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Review

Nanoscale copper in the soil–plant system – toxicity and underlying potential mechanisms

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ABSTRACT

Nanoscale copper particles (nano-Cu) are used in many antimicrobial formulations and products for their antimicrobial activity. They may enter deliberately and/or accidentally into terrestrial environments including soils. Being the major 'eco-receptors' of nanoscale particles in the terrestrial ecosystem, soil–microbiota and plants (the soil–plant system) have been used as a model to dissect the potential impact of these particles on the environmental and human health. In the soil–plant system, the plant can be an indirect non-target organism of the soil-associated nano-Cu that may in turn affect plant-based products and their consumers. By all accounts, information pertaining to nano-Cu toxicity and the underlying potential mechanisms in the soil–plant system remains scanty, deficient and little discussed. Therefore, based on some recent reports from (bio)chemical, molecular and genetic studies of nano-Cu versus soil–plant system, this article: (i) overviews the status, chemistry and toxicity of nano-Cu in soil and plants, (ii) discusses critically the poorly understood potential mechanisms of nano-Cu toxicity and tolerance both in soil–microbiota and plants, and (iii) proposes future research directions. It appears from studies hitherto made that the uncontrolled generation and inefficient metabolism of reactive oxygen species through different reactions are the major factors underpinning the overall nano-Cu consequences in both the systems. However, it is not clear whether the nano-Cu or the ion released from it is the cause of the toxicity. We advocate to intensify the multi-approach studies focused at a complete characterization of the nano-Cu, its toxicity (during life cycles of the least-explored soil–microbiota and plants), and behavior in an environmentally relevant terrestrial exposure setting. Such studies may help to obtain a deeper insight into nano-Cu actions and address adequately the nano-Cu-associated safety concerns in the 'soil–plant system'.

1. Introduction

1.1. Background

Nanoparticles are ultrafine hard or soft materials with dimensions measured in nanometers (nm; billionths of a meter). Although they exist naturally in the environment, they can also be produced/ engineered intentionally (reviewed by Bhatt and Tripathi, 2011). Thus, compared to non-nanoscale particles (with the same chemical composition), the engineered nanoparticles exhibit a unique characteristic dimension of 1–100 nm (Royal Society, 2004; US-NSTC, 2004; cited in Auffan et al., 2009). However, it has been advocated to consider primarily the size-dependent novel properties of nanoparticles (rather than particle size) when they are defined and/or studied in context with their role in the environment, health and safety issues (Auffan et al., 2009). Given the wide-range commercial, environmental and medical utility of nanoparticles, their production has reached the highest industrial scale. The global production of engineered nanoparticles was estimated to be 260,000–309,000 metric tons in the year 2010; of which about 8–28%, 0.4–7%, and 0.1–1.5% were estimated to end into soils, water bodies and atmosphere respectively (Keller et al., 2013). The world-wide production of Cu-based nanoparticles in

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particular was estimated to be ~200 t per year in 2010, and has since been is increasing (Keller et al., 2013). The rapidly increasing multifarious use of metallic nanoparticles in electronics, optics, textiles, medicine, cosmetics, food packaging, water-treatment technology, fuel cells, catalysts, biosensors and environmental remediation has necessitated evaluation of their impact on environmental, biotic (microbes/plants/animals) and human health (Handy et al., 2008; Gerloff et al., 2012; Piccinno et al., 2012). Once in the environment, nanoparticles may persist for long or be taken up by organisms and transferred between organisms of different trophic levels, act as an eco-toxicological hazard, and undergo biodegradation or bioaccumulation in the food chain (Handy et al., 2008; Jones and Grainger, 2009; Ma et al., 2010a; Anjum et al., 2013a; Keller et al., 2013; Conway et al., 2014). However, due to lack of information on their toxicity, behavior and fate even under laboratory conditions, it is not easy to estimate the severity of nanomaterial impacts on the ecosystem and human health. This is why the invisible pollution caused by nanoparticles (nano-pollution) is considered to be the most difficult type of pollution to manage and control (Gao et al., 2013).

1.2. Nanoscale copper and its toxicity in the soil–plant system

Nanoscale copper (hereafter termed as nano-Cu, if not specified) belongs to the metal-based nanometer materials. Nano-Cu particles of < 50 nm size are considered as a super hard material that does not exhibit the same malleability and ductility as the bulk Cu (Science Daily, 2014). Because of its ultrafine size, nano-Cu is widely used in the solar cells and lithium-ion batteries (Guo et al., 2002, 2009; Sau et al., 2010), lubricant oils (Liu et al., 2004), polymers/plastics, inks/ceramic pigments, gas sensors, catalysts and electronics (Li et al., 2007; Ebrahimnia-Bajestan et al., 2011). In particular, nano-CuO is being used increasingly in antimicrobial formulations and products because of its antimicrobial nature (Gabbay et al., 2006; Borkow et al., 2009; Abramova et al., 2013). Nano-Cu can be synthesized through a number of routes such as (i) chemical (Zhang et al., 2010; Liu et al., 2012), (ii) physical (Kim et al., 2006; Blosi et al., 2011) and (iii) biological (Ramanathan et al., 2011; Lee et al., 2013; Ingle et al., 2014). Detailed information on synthesis, characterization, growth mechanisms, fundamental properties, and applications of CuO nanostructures/materials can be found in Zhang et al. (2014) and Ananth et al. (2015). Owing to its multifarious uses (Ben-Sasson et al., 2014; Ingle et al., 2014) and high potential to enter the environmental compartments, such as soil (Chang et al., 2012; Xu et al., 2012), nano-CuO has been the major focus in bio-toxicity studies (Navarro et al., 2008; Aruoja et al., 2009; Dimkpa et al., 2011, 2012a, 2012b, 2012c, 2013; Bondarenko et al., 2013; Hu et al., 2014).

In the terrestrial ecosystem, the soil–microbiota and plants are among the major eco-receptors of nanoparticles; especially, the soil–microbial biomass serves as a pool of nutrients and is a sensitive indicator of microbial changes in soils (Atlas, 1984). Therefore, it is not surprising that the protection of soil–microbial biomass and diversity is one of the major challenges for a sustainable use of resources (Torsvik and Øvreås, 2002). In the 'soil–plant system', both soil and plants are closely linked, where a potential direct impact of soil-associated nanoparticles can harm plants, which may then affect consumers such as animals/humen (Anjum et al., 2013a) (Fig. 1). Thus, to understand the potential environmental impacts of manufactured nanoparticles, exploring the potential toxicity of nanoparticles in soil–microbiota (Dinesh et al., 2012; Frenk et al., 2013) and plants (Handy et al., 2008; Ma et al., 2010a) and unveiling of the mechanisms involved have become important. Although nano-CuO is not in the list of 'Organization for Economic Co-operation and Development (OECD)', studies on its potential toxicity to flora, fauna and environmental health have been emphasized upon (Saison et al., 2010; Buffet et al., 2011). However, in contrast to the huge amount of research done on the bulk chemicals, as environmental hazard, the research on nanoparticles toxicity is markedly meager (Oberdörster et al., 2007; Kahru and Ivask, 2013; Hu et al., 2014), and whatever little has been done on nano-Cu types (such as nano-CuO), is confined mainly to animal system (Fahmy and Cormier, 2009; Griffitt et al., 2009; Gomes et al., 2011). Further, the studies available on the effect of nano-Cu on terrestrial plants (including agricultural crops) as the test model (Dimkpa et al., 2012a, 2012b, 2012c, 2012d; Wang et al., 2012; Hawthorne et al., 2012; Shaw and Hossain, 2013), rarely encompass the status and fate of nano-Cu and its impact on the soil–plant system.

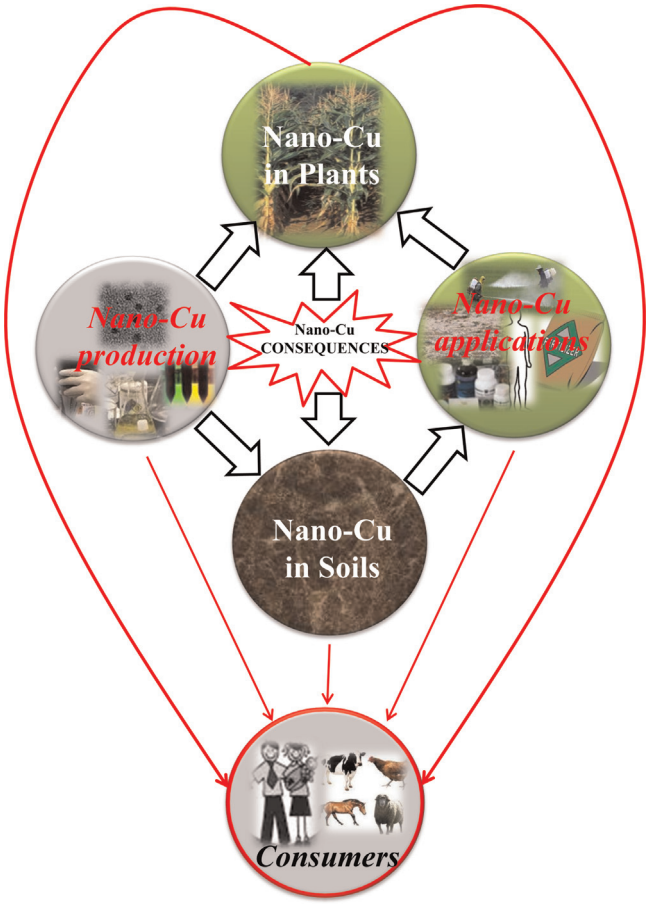

Fig. 1. Schematic diagram highlighting the interrelationships among nanoparticle production, its applicability and entry into 'soil–plant system' and potential consumers.

In order to expand safely the multidisciplinary use of nano-Cu, and considering the recent information obtained through (bio) chemical, molecular and genetic studies, this paper (a) overviews the nano-Cu status and chemistry in soil and plants, (b) discusses critically the poorly understood mechanisms of nano-Cu toxicity and tolerance in soil–microbiota and plants, and (c) suggests prospective research directions. The discussion is expected to enhance our current understanding of nano-Cu in the soil–plant system, and elucidate the road to future research on this specific subject.

2. Nanoscale copper in the soil — with focus on soil–microbiota

Soil sustains the plant and animal productivity, maintains the water and air quality, and supports human health and habitation

(Karlen et al., 2003). Exposure modeling suggests that the soil could be a major sink of engineered nanoparticles released into the environment and that their concentrations in soils would be higher than in water or air (Boxall et al., 2007; Klaine et al., 2008; Shah and Belozerova, 2009; Dinesh et al., 2012). Notably, deliberate releases (*via* soil and water remediation technologies), potential agricultural uses (such as fertilizers), and also unintentional releases (*via* air, water and sewage sludge) may enhance the background concentration of Cu and/or nano-Cu (Peralta-Videa et al., 2009; Trujillo-Reyes et al., 2014). Thus, in addition to increasing geogenic level of nano-Cu in soils, their physico-chemical properties (size distribution, agglomeration and purity state, surface reactivity) may significantly cause several environmental and human health concerns (Gottschalk et al., 2013).

