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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

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Abstract

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

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

Contents

Introduction

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

Copper (Cu) is a naturally occurring ubiquitous element present in the environment with a concentration around 60 g per ton in the Earth's crust (Ojha et al. 2017) and essential micronutrient for plant growth at certain concentrations and is known to play important roles in mitochondrial respiration, hormone signalling, cell wall metabolism, iron mobilization, and electron transport (Yruela 2009). However, at higher concentrations, Cu is generally toxic to plants and other organisms including algae, mussels, crustaceans, and fish (Aruoja et al. 2009; Braz-Mota et al. 2018; Katsumiti et al. 2018; Ruiz et al. 2015). While there is no data available on the concentration of CuO-NPs in the soil total Cu could range from 2-100 mg kg^{-1} in unpolluted soils (Nagajyoti et al. 2010). Soil receives Cu-based NPs from direct application of agricultural nano-products and industrial wastes (Adeleye et al. 2016; Rajput et al. 2017b, 2018b). The toxic action of pesticides specifically Cu-based NPs and Cu-based nano pesticides (e.g., Kocide 3000) makes them appropriate to be used for the control of plant pathogens and pests (Anjum et al. 2015; Shahid and Khan 2017). Cu-based fungicides have been used for more than a century contributing to soil contamination based on their Cu^{2+} content, allowing them to function as a reducing or oxidizing agent in biochemical reactions. Terrestrial species can have more interactions with NPs because up to 28% of the total NPs production ends into soils (Keller and Lazareva 2013). Substantially increased production of Cu-based NPs in the last decade emphasizes the need of thorough and systematic investigation of nano-Cu release, environmental fate, bioavailability, dissolution of Cu^{+}/Cu^{2+} ions from Cu-based NPs, exposure routes, and their toxic impacts on non-target organisms (Keller et al. 2017).

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

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

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

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

Owing to diverse applications of Cu-based NPs in the nanotechnology industry, the release of nanoscale-Cu in a different sphere of the environment is expected (Qiu and Smolders 2017). Sources of NPs include both the point and non-point sources. Point sources are comprised of production and storage units, research laboratories, disposal of nanomaterial-containing consumer products and wastewater treatment plants etc., whereas Cu discharge through non-point sources occurs through wear and tear of Cu-based NPs containing paints, cosmetic products, and cleaning agents (Rajput et al. 2018b). The Cu-NPs have potential to enter water, soil, and sediments during and at the end of their life cycle (Keller et al. 2013; Slotte and Zevenhoven 2017). Soil can receive NPs through various channels, for example, agricultural amendments of sewage sludge, atmospheric deposition, landfills, or accidental spills during industrial production (Simonin and Richaume 2015). The Cu-based NPs are available with various morphologies like Cu, CuO, Cu_2O, Cu_3N exhibiting various oxidation states, for instance, Cu^0, Cu^I, Cu^{II}, and Cu^{III}, Cu^+ (Cu^2O) or Cu^{2+} (CuO) (Ojha et al. 2017). In soil, nanoscale-Cu might be present in various forms like complexes with soil organic matters such as natural organic matter, humic acid, fulvic acid etc., Cu-NPs containing pesticides including Kocide 3000 [$nCu(OH)_2$], as complex with other metal components/plant exudates etc. (Conway et al. 2015; Gao et al. 2018; Peng et al. 2017; Servin et al. 2017a).

