adult
1383 zebrafish after exposure to CuO nanoparticles or ionic copper. *Ecotoxicology* 27:89-101
- 1384 Vishnevetsky M, Ovadis M, Vainstein A (1999) Carotenoid sequestration in plants: the role of carotenoid-associated
1385 proteins. *Trends Plant Sci* 4:232-235
- 1386 Wang S, Li Z, Gao M, She Z, Ma B, Guo L, Zheng D, Zhao Y, Jin C, Wang X, Gao F (2017) Long-term effects of
1387 cupric oxide nanoparticles (CuO NPs) on the performance, microbial community and enzymatic activity of
1388 activated sludge in a sequencing batch reactor. *J Environ Manage* 187:330-339
- 1389 Wang T, Long X, Cheng Y, Liu Z, Yan S (2014) The potential toxicity of copper nanoparticles and copper sulphate
1390 on juvenile *Epinephelus coioides*. *Aquat Toxicol* 152:96-104
- 1391 Wang Z, Li J, Zhao J, Xing B (2011) Toxicity and internalization of CuO nanoparticles to prokaryotic alga
1392 *Microcystis aeruginosa* as affected by dissolved organic matter. *Environ Sci Technol* 45:6032-6040
- 1393 Wang Z, von dem Bussche A, Kabadi PK, Kane AB, Hurt RH (2013) Biological and environmental transformations
1394 of copper-based nanomaterials. *ACS Nano* 7:8715-8727
- 1395 Wang Z, Xie X, Zhao J, Liu X, Feng W, White JC, Xing B (2012). Xylem- and phloem-based transport of CuO
1396 nanoparticles in maize (*Zea mays* L.). *Environ Sci Technol* 46:4434-4441
- 1397 Wang Z, Xu L, Zhao J, Wang X, White JC, Xing B (2016) CuO nanoparticle interaction with *Arabidopsis thaliana*:
1398 toxicity, parent-progeny transfer, and gene expression. *Environ Sci Technol* 50:6008-6016
- 1399 Woo-Mi L, Youn-Joo A, Hyeon Y, Hee-Seok K (2008) Toxicity and bioavailability of copper nanoparticles to the
1400 terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): Plant agar test for water-
1401 insoluble nanoparticles. *Environ Toxicol Chem* 27:1915-1921
- 1402 Xiong T, Dumat C, Dappe V, Vezin H, Schreck E, Shahid M, Pierart A, Sobanska S (2017) Copper oxide
1403 nanoparticle foliar uptake, phytotoxicity, and consequences for sustainable urban agriculture. *Environ Sci*
1404 *Technol* 51:5242-5251
- 1405 Xiong ZT, Wang H (2005) Copper toxicity and bioaccumulation in Chinese cabbage (*Brassica pekinensis* Rupr.).
1406 *Environ Toxicol* 20:188-194
- 1407 Xu C, Peng C, Sun L, Zhang S, Huang H, Chen Y, Shi J (2015) Distinctive effects of TiO₂ and CuO nanoparticles
1408 on soil microbes and their community structures in flooded paddy soil. *Soil Biol Biochem* 86:24-33
- 1409 You T, Liu D, Chen J, Yang Z, Dou R, Gao X, Wang L (2017) Effects of metal oxide nanoparticles on soil enzyme
1410 activities and bacterial communities in two different soil types. *J Soils Sediments* 18:211-221
- 1411 Yruela I (2009) Copper in plants: acquisition, transport and interactions. *Funct Plant Biol* 36:409-430
- 1412 Yu S, Liu J, Yin Y, Shen M (2018). Interactions between engineered nanoparticles and dissolved organic matter: A
1413 review on mechanisms and environmental effects. *J Environ Sci (China)* 63:198-217
- 1414 Yuan J, He A, Huang S, Hua J, Sheng GD (2016) Internalization and phytotoxic effects of CuO nanoparticles in
1415 *Arabidopsis thaliana* as revealed by fatty acid profiles. *Environ Sci Technol* 50:10437-10447
- 1416 Zakharova OV et al. (2018) Biological effects of freshly prepared and 24-h aqueous dispersions of copper and
1417 copper oxide nanoparticles on *E. coli* bacteria. *Nanotechnol Russ* 13.3-4:173-181
- 1418 Zhang Z, Ke M, Qu Q, Peijnenburg W, Lu T, Zhang Q, Ye Y, Xu P, Du B, Sun L, Qian H (2018) Impact of copper
1419 nanoparticles and ionic copper exposure on wheat (*Triticum aestivum* L.) root morphology and antioxidant
1420 response. *Environ Pollut* 239:689-697
- 1421 Zhao J, Ren W, Dai Y, Liu L, Wang Z, Yu X, Zhang J, Wang X, Xing B (2017a) Uptake, distribution, and
1422 transformation of CuO NPs in a floating plant *Eichhornia crassipes* and related stomatal responses.
1423 *Environ Sci Technol* 51:7686-7695
- 1424 Zhao L, Hu J, Huang Y, Wang H, Adeleye A, Ortiz C, Keller AA (2017b) NMR and GC-MS based metabolomics
1425 reveal nano-Cu altered cucumber (*Cucumis sativus*) fruit nutritional supply. *Plant Physiol Biochem*
1426 110:138-146
- 1427 Zhao L, Huang Y, Zhou H, Adeleye AS, Wang H, Ortiz C, Mazer SJ, Keller AA (2016a) GC-TOF-MS based
1428 metabolomics and ICP-MS based metallomics of cucumber (*Cucumis sativus*) fruits reveal alteration of
1429 metabolites profile and biological pathway disruption induced by nano copper. *Environ Sci-Nano* 3:1114-
1430 1123
- 1431 Zhao L, Ortiz C, Adeleye AS, Hu Q, Zhou H, Huang Y, Keller AA (2016b) Metabolomics to detect response of
1432 lettuce (*Lactuca sativa*) to Cu(OH)₂ nanopesticides: oxidative stress response and detoxification
1433 mechanisms. *Environ Sci Technol* 50:9697-9707
- 1434 Zhu Y, Xu J, Lu T, Zhang M, Ke M, Fu Z, Pan X, Qian H (2017) A comparison of the effects of copper
1435 nanoparticles and copper sulfate on *Phaeodactylum tricornutum* physiology and transcription. *Environ*
1436 *Toxicol Pharmacol* 56:43-49
- 1437 Zuverza-Mena N, Medina-Velo IA, Barrios AC, Tan W, Peralta-Videa JR, Gardea-Torresdey JL (2015) Copper
1438 nanoparticles/compounds impact agronomic and physiological parameters in cilantro (*Coriandrum*
1439 *sativum*). *Environ Sci Process Impacts* 17:1783-1793