Extensive reports are available on the use of the nano-CuO- and nano-$CuCO_3$-based biocides for the protection of wood products against the fungi- and insect-caused biodegradation (reviewed by Evans et al., 2008). The North American wood preservation market has already captured 50% of the global market for wood preservatives, where the annual consumption of Cu salts was estimated to be 79,000 t (Vlosky 2006; Evans et al., 2008; Amorim and Scott-Fordsmand, 2012). Considering the above points and also due to expected global increase in the nano-Cu-based products (Keller et al., 2013), a direct exposure of nano-Cu to soils and the subsequent consequences therein cannot be ignored. In the terrestrial ecosystem, soil–microbiota, with its immense ecological significance, has been the subject of extensive ecotoxicological studies (Torsvik and Øvreås, 2002; Dinesh et al., 2012; Frenk et al., 2013). Further, since a close association exists between plants and soil–microbiota, any change in the status of microbiota of plant–rhizosphere can impact the growth, development and productivity of plants (Kamnev, 2008). Considering the presence of nanoparticles in the soil, interests in studies on the nanoparticles exposure effects on soil microbes, 'non-target' organisms (Bondarenko et al., 2013) and 'plant–microbe interactions' has gone high (Frenk et al., 2013). However, soil-disease suppression, an indicator of the soil health (Janvier et al., 2007), and the consequent potential impacts on trophic balances are particularly worrisome in view of the nanoparticle's impact on soil–microbiota (Suresh et al., 2013). Moreover, the information available on the mechanisms underlying the nanoparticle impact on the soil–microbial biomass is negligible (Dinesh et al., 2012; Jin et al., 2014). Thus, a critical appraisal of the major outcomes of the 'bacteria–nanoparticle–interaction' studies may help us get a greater insight into the potential impact of nanoparticles (such as nano-Cu) released into the ecosystem.

Microbial toxicity of nano-Cu has been reported extensively (Mahapatra et al., 2008; Wang et al., 2010, 2011; Baek and An, 2011). In most of the studies, Cu ions, released from the nano-CuO were considered as a major cause of nano-CuO lethality to both the pathogenic and beneficial bacteria (Gajjar et al., 2009; Dimkpa et al., 2011, 2012a; Gunawan et al., 2011). Information is scanty on the potential impact of sub-lethal levels of nano-CuO on the secondary metabolism, a driver of the fitness, survival, and benefit of bacteria to the environment (Dimkpa et al., 2012a, 2012b). A dose-dependent toxicity of nano-CuO to bacteria can be possible, where sub-lethal concentrations of nano-CuO can disrupt the bacterial metabolism (Dimkpa et al., 2012c). Nano-CuO particles (size: 80–160 nm) exhibit antibacterial activity against the plant-growth–promoting *Klebsiella pneumoniae*, *Pseudokirchneriella aeruginosa*, *Salmonella paratyphi* and *Shigella* strains (Mahapatra et al., 2008). The nano-CuO-mediated growth inhibition can also be possible in *Pseudomonas putida* (Gajjar et al., 2009), *P. chlororaphis* O6 (Dimkpa et al., 2012a, 2012b), *Bacillus subtilis* and *Streptococcus aureus* (Baek and An, 2011). The effect of engineered metal nanoparticles on the terrestrial microbial communities has been tested under laboratory conditions (Shah and Belozerova, 2009; Hänsch and Emmerling, 2010; Kumar et al., 2011), but the effect of nano-Cu on the soil–microbial community in pots under field conditions is little explored (Collins et al., 2012). Nonetheless, ample evidence indicates that nano-CuO toxicity to microbiological community is dependent on several factors such as incubation time, soil type, oxidation state and the extent of ion-release. In aqueous suspension, nano-CuO exhibits a low stability and tends to aggregate rapidly (Ben-Moshe et al., 2010). Once released to the environment, large quantities of nano-CuO are retained in the soil because of its low mobility (Ben-Moshe et al., 2013). Reports on the potential effects of nano-Cu or nano-CuO-contaminated soils on soil-microbes are contradictory. The impact of nano-Cu (nano-size activated Cu powder) on the bacterial community size (measured as colony forming units) was in significant (Shah and Belozerova, 2009). In contrast, a significant (40%) reduction in substrate utilization has been reported in Arctic soils exposed to nano-Cu (size: 20 nm) (Kumar et al., 2011). Siderophores are a significant part of the chemical communication between soil microbes and plants, and help in soil–microbe survival and interaction with other organisms and metals (Dimkpa et al., 2012c, 2012d). Thus, siderophore responses to nanoparticles can modulate the outcome of plant–microbe interactions (Dimkpa et al., 2012c, 2012d). Both the bulk CuO and Cu ions at concentrations equivalent to those released from nano-CuO were unable to modify the production of fluorescent siderophore pyoverdine (PVD) in *Pseudomona chlororaphis* O6 (Dimkpa et al., 2012b). However, the sub-lethal level of nano-CuO can impair expression of genes encoding proteins involved in the periplasm-located PVD maturation and modify the production of the fluorescent siderophore PVD (Dimkpa et al., 2012b). Compared to their ionic counterparts, nano-CuO particles can inhibit CH_4 oxidation activity in tropical agricultural soil namely vertisol (soils with high clay and a moisture content-controlled shrinking and swelling property) (Mohanty et al., 2014) (Table 1).

2.1. Potential mechanisms underlying the nanoscale copper toxicity

Bacteria-based tests are significant in the assessment of environmental fate and potential ecological toxicity of manufactured nanoparticles (reviewed by Holden et al., 2014). Despite the availability of a well-done documentation of the antibacterial effect of nano-Cu (Heinlaan et al., 2008; Ruparelia et al., 2008; Gajjar et al., 2009), information on basic mechanisms underlying the nanoparticle mode of action and differential bacterial cell-killing potential is very limited (Deryabin et al., 2013; Chatterjee et al., 2014). Oxidative stress *via* elevated reactive oxygen species (ROS) generation (Kohen and Nyska, 2002), and the nanoscale particle-photosensitivity (Jiang et al., 2009) have been considered as the main mechanisms of nanoscale particle toxicity, which damages lipids, carbohydrates, proteins and DNA (Kelly et al., 1998). Being a transition metal, Cu is involved in ROS generation *via* the Fenton ($Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{\bullet} + OH^{-}$) or the Haber–Weiss ($H_2O_2 + O_2^{\bullet -} \rightarrow OH^{\bullet} + OH^{-} + O_2$) reactions (Letelier et al., 2010). The bactericidal potential of these ROS is estimated as being $OH^{\bullet} > O_2^{-} > H_2O_2$. They cause significant impairments to the cell-membrane architecture (such as the loss of respiratory activity) *via* lipid peroxidation (LPO), leading to alterations in cell-membrane properties, which in turn disrupt the vital cellular functions (Maness et al., 1999). ROS can impact the activity of metallo-enzymes and damage the integrity of DNA in *E. coli* (reviewed by Imlay 2013). The bacterial susceptibility to nanoparticles may be controlled by the difference in the bacterial cell-wall structure (Gram-positive: thick wall with 20–50 nm layer of peptidoglycan, which is attached to teichoic acids; Gram-negative: structurally and chemically more complex cell walls) (Hajipour et al., 2012).

Table 1
Summarized outcomes of the representative studies investigating the impact of nanoscale copper particle types on plants and soil-microbes.

Particle size/range (nm) and concentration(s) used	Plant species/Soil–microbes	Endpoint(s) tested	Exposure condition and incubation period	Effect(s)/remarks	References
PLANTS					
Growth, development and photosynthesis and related variables					
Nano-CuO; Size: < 50 nm; Concentrations: 5, 15, 30, 45, 60, 100, 200, 400, 600 mg L^{-1}	Soybean (*Glycine max*) and chickpea (*Cicer arietinum*) seedlings	Seed germination and root elongation	Petri dishes with filter paper soaked with distilled water-dissolved nano-CuO; 5 days	In both *Glycine max* and *Cicer arietinum*, germination was not checked up to 2000 mg L^{-1} nano-CuO but the root growth was prevented above 500 mg L^{-1} nano-CuO; the elongation of the roots was severely inhibited with increasing concentration of nano-CuO as compared to control; root necrosis was also observed	Adhikari et al. (2012)
Nano-CuO; Size: < 100 nm; Concentrations: 10, 100, 500 and 1000 mg L^{-1}	*Raphanus sativus*	Plant root/shoot elongation and biomass	Petri dishes with filter paper soaked with distilled water-dissolved nano-CuO; 6 days	Strongly inhibited seedling growth over the entire treatment range; root growth was inhibited 97% and that of shoot growth was inhibited 79%	Atha et al. (2012)
Nano-CuO; size: < 50 nm; concentrations: 500 mg kg^{-1} sand	*Triticum aestivum*	Root and shoot length, and number of roots; chlorophyll contents	Sand matrix; 14 days	Shoot length was reduced significantly by 13%; reduced root length by 59%; In contrast to plant length (root and shoot), nano-CuO caused proliferation of the number of the roots significantly increasing the number of roots by 42%; Roots exhibited brown necrotic lesions, and were thinner and more brittle than the control plants; decreased chlorophyll levels compared to control	Dimkpa et al. (2012c)
Nano-CuO and nano-Cu; Size: 50 nm; Concentrations: 10, 50, 100, 500 and 1000 mg L^{-1}	*Cucumis sativus* seedlings	Biomass accumulation	Hydroponic culture; 5 days	The biomass level was 75% of that of control at 1000 mg L^{-1} of nano-CuO; the IC50 of nano-CuO was 376 mg L^{-1}; the biomass level was 33% of that of control at 1000 mg L^{-1} of nano-Cu; the IC_{50} of nano-Cu was 333 mg L^{-1}	Kim et al. (2012)
Nano-Cu; Size: < 50 nm; Concentrations: 100 and 500 mg L^{-1}	Zucchini (*Cucurbita pepo*)	Growth and photosynthetic traits	Hydroponics Assay; 14 days	The biomass of plants exposed to 100 and 500 mg L^{-1} nano-Cu was 2.5 and 1.9 g, respectively; these values represented 93 and 99% reductions in normalized plant growth relative to untreated controls; transpiration volume was reduced by 51% in plants exposed to 100 mg nano-Cu L^{-1} and 61% in plants treated with 500 mg nano-Cu L^{-1}	Musante and White (2012)
Nano-CuO; Size: 20–40 nm; Concentrations: 2.0–100 mg L^{-1}	Maize (*Zea mays*) seedlings	Germination; Root elongation; Biomass	Hydroponic culture; 15 days	Inhibition of root elongation; chlorotic symptoms in seedlings; reduced biomass and root elongation	Wang et al. (2012)
Nano-CuO; Size: 30–50 nm; Concentration: 1000 mg L^{-1}	Lettuce (*Lactuca sativa*), radish (*Raphanus sativus*) and *Cucumis sativus* seedlings	Seed germination and root elongation	Petri dishes with filter paper soaked with distilled water-dissolved nano-CuO; 3 days	The measured effective concentrations (EC_{50}) for seed germinations were 13 mg CuO L^{-1}), 398 CuO mg L^{-1} and	Wu et al. (2012)

Table 1 (*continued*)