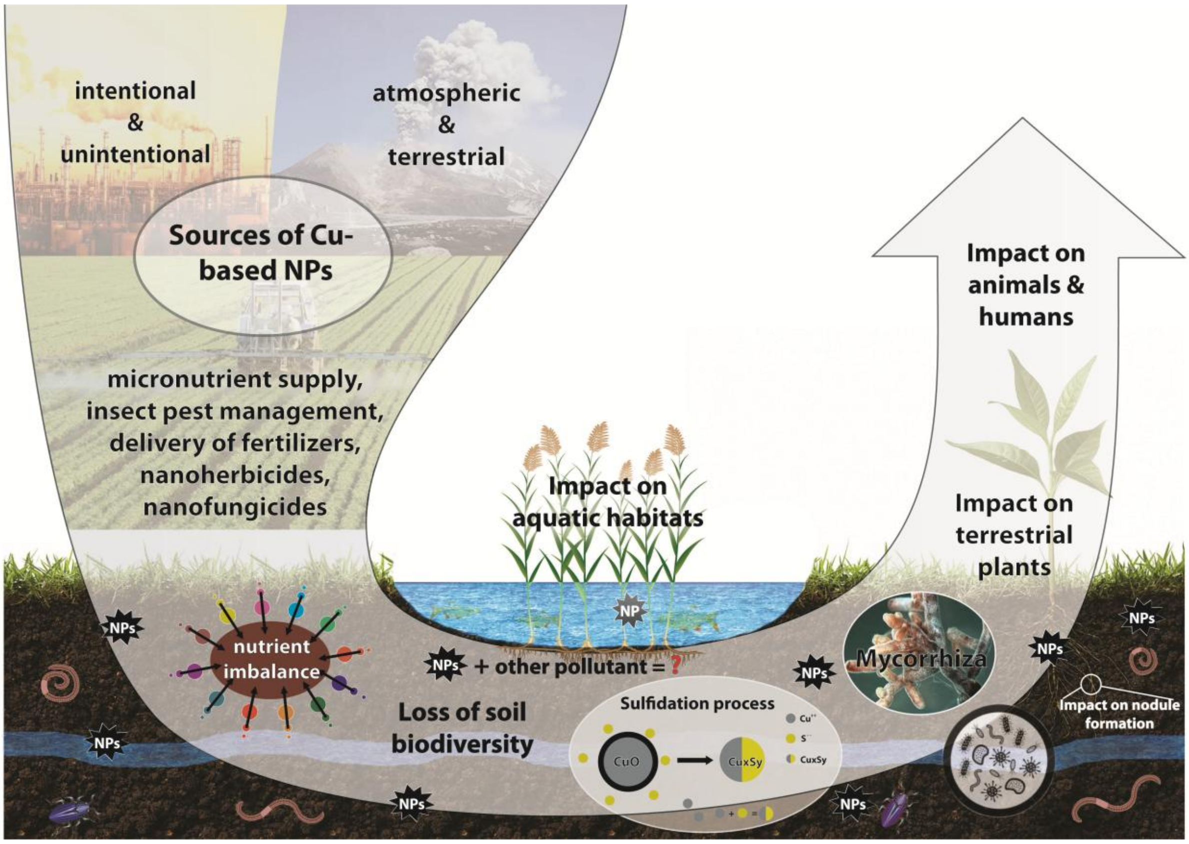

Fig 1. Schematic of CuO NPs sources to environment and their effects on different ecosystems

Due to their high density, Cu-NPs tend to settle rapidly from nano to micro scale. The Cu-NPs, both in the presence and absence of organisms may undergo micro scale aggregation with high polydispersity in water and simple salt solutions (Adeleye et al. 2014; Conway et al. 2015; Griffitt et al. 2007). In a study by Adeleye et al. (2014), only 20% Cu-NPs was detected after 6 h at pH 7.0 in NaCl (10mM) which suggested rapid aggregation of Cu-NPs leading to sedimentation. On the other hand, natural organic matter released in the environment may reduce the Cu-NPs sedimentation; for instance, approximately 40% of Cu-NPs remained stabilized by organic matter released by fish even after 48 h (Griffitt et al. 2007). Indeed, the dissolution of CuO-NPs in aqueous medium is too slow; so much so that a within concentration range of 0.01-10 mg L^{-1}, CuO-NPs showed as little as ≤1% dissolution after weeks in freshwater and after a month in seawater (Adeleye et al. 2014; Atha et al. 2012; Buffet et al. 2013; Conway et al. 2015; Hanna et al. 2013). A month after soil contaminated by CuO-NPs, an increase in labile fraction of the Cu was noted, which had negative effects on the *T. aestivum* growth (Gao et al. 2018).