Particle size/range (nm) and concentration(s) used PLANTS	Plant species/Soil–microbes	Endpoint(s) tested	Exposure condition and incubation period	Effect(s)/remarks	References
				228 mg CuO L^{-1} for seeds of *Lactuca sativa*, *Raphanus sativus* and *Cucumis sativus*, respectively; role of the surface area-to-volume ratio of seeds in nano-CuO-mediated phytotoxicity was revealed; small seeds (that of *Lactuca sativa*) were the most sensitive to nano-CuO	
Nano-CuO; size: < 50 nm; concentration: 500 mg kg^{-1} sand	Wheat (*Triticum aestivum*)	Root phytotoxicity	Sand matrix; 14 days	Reduced root length by ~64% from control levels; browning of the root surface	Dimkpa et al. (2013)
Nano-CuO; Size: < 50 nm; Concentrations: 50, 500, 2000 and 4000 mg L^{-1}	Buckwheat (*Fagopyrum esculentum*)	Seedling growth; root tissue morphology	Petri dishes with filter paper soaked with distilled water-dissolved nano-CuO; 7 days	High doses of nano-CuO (2000 and 4000 mg L^{-1}) significantly inhibited root length relative to that in the control; seedling biomass decreased only in the treatments with 4000 mg L^{-1} of nano-CuO	Lee et al. (2013)
Nano-CuO; Size: < 50 nm; Concentrations: 0.5 mM, 1.0 mM and 1.5 mM	Rice (*Oryza sativa*) seedlings	Seed germination and seedlings growth; carotenoids content	Plastic trays with cotton pads soaked with double distilled water-dissolved nano-CuO; 14 days	Inhibition of seed germination and seedlings growth; declined carotenoids content	Shaw and Hossain (2013)
Nano-CuO; size: < 50 nm; concentrations: 100, 250 and 500 mg kg^{-1} sand	Bean (*Phaseolus vulgaris*)	Growth traits	Sand matrix; 7 days	Inhibition of growth was more apparent in roots (10–66%) than shoots (9–25%)	Dimkpa et al. (2015)
Nano-CuO; Size:..; Concentrations: 100, 200, 400, and 600 mg L^{-1}	Cucumber (*Cucumis sativus*) seedlings	Seed germination and root elongation	Petri dishes with filter paper soaked with distilled water-dissolved nano-CuO; 7 days	Significant inhibition of seed germination and root elongation; differential expression of 34 proteins in *C. sativus* seeds where, the expression patterns of at least 9 proteins highly differential; one novel biomarker candidate protein (5977-m/z) was also identified	Moon et al. (2014)
Nano-CuO; Size: < 50 nm; Concentrations: 50, 100, 200, 400 and 500 mg L^{-1}	*Glycine max* seedlings	Shoot and root development, total chlorophyll content	Murashige and Skoog medium (1/2 strength); 14 days	Exposure to 500 mg L^{-1} of nan-CuO significantly reduced the shoot growth, weight, and total chlorophyll content; root length and fresh weights were significantly reduced at all concentrations of nano-CuO exposure	Nair and Chung (2014a)
Nano-CuO; Size: 30 nm; Concentrations: 0.5, 1.0, 2.0, 5.0, 10, 20, 50 and 100 mg L^{-1}	*Arabidopsis thaliana*	Plant biomass; total chlorophyll content; anthocyanin content; root elongation	Murashige and Skoog medium (1/2 strength); 21 days	Plant biomass and total chlorophyll content were significantly reduced under all the tested concentrations (2.0, 5.0, 10, 20, 50 and 100 mg L^{-1}; the anthocyanin content significantly increased upon exposure to 10, 20, 50 and 100 mg L^{-1}; significant reduction in root elongation was observed upon exposure to	Nair and Chung (2014b)

				0.5–100 mg L^{-1}; retarded primary root growth, enhanced lateral root formation, and loss of root gravitropism were also noted	
Nano-CuO; Size: < 50 nm; Concentrations: 0.5, 1.0 and 1.5 mM	Syrian barley (*Hordeum vulgare*)	Growth traits, chlorophyll fluorescence and the contets of chlorophyll and epidermal flavonols	Plastic trays with cotton pads soaked with double distilled water-dissolved nano-CuO; 20 days	Significant gradual decreases in shoot length as well as shoot and root weight with increasing nano-CuO concentration; significant changes in the chlorophyll, epidermal flavonols contents; nano-copper induced increase in quantum efficiency of photosystem (PS II; ΦPSII) was only recorded in highest nano-CuO concentration (1.5 mM); nano-CuO had no significant effects on maximal quantum yield of PSII (Fv/Fm) irrespective of nano-copper concentration	Shaw et al. (2014)
Nano-CuO; Size: 20–30 nm; Concentrations: 10 and 20 mg L^{-1}	*Lactuca sativa* seedlings	Plant root/shoot elongation and biomass; chlorophyll content	Hydroponic system using magenta boxes; 15 days	Reduced root length by 51%; no effect were observed in leaf length; reduced water content in roots by 61%; reduced biomass accumulation of roots and leaves by 69% and 52% respectively; reduced chlorophyll content by 14% compared with the control	Trujillo-Reyes et al. (2014)
Nano-CuO; Size: 10–100 nm; Nano-Cu; Size: up to 10 μm; Concentrations for both nanoparticle types: 5.0, 10 and 20 mg L^{-1}	*Lactuca sativa* and alfalfa (*Medicago sativa*)	Growth traits	Hydroponic system using magenta boxes; 15 days	The shortest root in *Lactuca sativa* (15.9 ± 2.4 cm) and *Medicago sativa* (16.2 ± 0.2 cm) occurred in plants treated with 20 mg L^{-1} nano-CuO and nano-Cu	Hong et al. (2015)
Oxidative stress and its metabolism					
Nano-CuO; Size: < 50 nm; Concentration: 500 mg kg^{-1} sand	*Triticum aestivum*	Lipid peroxidation; oxidized glutathione; activity of peroxidase (POD) and CAT enzymes	Sand matrix; 14 days	Elevated activity of POD and CAT in roots; more glutathione was present as the oxidized form, GSSG, in the roots than the shoots of the control plants on a fresh weight basis; the level of GSSG in the shoots of plants grown with nano-CuO significantly increased compared to control plants; increases in GSSG in roots were not significant	Dimkpa et al. (2012c)
Nano-CuO; Size: 50 nm; Concentrations: 10, 50, 100, 500 and 1000 mg L^{-1}	*Cucmis sativus*	Activity of reactive oxygen species (ROS)-metabolizing enzymes (superoxide dismutase, SOD), catalase, CAT), and peroxidase, POD)	Hydroponic culture; 5 day	Enzyme activities of SOD, CAT, and POD in the root cells increased compared to that of control, where significant differences in their activity were noted at 100 mg L^{-1}; in particular, SOD and POD activities in root cells increased to higher than 50% of that of control, whereas CAT activity did not significantly change	Kim et al. (2012)
Nano-CuO; Size: < 50 nm; Concentrations: 50, 100, 200, 400 and 500 mg L^{-1}	*Glycine max* seedlings	H_2O_2 generation, peroxidase (POD) enzyme activity; lignification of root cells	Murashige and Skoog medium (1/2 strength); 14 days	Exposure to 100, 200, 400 and 500 mg L^{-1} of nano-CuO significantly increased the H_2O_2 level, POD activity, and lignin contents of roots; staining with	Nair and Chung (2014a)

Table 1 (*continued*)

Particle size/range (nm) and concentration(s) used PLANTS	Plant species/Soil–microbes	Endpoint(s) tested	Exposure condition and incubation period	Effect(s)/remarks	References
				phloroglucinol-HCl revealed a concentration dependent increase in lignification of root cells	
Nano-CuO; Size: 30 nm; Concentrations: 0.5, 1.0, 2.0, 5.0, 10, 20, 50 and 100 mg L^{-1}	*Arabidopsis thaliana*	$O_2^{\bullet -}$; H_2O_2 formation; antioxidant metabolism	Murashige and Skoog medium (1/2 strength); 21 days	Nano-CuO concentration-dependent increase in $O_2^{\bullet -}$ and H_2O_2 formation in leaves and roots; induced antioxidant, sulfur assimilation, GSH biosynthesis genes	Nair and Chung (2014b)
Nano-CuO; Size: < 50 nm; Concentrations: 0.5 mM, 1.0 mM and 1.5 mM	*Oryza sativa* seedlings	Oxidative stress traits and metabolizing enzymes	Plastic trays with cotton pads soaked with double distilled water-dissolved nano-CuO; 14 days	Loss of root cells viability; severe oxidative burst; high membrane lipid peroxidation; severe oxidative burst; incapacity of elevated APX and GR activity in the protection of stressed cells against nano-CuO accrued oxidative damage; decline in DHAR rendered stressed cells in futile recycling of AsA pool	Shaw and Hossain (2013)
Nano-CuO; Size: < 50 nm; Concentrations: 0.5, 1.0 and 1.5 mM	Syrian barley (*Hordeum vulgare*)	Oxidative stress traits and metabolizing enzymes	Plastic trays with cotton pads soaked with double distilled water-dissolved nano-CuO; 20 days	Severe oxidative burst was revealed measured as H_2O_2 deposits evident in all stressed leaves irrespective of CuO concentration; intensity and the occurrence of spots were found to be more in high concentrations (1.0 and 1.5 mM) as compared to 0.5 mM nano-CuO; maximum cell death was observed with 1.0 and 1.5 mM nano-CuO exposed roots; maximum increase (~1.8-fold) in foliar lipid peroxidation (measured as malondialdehyde, MDA) was noticed in 1.5 mM CuO treatment irrespective of stress period; decreased GSH and the GSH/GSSG ratio; elevated SOD, APX and GR activity; declined activity of DHAR and MDHAR, and the recycling of AsA pool indicated as elevated DHA level	Shaw et al. (2014)
Nano-CuO; Size: 20–30 nm; Concentrations: 10 and 20 mg L^{-1}	*Lactuca sativa* seedlings	Activity of catalase (CAT) and ascorbate peroxidase (APX) enzymes	Hydroponic system using magenta boxes; 15 days	At 10 mg L^{-1}, CAT and APX activity respectively increased and decreased in roots and leaves; however, the roots and leaves at 20 mg L^{-1} exhibited a lower CAT activity compared to the 10 mg L^{-1} treatment	Trujillo-Reyes et al. (2014)
Nano-CuO; Size: 10–100 nm; Nano-Cu; Size: up to 10 μm; Concentrations for both nanoparticle types: 5.0, 10 and 20 mg L^{-1}	*Lactuca sativa* and *Medicago sativa*	Assays for CAT and APX enzymes	Hydroponic system using magenta boxes; 15 days	CAT and APX activity was plant types and plant-organ dependent irrespective of nano-Cu types; the down regulated and up regulated activity of respectively CAT and APX in Lactuca sativa and Medicago sativa roots indicated a differential ROS-generating	Hong et al. (2015)

				potential of nano-CuO and nano-Cu, and also ROS-metabolizing capacity in root cells of the test plants	
Cyto/genotoxicity					
Nano-CuO; Size: <100 nm; Concentrations: 10, 100, 500 and 1000 mg L^{-1}	*Raphanus sativus*, perennial ryegrass (*Lolium perenne*) and annual ryegrass (*Lolium rigidum*)	DNA damage	Petri dishes with filter paper soaked with distilled water-dissolved nano-CuO; 6 days	Of the three plant species examined, *Lolium rigidum* was the most resistant to nano-CuO induced DNA damage; Under similar nano-CuO concentrations (10, 100, 500 and 1000 mg L^{-1}), accumulation of FapyGua and 8-OH-Gua was $\approx$2-times lower, and that of FapyAde $\approx$10-times lower in *L. perenne* than in *R. sativus*; only high doses of nano-CuO were evidenced to cause accumulation of FapyAde, FapyGua, and 8-OH-Gua in *L. rigidum*	Atha et al. (2012)
Nano-CuO; Size: <50 nm; Concentrations: 50, 500, 2000 and 4000 mg L^{-1}	Buckwheat (*Fagopyrum esculentum*)	Random amplified polymorphic (RAPD) DNA assays	Petri dishes with filter paper soaked with distilled water-dissolved nano-CuO; 7 days	RAPD assay conducted at high nano-CuO concentrations (2000 and 4000 mg L^{-1}) revealed four random 10-mer primers generated specific and reproducible results; of 87 bands, 58 showed changes as compared to the controls and ranged in size from 200 to 1600 base pair; DNA damage effect displayed for averaged RAPD pattern by four random amplifications. The average Nei's genetic identity (NGI) of seedlings exposed to nano-CuO at 2000 mg L^{-1} decreased significantly; at the highest dose tested (4000 mg L^{-1}, the reduction in NGI was significantly more pronounced than that for the control for DNA damage; the band changes tend to increase with an increase in nano-CuO concentrations	Lee et al. (2013)
Nano-CuO; Size: <50 nm; Concentrations: 50, 100, 200, 400 and 500 mg L^{-1}	*Glycine max* seedlings	The mRNA expression of different genes involved in lignin biosynthesis viz. phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), cinnamyl alcohol dehydrogenase (CAD), peroxidase 2 (POD2), peroxidase 4 (POD4), and peroxidase 7 (POD7) was studied using real-time polymerase chain reaction	Murashige and Skoog medium (1/2 strength); 14 days	The expression levels of PAL, C4H, and CAD genes were significantly up-regulated upon exposure to 100, 200 and 400 mg L^{-1}; significant up-regulation in the expression levels of POD2 and POD4 genes was observed upon exposure to 100, 200, 400 and 500 mg L^{-1}; exposure to 200, 400 and 500 mg L^{-1} of nano-CuO resulted in significant up-regulation of POD7 gene	Nair and Chung (2014a)
Nano-CuO; Size: 30 nm; Concentrations: 0.5, 1.0, 2.0, 5.0, 10, 20, 50 and 100 mg L^{-1}	*Arabidopsis thaliana*	Cell viability	Murashige and Skoog medium (1/2 strength); 21 days	Root cell death was not observed in roots of plants exposed to 1.0 mg L^{-1}; however, propidium iodide staining showed a dose-dependent increase in cytotoxicity in lateral root tips of plants which were exposed to 2.0, 5.0, 10 and 20 mg L^{-1}	Nair and Chung (2014b)

Table 1 (*continued*)

Particle size/range (nm) and concentration(s) used	Plant species/Soil–microbes	Endpoint(s) tested	Exposure condition and incubation period	Effect(s)/remarks	References
PLANTS					
Uptake/ accumulation and localization					
Nano-CuO; Size: 25–80 nm; Concentrations: 5.0, 10, 100, 500 and 1000 mg L^{-1}	*Triticum aestivum*	Adsorption and uptake of nano-CuO on the root	Agar culture media; 48 h	Some of nano-CuO was strongly adsorbed on the plant root surface, and part of them by mechanical adhesion; the uptake and adsorption of nano-CuO increased with increasing exposure concentrations in the range of 5.0–200 mg L^{-1}; the amount of nano-CuO-adsorption was always lower than that of their uptake	Zhou et al. (2011)
Nano-CuO; Size: $<$ 100 nm; Concentrations: 10, 100, 500 and 1000 mg L^{-1}	*Raphanus sativus*, *Lolium perenne* and *Lolium rigidum*	Accumulation and localization in root and shoot tissues	Petri dishes with filter paper soaked with distilled water-dissolved nano-CuO; 6 days	In the 10, 100, 500 and 1000 mg L^{-1} nano-CuO and bulk-CuO exposed *R. sativus*, the approximately three times greater total Cu accumulation in nano-CuO-treated shoot, compared to bulk CuO); For *L. perenne*, the measured background level of Cu was 15.8 μg Cu g^{-1} plant shoots and the total Cu uptake from nano-CuO was only 1.5 times the background level; the measured background level of Cu in the *L. rigidum* shoots was 21.0 μg Cu g^{-1} plant shoots and the total Cu uptake (24.0 $\pm$ 23.1 μg Cu g^{-1} plant shoots from nano-CuO was equivalent to the background level	Atha et al. (2012)
Nano-CuO; Size: $<$ 50 nm; Concentration: 500 mg kg^{-1} sand	*Triticum aestivum*	Accumulation and speciation in shoot	Sand matrix; 14 days	*T. aestivum* shoots exhibited 375 $\pm$ 115 mg Cu kg^{-1} shoot dry weight; the XANES data showed that nano-CuO was detected in the shoots of *T. aestivum* seedlings grown from roots exposed to the nan-CuO; LC analysis of the XANES spectra from plants grown with nano-CuO revealed that at the majority (64 $\pm$ 10%) of the Cu was in the original form as CuO and the rest (36 $\pm$ 10%) was bound to sulfur as a reduced Cu (I)–S species	Dimkpa et al. (2012c)
Nano-CuO; Size: 50 nm; Concentrations: 10, 50, 100, 500 and 1,000 mg L^{-1}	*Cucmis sativus*	Bioaccumulation	Hydroponic culture; 5 d	Bioaccumulation of Cu was concentration-dependent; the Cu concentration in *C. sativus* increased steeply at 100 mg L^{-1} of Cu^{2+}, after which accumulated levels reached about 3000 μg g^{-1} at 1000 mg L^{-1} of Cu^{2+}; TEM images of root tissues revealed that nano-CuO entered into the endodermis of the *C. sativus* root	Kim et al. (2012)

				cells	
Nano-Cu; Size: < 50 nm; Concentrations: 100 and 500 mg L^{-1}	*Cucurbita pepo*	Uptake	Hydroponics Assay; 14 days	Cu shoot content in nano-Cu treatments at 100 mg L^{-1} was 3.9 μg g^{-1} (wet weight), and at 500 mg L^{-1}, this value was 4.8 μg g^{-1}	Musante and White (2012)
Nano-CuO; Size: 20–40 nm; Concentration: 2.0–100 mg L^{-1}	*Zea mays*	Uptake in roots and shoots	Hydroponic culture; 15 days	Plant tissue Cu contents increased with increasing nano-CuO concentrations; with the exception of 100 mg L^{-1} there were no significant differences in root Cu content across all treatments; the Cu content in the roots of plants exposed to 100 mg L^{-1} was 3.6 times higher than that of control; similarly, Cu content in the shoots at 100 mg L^{-1} was 7 times higher than the control; nano-CuO not only existed inside the cell wall of epidermal cell in root tips but also in the intercellular space and cytoplasm of cortical cells as well as in the nuclei	Wang et al. (2012)
Nano-CuO; Size: 20–30 nm; Concentrations: 10 and 20 mg L^{-1}	*Lactuca sativa*	Uptake	Hydroponic system using magenta boxes; 15 days	Root Cu concentration was 3362 mg kg^{-1} dry weight; 20 mg L^{-1} exposed plant leaves exhibited 376% increased Cu content when compared to the control	Trujillo-Reyes et al. (2014)
Nano-CuO; Size: 10–100 nm; Nano-Cu; Size: up to 10 μm; Concentrations for both nanoparticle types: 5.0, 10 and 20 mg L^{-1}	*Lactuca sativa* and *Medicago sativa*	Uptake of Cu	Hydroponic system using magenta boxes; 15 days	In *L. sativa* root, Cu accumulation from nano-Cu at 5.0 mg L^{-1} and 20 mg L^{-1} was higher, compared to the nano-CuO; In the case of *L. sativa* shoot, only nano-Cu at 10 and 20 mg L^{-1} significantly increased Cu accumulation, with respect to the control; exposure to nano-Cu at 20 mg L^{-1} produced significantly higher Cu concentration in ythe shoots of both *L. sativa* and *M. sativa*; under all treatments, *M. sativa* translocated about 3–5% of Cu from root to shoot, while only 0.5–0.6% was translocated in *L. sativa*	Hong et al. (2015)
SOIL–MICROBES					
Nano-CuO; Size: 33 nm; Concentration: 10,000 mg L^{-1}	*Pseudomonas putida* KT2440 (KT2440 construct with a plasmid bearing the luxAB reporter genes	Antimicrobial activity	Agar culture medium; 60 min treatment	Cell death accompanied loss in Lux activity	Gajjar et al. (2009)
Nano-CuO (zero valent); Size: < 10–200 nm; Concentration: 550 mg kg^{-1} soil	Members of the orders Rhizobiales, Flavobacteriales and Sphingomonadales	Fate of nano-CuO	Soil in pots (Plastic planting pots) kept in field at 40.72°N, 73.09°W; 160 days	Altered microbial community structure as they migrated through the matrix; in particular, two orders of organisms found in rhizosphere, Flavobacteriales and Sphingomonadales, appeared to be particularly susceptible to the presence of nano-CuO; leaching of Cu ions from the parent nano-CuO was also observed as a function of time	Collins et al. (2012)

Table 1 (*continued*)

Particle size/range (nm) and concentration(s) used	Plant species/Soil–microbes		Endpoint(s) tested	Exposure condition and incubation period	Effect(s)/remarks	References
PLANTS						
Nano-CuO; Size: < 50 nm; Concentrations: 0.1 and 1.0% wet weight of experimental/collected soils	Soil bacterial community from the order such as Rhizobiales and family Sphingobacteriaceae		Bacterial community activity through assays that of enzymes and 16S rRNA gene copies	Soil types collected at Bet–Dagan, Israel (collected from a site located at 31°59′N34°49′E) and Yatir, Israel (located at 31°21′ N35°1′E)	Negative effects on soil bacterial groups including Rhizobiales and Sphingobacteriaceae; nano-CuO-treated sandy loam soil exhibited strong effect on the bacterial hydrolytic activity, oxidative potential, community composition and size in comparison to sandy clay loam soil; a differential interaction of nano-CuO with clay fraction and organic matter was argued to be a major factor significantly modulating the nano-CuO toxicity	Frenk et al. (2013)

However, several instances of a differential susceptibility of bacterial strains to nano-Cu support the insignificance of difference in the bacterial cell wall composition. For example, Baek and An (2011) revealed a low susceptibility of *E. coli* (−), *Staphylococcus aureus* (+) and *Bacillus subtilis* (+) to nano-CuO (20–30 nm, black in color). However, the antibacterial effect of silver nanoparticles against *E. coli* (−) and *S aureus* (+) bacteria was higher, compared to that of nano-CuO (Baek and An, 2011). Thus, the toxicity of the nano-Cu forms (such as nano-CuI; size: 8 nm) to bacteria (such as *E. coli*) may be modulated by a combination of several other factors such as size, temperature, aeration, pH, and concentration of both nanoparticles and bacteria (Pramanik et al., 2012). The antibacterial activity of nano-CuO (size: 22 nm) was particle size dependent (Azam et al., 2012). Pramanik et al. (2012) reported that a decreased agglomeration due to high temperature, high aeration, and low pH may provide more surface area for interaction with bacterial membranes, and subsequently for solubilization of Cu ions and higher toxicity of nano-CuI (size: 8 nm).

In addition, the uptake of Cu ions released from nano-CuO by the bacteria and/or the interactions of nano-CuO (size: 30 nm) with microbial organics and the ROS generation can be responsible for the nano-CuO toxicity (Mortimer et al., 2011). Nano-CuO (size: 42 nm) can efficiently penetrate the cell membrane, release Cu ions inside the cell, and cause toxicity (Karlsson et al., 2008). However, in bacterial strains such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Shigella*, nano-CuO (size: 80–160 nm) itself may form stable complexes with intracellular enzymes, which in turn alter various biochemical pathways leading to cell death (Mahapatra et al., 2008). The accumulation and dissolution of nano-CuO (size: 22 nm) in the bacterial membrane can bring changes in its permeability, and subsequently causes release of lipopolysaccharides, membrane proteins and intracellular biomolecules and dissipation of the proton motive force across the plasma membrane (Azam et al., 2012). In fact, nano-CuO (size: ~30 nm) can change the local microenvironment near bacteria, which in turn can induce bacterial damage *via* increasing the solubility of nano-CuO and subsequently the production of ROS (Heinlaan et al., 2008). Similar to the effect of Cu ions on DNA such as Cu ion binding to DNA molecules, disruption of biochemical processes (Stohs and Bagchi, 1995; Kim et al., 2000) and damage to the helical structure through cross-linking within and between the nucleic acid strands (Ruparelia et al., 2008), nano-CuO can also bring cell membrane damages *via* the specific or nonspecific interactions or membrane wrapping of the nanoparticles (Nel et al., 2009). Moreover, the depletion of intracellular ATP production, generation of ROS and oxidative damage to cellular structures can be caused by nano-CuO (size: 2–30 nm) (Applerot et al., 2012); whereas disruption of DNA replication can also be caused by the cellular uptake of nano-CuO (size: ~40–80 nm) or the released metallic ions (Lu et al., 2013). The sequence of major events in the nano-Cu toxicity in *E. coli* bacterial strains was reported by Deryabin et al. (2013).

The potential mechanisms underlying the toxicity induced by the majority of nanoparticles have not yet been completely elucidated. However, based on earlier reports (Heinlaan et al., 2008; Klaine et al., 2008; Gajjar et al., 2009; Bhatt and Tripathi, 2011; Wang et al., 2011; Dinesh et al., 2012; Pramanik et al., 2012; Deryabin et al., 2013), Fig. 2 summarizes the major events that are assumed to occur during the nano-Cu microbial toxicity.

2.2. Modulation of nanoscale copper toxicity to microbes by soil-associated factors

Assessment of the ability of nanoparticles to aggregate or interact with other particles can be of great interest while assessing their stability (Zhu et al., 2006; Dinesh et al., 2012). The role of soil

organic matter (SOM) in modulating the toxicity of nanoparticles has been credibly evidenced (Dinesh et al., 2012). Contingent upon the types of nanoparticle as well as soil, adsorption of nanoparticles by SOM reduces the mobility of nanoparticles in soil matrix, which in turn influences the severity of nanoparticle toxicity to the microbial populations in terms of survival and population growth. Nevertheless, nano-CuO was unable to cause any change in the total amount of SOM; however, changes in humic-like substances in the dissolved organic matter were observed due to nano-CuO exposure (Ben-Moshe et al., 2013). Since the complexation of Cu with different organic functional groups (carboxyls, −COOH; phenols, −OH; thiols, −SH; amines, $-NH_2$) of SOM-organic substances has been evidenced (Alacio et al., 2001; Smith et al., 2002; cited in Karlsson et al., 2006), modulation of nano-Cu toxicity to microbes can be envisaged. Soil type has been considered as a deterministic factor dictating the vulnerability of soil organisms to metal oxide engineered nanoparticles (Frenk et al., 2013). Nano-CuO may impact the structure of soil microbial community differently, when present in soils differing in textures and characteristics (Frenk et al., 2013). For example, nano-CuO (<50 nm)-treated sandy loam strongly affected the bacterial hydrolytic activity, oxidative potential, community composition and size in comparison to sandy clay loam soil (Frenk et al., 2013). A differential interaction of nano-CuO with clay fraction and organic matter was argued to be a major factor modulating significantly the nano-CuO toxicity. In fact, transformation of engineered nanoparticle such as crystal growth, dissolution, aggregation and aging may also cause changes to the micro- or nano-environment surrounding the engineered nanoparticles (Qafoku, 2010). A higher detainment rate for zero-valent nano-CuO (size: <10 and 200 nm) (vs. nano-ZnO) in soil matrix may be considered as a major factor responsible for high nano-CuO-susceptibility of rhizospheric microbial community (Flavobacteriales and Sphingomonadales) (Collins et al., 2012). Nevertheless, leaching of zero-valent Cu ions from the parent nano-CuO (size: <10 and 200 nm) can be a function of time rather than a function of depth or nanoparticle speciation (Collins et al., 2012). Data on the fate, transport and mobility of nanoparticles in the soil are crucial for a better assessment of potential consequences of soil-associated nanoparticles on the soil biological community and the plants and human/animal systems. However, our knowledge about the possible effects of nanoparticles on the chemical, physical and biological properties of the soil, and about the significance of environmental conditions in this context is limited (Ben-Moshe et al., 2013).

3. Nanoscale copper in plants

Since plants are critical to both ecosystem function and food supply, and the plants and soils are closely linked in the soil–plant system', the impact of soil-associated nanoparticles poses a threat to plants and through plant products, to consumers (Anjum et al., 2013a) (Fig. 1). Being an essential micronutrient for plants, Cu at low concentration participates in photosynthetic electron transport, mitochondrial respiration, cell-wall metabolism, hormone signaling, protein trafficking and iron mobilization, and significantly improves plant growth and development (Raven et al., 1999; Yruela, 2005, 2009). However, 20–30 μg Cu g^{-1} leaf dry weight was considered as a critical toxicity level of Cu for most crop species (reviewed by Yruela, 2005, 2009 and Anjum et al., 2015). Owing to the redox-active nature of high Cu concentrations, Cu ions can: (a) elevate the generation of ROS through the Fenton or Haber–Weiss reactions (Halliwell and Gutteridge, 1999), (b) cause enzyme inactivation as a result of its interaction with the sulfhydryl groups of proteins, leading to protein dysfunctioning, and (c) lead to chlorosis, necrosis, stunting, and root-growth inhibition (Xiong, 2005; Yruela, 2005; Manceau et al., 2008). Plant response to Cu (bulk or ion) stress has been explored at the physiological, molecular and proteomic levels (Ahsan et al., 2007; Zhang and Shen, 2009; Ritter et al., 2010; Atha et al., 2012; Thounaojam et al., 2012; Ansari et al., 2013), but soil bacteria (Rousk et al., 2012) and algae (Aruoja et al., 2009; Wang et al., 2011) have been the major focus of nano-Cu-impact studies. In contrast, studies on nano-Cu interaction with the terrestrial plant system are rare (Dimkpa et al., 2012a, 2012b, 2012c, 2012d, 2012e; Shaw and Hossain, 2013). In view of the above and for the paucity of information, bioaccumulation/uptake and toxicity of nano-Cu and the invoked response of plant defense system (potential toxicity mechanisms) will be discussed hereunder in separate subsections, with a hope that this may fill the knowledge gaps about interactions of plant system with engineered nanoparticles (including nano-Cu and/or nano-CuO).

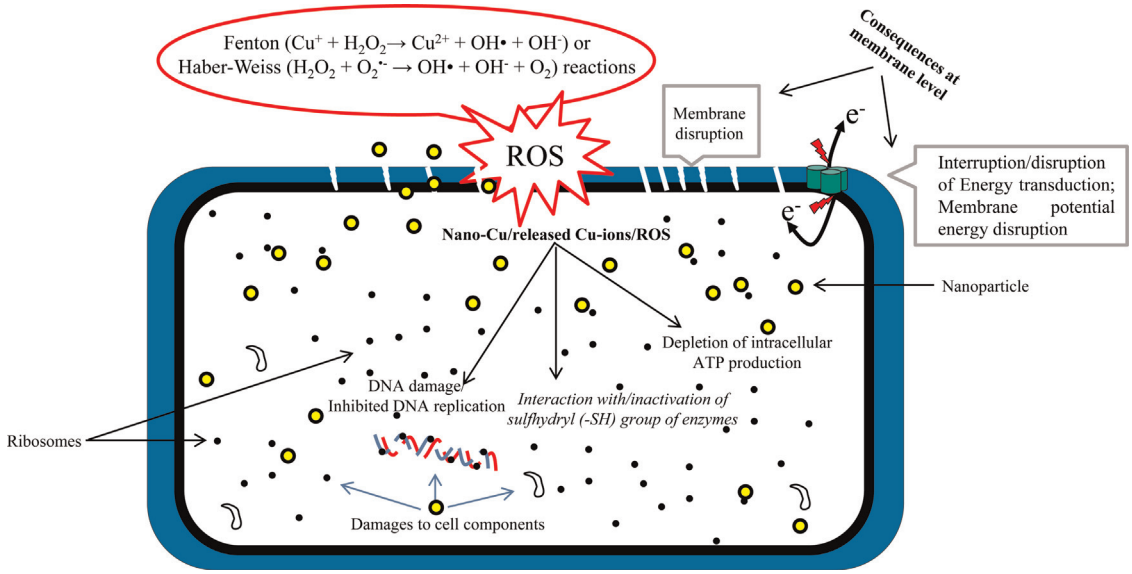

Fig. 2. Simplified diagram highlighting potential mechanisms of antibacterial activity of nano-copper. (Heinlaan et al., 2008, Gajjar et al., 2009, Wang et al., 2011, Pramanik et al., 2012, Deryabin et al., 2013).

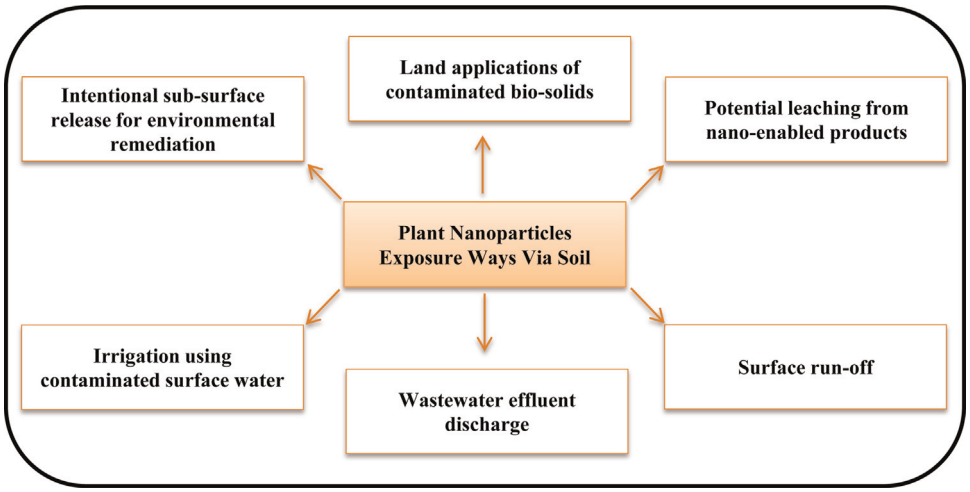

Fig. 3. Simplified diagram highlighting terrestrial plant nanoparticle-exposure ways via soil. (Pokhrel and Dubey, 2013).

3.1. Accumulation

Owing to their strong interaction with the surrounding environment, higher plants are vulnerable to effects of the available contaminants including the nanoparticles (Fig. 3). Hence, a good understanding of the nanoparticles' interactions with plant system is of paramount importance for assessing their toxicity and trophic transport (Sabo-Attwood et al., 2012; Anjum et al., 2013a). However, the nanotoxicology research on plant uptake and accumulation of nanoparticles has generated new and sometimes controversial data (Ma et al., 2010a). Zhou et al. (2011) have reported adsorption of nano-CuO (size: 55 nm) to the *Triticum aestivum* root surface. The accumulation profiles of three forms of Cu namely nano-CuO (size: <100 nm), bulk-CuO (10, 100, 500, and 1000 mg L^{-1}) and Cu^{2+} (10 and 50 mg L^{-1}) were differential in radish (*Raphanus sativus*) and rye grass (*Lolium perenne*), where the uptake of Cu was substantially greater from Cu^{2+} than from the nano or bulk Cu (Atha et al., 2012). The overall Cu-uptake observed in *Lolium perenne* (23.2 μg Cu g^{-1} plant shoots) was approximately 17 times lower than in *Raphanus sativus* (400 μg Cu g^{-1} plant shoots), possibly due to a putative active Cu-uptake mechanism present in *Raphanus sativus* Cu-stimulated potential protein transporters (Yruela, 2005, 2009; Atha et al., 2012). In bean (*Phaseolus radiatus*) and *Triticum aestivum* cultured on agar media, Lee et al. (2008) revealed a relationship of the uptake and accumulation of nano-Cu with its bioavailability. In *T. aestivum*, the occurrence of a larger surface area of thin and numerous roots was supposed to facilitate penetration and subsequent accumulation of nano-Cu in the cells. In the roots of hydroponically grown lettuce (*Lactuca sativa*), application of nano-Cu/nano-CuO (size: ~20–30 nm) (10 and 20 mg L^{-1}) led to a higher accumulation of Cu, compared with application of Cu ions (Trujillo-Reyes et al., 2014). In sand matrix, bioaccumulation of Cu, mainly as CuO and Cu(I)-sulfur complexes, was detected in the shoots of *Triticum aestivum* exposed to nano-CuO (size: <50 nm) (Dimkpa et al., 2012c). The total Cu level in the shoot of nano-CuO-exposed *T. aestivum* was similar under both the nano- or the bulk-material exposures (Dimkpa et al., 2012c). Regarding the absorption of nano-Cu in roots and its subsequent translocation to shoots, maize (*Zea mays*) roots exposed to 100 mg L^{-1} nano-CuO (size: 20–40 nm) had a 3.6 times higher Cu content than the control. It was 2 times and 1.8 times higher than in roots exposed to Cu^{2+} and CuO bulk particles respectively. Additionally, in shoots of the 100 mg L^{-1} nano-CuO-treated *Zea mays*, Cu content was 7, 1.2 and 1.8 times higher in comparison to the control, Cu^{2+}-treated and CuO bulk treated plants respectively (Wang et al., 2012). Notably, nano-CuO particles accumulated in *Z. mays* root cell, intracellular space, and the cytoplasm, and nuclei of cortical cells and xylem cells (Wang et al., 2012). The authors observed a xylem- and phloem-based transport and biotransformation of nano-CuO (20–40 nm) and revealed a nano-CuO transport from roots to shoots *via* xylem and its translocation back to roots *via* phloem. Additionally, these authors observed a reduction of nano-CuO from Cu (II) to Cu (I) during the course of translocation (Table 1).

3.2. Phytotoxicity

To date, investigations on 'plant-nanoparticle-interaction' with reference to growth, development, and gene expression in plants have been very few (Burklew et al., 2012). Bulk-Cu-phytotoxicity has been analyzed at physiological, molecular and proteomic levels (Ahsan et al., 2007; Zhang and Shen, 2009; Ritter et al., 2010; Atha et al., 2012; Thounaojam et al., 2012). However, the nano-CuO toxicity in plants remains little explored (Dimkpa et al., 2012a, 2012b, 2012c, 2012d, 2012e, 2013; Shaw and Hossain, 2013). Apart from the significance of biological model and toxicity assays, the nanoparticle physicochemical characterization (for traits like size/shape, distribution, agglomeration or aggregation state, crystal structure, surface chemistry/charge/area, stability over time/dissolution) and the nature of exposure media (such as solid matrix or solution) are important in the systematic nano-toxicity studies (Calder et al., 2012; Dimkpa et al., 2012e, 2013; Love et al., 2012; Zhao et al., 2012). Nanoparticle dissolution, aggregation, and surface-properties modulation may occur in solid matrices that in turn can modify their bioactivity (Calder et al., 2012; Dimkpa et al., 2012e; Zhao et al., 2012). Aggregation of nano-CuO (size: <50 nm) has been evidenced under sand-matrix condition, where the release of soluble metals and the rate of dissolution decreased with time (Dimkpa et al., 2012e). Information available on the nano-Cu-mediated toxicity to plant growth/development, photosynthesis and its variables is appraised in the following paragraphs with due emphasis on the potential mechanisms involved.

In general, nano-Cu can impair plant growth and development (Lee et al., 2008, 2013; Stampoulis et al., 2009; Fini et al., 2011; Shi et al., 2011; Atha et al., 2012; Kim et al., 2012; Wang et al., 2012; Wu et al., 2012; Shaw et al., 2014). Recently, nano-CuO (40, 80 and 120 mg L^{-1}; size: <50 nm)-dose-dependent reduction in the shoot and root growth has been recorded in *Hordeum vulgare* (Shaw et al., 2014). Nano-CuO (size: 43 nm; 1.0 mg L^{-1}) brought root-length-reduction in duckweed (*Landoltia punctata*) (Shi et al., 2011). The level of nano-Cu that can cause 50% inhibition in the test parameters may vary depending on the test plant models and

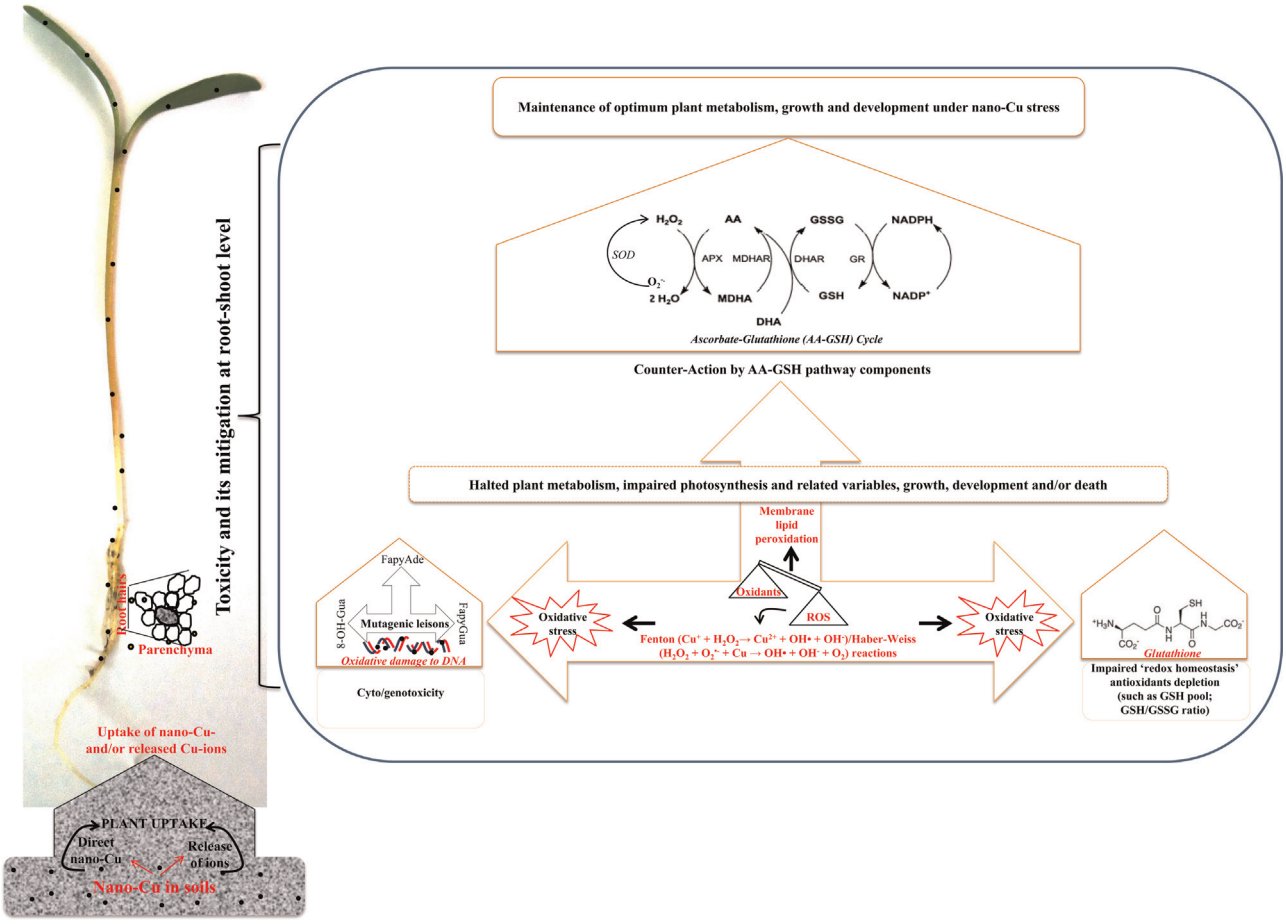

Fig. 4. Schematic representation of major events underlying nano-copper toxicity and plant tolerance strategies. (Atha et al., 2012, Wang et al., 2012, Lee et al., 2013, Shaw and Hossain, 2013, Shaw et al., 2014). [ROS, *reactive oxygen species*; 8-OH-dGuo, *the 2′-deoxynucleoside form of 8-OH-Gua*; FapyGua, *2,6-diamino-4-hydroxy-5-formamidopyrimidine*; FapyAde, *4,6-diamino-5-formamidopyrimidine*; SOD, *superoxide dismutase*; AA, *ascorbate*; APX, *ascorbate peroxidase*; MDHA, *mono dehydroascorbate*; MDHAR, *mono dehydroascorbate reductase*; DHA, *dehydro ascorbate*; DHAR, *dehydroascorbate reductase*; GSH, *reduced glutathione*; GSSG, *oxidized glutathione*; GR, *glutathione reductase*].

the nano-Cu concentrations. For example, inhibitory concentration values of 333 mg L^{-1} and 376 mg L^{-1} were recorded for nano-Cu and nano-CuO (size: 50 nm), respectively, in hydroponically cultivated *Cucumis sativus* seedlings (Kim et al., 2012), where both nano-Cu and nano-CuO aggregated more in the nutrient solutions than in the deionized water. Nano-CuO (size: 20–40 nm) itself can be redistributed from root to shoot and back to root, and bring about toxic consequences (Wang et al., 2012). The effects of Cu-based nanoparticles on nutrient quality of food crops or plant nutrition have been reported in few studies (Dimkpa et al., 2014; Hong et al., 2015). In bean (*Phaseolus vulgaris*), nano-CuO (size: <50 nm; at 100, 250 and 500 mg kg^{-1} sand) decreased the shoot Fe, Zn and Ca levels but not that of Mg; of the monovalent metals, K showed little change and Na increased (Dimkpa et al., 2014). On the other hand, both nano-CuO (size: 10–100 nm) and nano-Cu (size: up to 10 μm) at concentrations 5.0, 10, and 20 mg L^{-1} increased Cu, P, and S ($>100\%$, $>50\%$, and $>20\%$, respectively) in alfalfa (*Medicago sativa*) shoots and decreased P and Fe in lettuce (*Lactuca sativa*) shoot ($>50\%$ and $>50\%$, respectively) (Hong et al., 2015). In addition, the shortest root in *L. sativa* (15.9 ± 2.4 cm) and *M. sativa* (16.2 ± 0.2 cm) occurred in plants treated with 20 mg L^{-1} nano-CuO and nano-Cu, respectively (Hong et al., 2015). Nanoparticle toxicity may vary with plant type. For example, the nano-CuO (size: 30–50 nm)-exposed seeds of *Lactuca sativa*, *Raphanus sativus* and *Cucumis sativus* displayed 13 mg L^{-1}, 398 mg L^{-1} and 228 mg L^{-1} nano-CuO, respectively, as the effective concentration (EC50) for seed germinations (Wu et al., 2012). The smaller seeds were more sensitive to nano-CuO toxicity, and the surface-area-to-volume ratio of seeds was a major factor. Additionally, the phytotoxicity of Cu ($CuCl_2$) metal ions and nano-CuO may also differ and exhibit different EC50 concentrations such as 5∼8 mg L^{-1} for Cu^{2+} and less than 2.0 mg L^{-1} for nano-CuO. It was concluded that apart from the metal oxide nanoparticles-sourced dissolved metals ions, interaction of nanoparticles with the seed/root surface can also cause toxicity (Wu et al., 2012). The nano, bulk and ionic Cu may exhibit a differential impact on plants (Atha et al., 2012). In the 10, 100, 500 and 1000 mg nano-CuO and bulk-CuO exposed *Raphanus sativus*, the approximately three-fold total Cu accumulation in nano-CuO-treated shoot (vs. bulk CuO) and the strong plant-growth inhibition were credited to nano-CuO (Atha et al., 2012).

Owing to their insolubility in water, nanoparticles in general, have a limitation for toxicity experiments. Therefore, there are contradictions on major factors (nano-Cu, nano-CuO or ions released from these nanoparticles types) responsible for the nano-Cu phytotoxicity. Agar media, prepared by dissolving Phytagel powder in ultrapure water, was argued to provide a homogeneous exposure of nano-CuO particles to test plants namely mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*) (Lee et al., 2008). These authors observed a negligible contribution of Cu ions to toxicity in the test plants, and attributed the exhibited toxic consequences to nano-Cu (Lee et al., 2008). In a recent study on *Glycine max* seedlings, MS medium (1/2 strength) was used in order to avoid precipitation of less water insoluble nano-CuO

(size: <50 nm) (Nair and Chung, 2014a). Free ions released from nano-CuO and subsequently localized in the root parenchyma were considered as a major factor causing drastic changes in root morphological features and inhibiting root growth in buckwheat (*Fagopyrum esculentum*) (Lee et al., 2013). Since the effects of free-ion dissolution on plant growth may differ from those of nanoparticles, the potential association of particle toxicity with growth inhibition should also be determined in both cases. In this context, nano-CuO suspensions of 50, 500, 2,000 and 4000 mg L^{-1} were reported to release metallic ion concentrations of 2.6, 2.6, 1.2, and 4.6 mg L^{-1} respectively (Lee et al., 2013). However, these authors could detect no relation between the nano-CuO-accrued inhibition of the *Fagopyrum esculentum* root growth and biomass and the toxicity caused by the released free ions. The treatment of *Landoltia punctata* with nano-CuO (size: 43 nm) (1.0 mg L^{-1}; that released only 0.16 mg L^{-1} soluble Cu into growth medium) and comparable doses of soluble Cu (0.6 mg L^{-1}) caused 50% inhibition of growth (Shi et al., 2011). Dissolution from bulk materials alone cannot be linked to the observed phytotoxicity of nano-Cu (<50 nm) (Stampoulis et al., 2009). In this context, Cu-bulk material reduced the biomass of zucchini (*Cucurbita pepo*) in comparison with the controls, but the nano-Cu-exposed *C. pepo* demonstrated a higher degree of reduction, indicating that at least a part of the toxicity is due to elemental metal nanoparticle (Stampoulis et al., 2009). Moreover, the dissolved Cu from the nano-CuO was reported to contribute to its phytotoxicity in the nano-CuO (<50 nm) exposed *Triticum aestivum* (Dimkpa et al., 2012e). In another instance, nano-CuO (100 mg L^{-1}) did not affect germination, but inhibited growth of *Zea mays* seedlings (Wang et al., 2012). In contrast, the dissolved Cu^{2+} ions and CuO bulk particles could not affect the *Zea mays* growth. Simple growth traits such as seed germination and root elongation have also been considered in extensive nanoparticle-plant interaction studies (Lin and Xing, 2007; Ma et al., 2010a, 2010b; Wu et al., 2012).

Nano-CuO can severely impair photosynthesis and its related variables (Nekrasova et al., 2011; Dimkpa et al., 2012e; Shaw et al., 2014). In addition to impacting the root length and biomass of zucchini (*Cucurbita pepo*), nano-Cu (size: <50 nm) was reported to reduce the transpiration volume by 51% in plants exposed to 100 mg nano-Cu L^{-1} and 61% in plants treated with 500 mg nano-Cu L^{-1} (Musante and White, 2012). Nano-CuO (size: <30–50 nm) was reported to decrease chlorophyll content significantly (Dimkpa et al., 2012c; Nair and Chug, 2014a,b). In a recent study on *Glycine max*, nano-CuO concentration (500 mg L^{-1}) caused a significant reduction in the total chlorophyll content (Nair and Chung, 2014a). Similar observations were made on *Arabidopsis thaliana* exposed to nano-CuO (size: 30 nm) concentrations (2.0, 5.0, 10, 20, 50 and 100 mg L^{-1}) (Nair and Chung, 2014b). Compared to bulk Cu (500 mg Cu kg^{-1} sand), nano-CuO (size: <50 nm) impaired the chlorophyll content to a higher extent (Dimkpa et al., 2012c). The impact on chlorophyll and flavonol contents might depend nano-CuO (size: <50 nm) exposure-duration; the maximum reduction occurred during the initial period (such as 10 days) of exposure. However, a prolonged exposure (such as >20 days) could decrease the chlorophylls but increase the epidermal flavonols significantly (Shaw et al., 2014). Considering the key role of flavonols in H_2O_2 metabolism (Fini et al., 2011), inefficiency of elevated epidermal flavonols in controlling H_2O_2 was highlighted in the nano-CuO (40, 80 and 120 mg L^{-1}; size: <50 nm)-exposed Syrian barley (*Hordeum vulgare*) (Shaw et al., 2014). Anthocyanins are the flavonoids known for their role in protection of plant cells against oxidative stress caused by the elevated ROS level (Solfanelli et al., 2006; Tahara, 2007; Gill and Tuteja, 2010). Significance of elevated anthocyanin level was in protecting *Arabidopsis thaliana* against the impact of nano-CuO concentrations (10, 20, 50 and 100 mg L^{-1}) has recently been discussed by Nair and Chung (2014b). Modulation of the fluorescence kinetics of chlorophyll *a* has been used earlier to investigate the function of PSII and its reaction with changes in the environment and plant growth conditions (Kalaji et al., 2012). In this context, nano-CuO (40, 80 and 120 mg L^{-1}; size: <50 nm) can bring a significant decline in the performance-index parameters, irrespective of the stress level and the treatment period, and nano-CuO may not be able to affect significantly the maximal quantum yield of PSII (*Fv/Fm*) (Shaw et al., 2014). Application of nano-CuO (1.0 mg L^{-1}; size: ≈ 30 nm) and Cu ions (0.5 mg L^{-1}) led to suppression of photosynthesis in *Elodea densa* (Nekrasova et al., 2011). On the contrary, seed germination and shoot-to-root ratio were enhanced by nano-Cu application (Shah and Belozerova, 2009). Lignin, comprising of phenolic hetero polymers, is a complex component of the cell wall (Lin et al., 2005). To this end, nano-CuO concentrations (such as 100, 200, 400 and 500 mg L^{-1}) have been reported to enhance the lignification of root cells, thereby affecting the root development in plants such as soybean (*Glycine max*) (Nair and Chung, 2014a). Additionally, nano-CuO (above 2.0 mg L^{-1})-accrued retardation in primary root growth, enhancement in lateral root formation, and also a loss of root gravitropism have been evidenced in *Arabidopsis thaliana* (Nair and Chung, 2014b). However, the actual mechanisms, which underpin the above-discussed reports on nano-Cu or nano-CuO, need to be ascertained.

3.3. Nanoscale copper phytotoxicity mechanisms

A good understanding of mechanisms of the nanoparticle toxicity is important for targeted application of nanoparticles (Rai et al., 2014). Nano-Cu toxicity mechanisms have been extensively studied in animal/human system (Rastogi and Sinha, 2009; Ahamed et al., 2010; Gomes et al., 2011; Minocha and Mumper, 2012; Bulcke et al., 2014; Piret et al., 2014). In plants, the underlying mechanisms are well known for toxicity of Cu and/or Cu ions (reviewed by Yruela 2005, 2009) but not for nano-Cu phytotoxicity. It may be advocated that nano-Cu-accrued oxidative stress, impaired antioxidant defense system and the damaged vital cyto/genetic endpoints could be the major factors underlying the nano-Cu-caused anomalies (Fig. 4). The following sections critically discuss these aspects in the light of recent reports.

3.3.1. Antioxidant defense system

In plants, non-metabolized-ROS-accrued consequences are avoided by a direct or indirect scavenging or by detoxification of the excess ROS (Gill and Tuteja, 2010; Anjum et al., 2012a, 2012b; Gill et al., 2013; Majid et al., 2014). Plant antioxidant defense system comprises of enzymes (such as superoxide dismutase, SOD; catalase, CAT; guaiacol peroxidase, GPX; glutathione sulfotransferase, GST; ascorbate peroxidase, APX; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione reductase, GR) as well as non-enzymes (such as ascorbate, AsA; glutathione, GSH; carotenoids; tocopherols; phenolics) (Gill and Tuteja, 2010; Anjum et al., 2010, 2012b, 2014a). The significance of these components of the antioxidant defense system for alleviation of the ROS-mediated oxidative stress has been evident in different plant species exposed to nanoparticles such as graphene oxide nano-sheet (Anjum et al., 2013b, 2014b), cerium oxide (Rico et al., 2013) and silver (reviewed by Anjum et al., 2013a). However, such information about the nano-Cu-exposed plants is scarce.

As mentioned earlier, nano-CuO can mediate significant elevations in ROS generation and its subsequent consequences (such as membrane damage), and the modulation of antioxidant defense system components and cellular redox homeostasis in plants (Nekrasova et al., 2011; Dimkpa et al., 2012c,2012e, 2013; Kim et al., 2012; Shaw et al., 2014). In fact, nano-CuO possesses redox

cycling properties with the capacity of intra- and extracellular generation of ROS due to a combination of the particle effect and the dissociation of Cu ions from the nanoparticle (Fahmy and Cormier, 2009; Gomes et al., 2013). The nano-CuO-mediated increase in membrane LPO may accompany significant increase in GSH oxidation and a high activity of H_2O_2-metabolizing enzymes such as peroxidase and catalase (Dimkpa et al., 2012c). The elevated LPO may also coincide with decreases in GSH and the GSH/GSSG ratio (Shaw and Hossain, 2013; Shaw et al., 2014) and increases in SODs that dismutate $O_2^{\bullet -}$ into H_2O_2 (Nekrasova et al., 2011; Kim et al., 2012). Nevertheless, nano-CuO concentration > 0.5 mM may trigger oxidative burst in terms of elevated levels of H_2O_2 and malondialdehyde (MDA), the indicators of ROS and membrane LPO respectively, and maximally disrupt the plant-defense system (Shaw et al., 2014). A complete analysis of major components of AsA-GSH pathway is still to be done in the nano-Cu-exposed plants. In the nano-CuO (0.5 mM, 1.0 mM and 1.5 mM; size: < 50 nm)-exposed *Hordeum vulgare*, Shaw et al. (2014) reported inefficiency of elevated APX activity for control over H_2O_2 level. In addition, concomitant declines in DHAR and MDHAR resulted into severely decreased recycling of AsA pool (Shaw et al., 2014). Earlier, Shaw and Hossain (2013) evidenced a similar trend in enhanced APX activity with concomitant increases in H_2O_2 and MDA levels in the leaves of nano-CuO-exposed *Oryza sativa*. Since SOD directly modulates the amount of $O_2^{\bullet -}$ and H_2O_2, the occurrence of high levels of H_2O_2 despite an elevated APX-activity level might be due to enhanced SOD activity in *Hordeum vulgare* (Shaw et al., 2014). Thus, the failure of an APX-mediated ROS (such as H_2O_2)-scavenging system is clear in the nano-CuO-exposed *O. sativa* and *H. vulgare* (Shaw and Hossain, 2013; Shaw et al., 2014). In *Lactuca sativa* roots and leaves, nano-CuO was reported to inhibit cellular H_2O_2-metabolizing potential by decreasing the APX activity (at 10 mg L^{-1}) and CAT activity (at 20 mg L^{-1}) (Trujillo-Reyes et al., 2014). In a similar recent report, the activity of CAT and APX has been found to be plant-type and plant-organ dependent under exposure to nano-CuO and nano-Cu concentrations (5.0 10, and 20 mg L^{-1}) (Hong et al., 2015). The down-regulated and up-regulated activity of CAT and APX respectively in *Lactuca sativa* and *Medicago sativa* roots is indicative of both a differential ROS-generating potential of nano-CuO and nano-Cu, and the ROS-metabolizing capacity in root cells of the test plants (Hong et al., 2015). GR is a rate-limiting enzyme of AsA-GSH cycle where it maintains the GSH/GSSG ratio favorable for AsA reduction. A high GR activity may lead to an increase (as in *Oryza sativa*) (Shaw and Hossain, 2013) or a decrease (as in *Hordeum vulgare*) (Shaw et al., 2014) in the level of GSH and the GSH/GSSG ratio. The nano-CuO concentrations (0.5, 1.0, 2.0, 5.0, 10, 20, 50, and 100 mg L^{-1}) can differentially impact both the dismutation of $O_2^{\bullet -}$ into H_2O_2 by modulating the expression patterns of genes of SODs (MnSOD gene: *MSD*; CuZnSOD genes: *CSD1* and *CSD2*) and the metabolism of H_2O_2 by modulating the expression patterns of genes of H_2O_2-metabolizing enzymes such as APX (*APX1* and *APX2*) and CAT (*CAT2* and *CAT3*) (Nair and Chung, 2014b). Apart from the role of the above-discussed components of AsA-GSH pathway components in nano-Cu tolerance, the role of amino acids, such as proline, in the tolerance of *Arabidopsis thaliana* to nano-CuO concentrations (10 and 20 mg L^{-1}) was evidenced recently, where the proline-biosynthesis genes (*P5CS1* and *P5CS2*) were significantly up-regulated under nano-CuO exposure (Nair and Chung, 2014b).

3.3.2. Cyto/genotoxicity mechanisms

Plants have been used as indicator organisms in studies of mutagenesis in higher eukaryotes (Plewa and Wagner 1981), and the use of physiologic, morphologic, microscopic, and molecular tools in plant-genotoxicology facilitates data interpretation for a complete understanding of the effect of nanoparticles (Kumari et al., 2009; Lee et al., 2013). Nevertheless, plant system has a variety of well-defined genetic endpoints (such as alterations in ploidy, chromosomal aberrations and sister-chromatid exchanges), and the estimation of cyto/genotoxicity in plants at the DNA level has the advantage of sensitivity and a short response time (Kumari et al., 2009; Lee et al., 2013). However, the mechanisms underlying the oxidative-stress-mediated damage to DNA and its repair in plants are poorly understood in comparison to our current knowledge on the mammalian system (Britt, 1996; Tuteja et al., 2001). Accumulation of mutagenic or cytotoxic DNA lesions can lead to genomic instability, reduced plant growth, and incidence of plant diseases (Britt, 1996, 1999). The extent to which the engineered nanoparticles may cause long-term toxic effects (such as genotoxicity) in plants is unknown (Rico et al., 2011). Although oxidative damage to plant DNA caused by high Cu^{2+} ion levels is known (Balestrazzi et al., 2009; Macovei et al., 2010), studies on nano-Cu-accrued cyto/genotoxicity in plants are rare (Lee et al., 2013; Perreault et al., 2014; Rai et al., 2014).

The most studied oxidative-stress-induced DNA lesions include 8-OH-dGuo, the 2′-deoxynucleoside form of 8-OH-Gua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde).Varying concentrations of nano-CuO and bulk-CuO (10, 100, 500 and 1000 mg L^{-1}) and Cu^{2+} (10 and 50 mg L^{-1}) were reported to damage DNA differentially (measured as oxidatively modified mutagenic DNA lesions (such as 8-OH-Gua; FapyGua; FapyAde) in agricultural and grassland plants including *Raphanus sativus*, *Lolium perenne*, and *L. rigidum* (Atha et al., 2012). Particularly in the *R. sativus* seedlings incubated with nano-CuO at the highest dose (1000 mg L^{-1}), the cited authors observed statistically significant increases (220, ≈ 260, and ≈ 450%) in the accumulated levels of FapyAde, FapyGua, and 8-OH-Gua, respectively. Nevertheless, compared to nano-CuO, much less induction of oxidative damage to radish DNA was depicted under the bulk CuO treatment. Analyses of the potential uptake and localization of nano-CuO in *R. sativus* seedling root and shoot tissues *via* STEM-EDS led Atha et al. (2012) to conclude that nano-CuO, as well as dissolved Cu^{2+} ions, that are able to enter the nucleus of plants, can mediate direct oxidative damage to duplex DNA *via* $^{\bullet}OH$ attack on the heterocyclic bases. Under similar nano-CuO concentrations (10, 100, 500, and 1000 mg L^{-1}), accumulation of FapyGua and 8-OH-Gua was ~2-times lower, and that of FapyAde ~10-times lower in *L. perenne* than in *R. sativus*. Only high doses of nano-CuO (500 and 1000 mg L^{-1}) were evidenced to cause accumulation of FapyAde, FapyGua, and 8-OH-Gua in *L. rigidum*. However, significant accumulation of all the three lesions due to the lowest nano-Cu and bulk CuO doses (10 mg L^{-1}) in *L. perenne* confirmed the susceptibility of this species to both nano-Cu and bulk CuO doses (10 mg L^{-1}) (Atha et al., 2012). Random amplified polymorphic DNA (RAPD) assay has been used as an alternative approach to monitor the potential genotoxic effects of nanoparticles in plant tissues (such as root nuclei) (López-Moreno et al., 2010). Employing this approach, Lee et al. (2013) provided the first clue to the genotoxic effects of nano-CuO (size: < 50 nm) on early growth of edible plants such as *Fagopyrum esculentum*, and reported different DNA polymorphisms at 4000 mg L^{-1} of nano-CuO compared to the controls. Nei's genetic identity (NGI) analyses revealed the average NGI value of 4000 mg nano-CuO L^{-1}, which was significantly lower than one for the controls. In a recent study on *Arabidopsis thaliana*, nano-CuO (size: 30 nm) was evidenced to cause a dose-dependent increase in cell death in the lateral root tips at 2.0, 5.0, 10 and 20 mg L^{-1} (Nair and Chung, 2014b). Changes in the genetic pattern (such as the appearance of new polymerase chain-reaction products) were nano-CuO-concentration dependent. Herein, the nano-CuO-accrued changes in genomic DNA-template stability were argued as being a

result of the nano-CuO-accrued mutations, large deletions, or homologous recombination in DNA (Lee et al., 2013). Hence, the consideration of the nano-CuO-mediated potential alteration in gene expression levels during agro-biotechnological applications is advocated.

4. Conclusions and prospective work plan

The world-wide use of nano-Cu can disturb the soil biological processes as well as the plant physiology/biochemistry, which in turn may affect human health. Very little is known about the behavior of nano-Cu in the soil–plant system and its effect on the environment. Interaction of nano-Cu with soil–microbial community in the field remains particularly unexplored. Additionally, the use of well-characterized model microorganisms could get the least focus in the nano-Cu toxicity studies. Many works on the nano-Cu toxicity on individual plants or soil–microbiota have ignored the soil–plant system altogether. As to the potential mechanisms underlying the nano-Cu toxicity in soil–microbiota and crop plants (through different reactions/pathways), the decrease of particle size in the nanoscale, the release of ions from nano-Cu, as well as the elevation in ROS can possibly make for the nano-Cu consequences or bio-toxic effects; this assumption, however, needs further evaluation. In conclusion, nano-Cu (nano-CuO) may be potentially capable of doing damage to plant DNA *via* direct redox interactions (Atha et al., 2012; Lee et al., 2013).

Because the soil bacterial community provides significant services to ecosystems and humankind, it is critical to work out the nanoparticle's impact on this community. Owing to the known modulatory role of the presence and absence of plants for the physiological state of the microbial populations (Lin and Xing, 2007), prospective studies on the potential effect of nano-Cu on the soil microbial communities should be conducted in the absence and presence of plants. The fate of metallic nanoparticles and their consequences in plants and their consumers should be examined thoroughly in order to elucidate the route for contamination of the food chain. The literature discussed herein reflects inconsistency about the cause of toxicity of nano-Cu and/or ion release. Hence, in order to address adequately the safety concerns associated with nano-Cu, studies focused at a complete characterization of the toxicity and behavior of nano-Cu must be intensified. The potential influence of nano-Cu aging on the modulation of its own physicochemical characteristics and reactivity should also be assessed with interdisciplinary approach while elucidating the nano-Cu-toxicity responses in the soil–plant system (Mudunkotuwa et al., 2012). In addition, significance of potential chemical transformations of nano-CuO should be examined under conditions relevant to living systems and the natural environment (Wang et al., 2013). To get more insight into nano-Cu-toxicity mechanisms, efforts should be made to develop the chemical, biochemical and geno-toxicity markers-based standard (and valid) methodologies to identify the nano-Cu levels that can induce toxicity. Investigations aimed at unveiling the potential mechanisms underlying the plant species- and genotype-specific differences in nano-Cu sensitivity must be intensified. Considering the information paucity on biological repertoire for DNA repair in plants (Brit, 1996, 1999; Kathe et al., 2009; Atha et al., 2012), future research should focus on nanoparticle effects on both the genomic machinery as well as the putative base and nucleotide excision repair processes in plants (Petersen and Nelson, 2010; Atha et al., 2012). Finally, efforts should be made to perform a comparative evaluation of nano-Cu-toxicity tests in natural vs. controlled conditions in order to understand fully the impact of nano-Cu on the soil–plant system, humen/animals and the environment.

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