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

3 Biotransformation of Cu-based NPs in soil

Being a less dynamic component of the biosphere, the soil system has a relatively high potential for pollutants accumulation in comparison to the atmosphere and hydrosphere. Soil not only acts as a depot for pollutants but also serves as a source of contaminant input into food chains. Additionally, the soil matrix is considered abundant in natural occurring NPs which exist in both forms; as primary particles and as agglomerates/aggregates. The natural organic matter of soil influences the bioavailability of NPs through a variety of mechanisms like electrostatic interactions, ligand-exchange, hydrophobic effect, hydrogen-bonding and complexation (Philippe and Schaumann 2014). The various soil processes such as homo/hetero-aggregation, oxidation, dissolution, sulfidation, sedimentation may impact NPs toxicity (Adeleye et al. 2016; Conway et al. 2015; Garner and Keller 2014; Lowry et al. 2012; Miao et al. 2015; Torres-Duarte et al. 2016). Aggregation and dissolution of NPs are generally influenced by a range of environmental factors such as pH, organic matter, ionic species and colloids. A passivation process frequently occurring under various environmental conditions is the sulfidation of CuO-NPs (Gogos et al. 2017; Ma et al. 2014). This process is expected to alter the speciation and properties of CuO-NPs significantly and might increase its apparent solubility resulting in increased bioavailability and thus eco-toxicity attributed to toxic Cu^{2+} (Ma et al. 2014).

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

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

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

The leaching and mobilization of nano-Cu ions from the source material followed by their complexation with humic acids or organic acids when secreted by fungi and contained in the plant root exudates influence the biotransformation. Although CuO-NPs are often considered as insoluble materials, the presence of organic acids such as citric and oxalic acid in the environment enhances the dissolution of Cu and CuO-NPs which in turn increases their mobility and bioavailability to plants and animals. In addition, the nature of the organic acids also affects NPs dissolution significantly (Mudunkotuwa et al. 2012). Other factors affecting NPs dissolution includes pH, dissolved organic matter, biomolecular ligands, ionic strength etc. (Yu et al. 2018). All these factors determine the toxicity of Cu-based NPs by influencing the total dissolved concentration of Cu in the concerned media. Among these factors, the pH has an inverse relationship with dissolution. The CuO-NPs have good solubility at lower pH which is turn down as the pH increases. However, the presence of ligands including those with amine functional groups, induce solubility of CuO-NPs at neutral pH (Wang et al. 2013). Recently, Kovacec et al. (2017) investigated potential efficacy of two phytopathogenic fungi namely *Botrytis cinerea* and *Alternaria alternate* for biotransformation of Cu^{2+} ions, micro and nanoparticulate forms of Cu and CuO. The study revealed that *B. cinerea* could transform micro and nanoparticulate forms of Cu and CuO into Cu-oxalate complex.

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

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

4 Interaction of Cu-based NPs with soil organisms

Deliberate administration of NPs into soils might have a significant impact on the living entities, as they are extremely resistant to degradation and have the potential to accumulate in the soil. The effect of NPs may also vary with varying concentration, soil properties, and enzymatic activity. Soil properties, such as pH, texture, structure, and organic matter content influence the structure of soil microbial community and the ability of pollutants to exert toxic effects on microorganisms (Simonin and Richaume 2015). As NPs have the ability to mobilize soil pollutants, comparison of the toxicity of the NPs in various soil types is much required. In order to understand the influence of soil physicochemical properties on Cu-based NPs toxicity, a number of predictive models have been developed; however, these models are not always effective for other region soils (Duan et al. 2016).

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

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

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

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

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

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

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

5 Uptake and bioaccumulation of Cu-based NPs in plants

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

In a study, the translocation and biotransformation of CuO-NPs in rice plants were explored. It was revealed that CuO-NPs get accumulated in epidermis and exodermis regions of the plants and get precipitated with citrate or phosphate ligands or get bound to amino acids forming Cu-cysteine, Cu-citrate, and $Cu_3(PO_4)_2$ kind of products or get reduced to Cu(I) (Peng et al. 2015). Cu(I) is a highly redox active species capable of producing hydroxyl radicals by Fenton-like reactions, and so its presence in even smaller quantities has significant biological importance. Servin et al. (2017a) compared bioaccumulation of un-weathered and weathered CuO-NPs, bulk and ions in lettuce plants after 70 days. In the case of CuO-bulk, weathered material was found to decrease Cu accumulation in plant roots, whereas, weathering had a positive impact on bioaccumulation of NPs. The authors further unearthed that in roots exposed to weathered NPs, the major 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 bind to sulfur in reduced form as Cu_2S. In contrast, roots exposed to un-weathered NPs showed negligible biotransformation. As the ageing/weathering have a profound effect on the particle-size, particle-size distribution, surface properties, composition, reactivity etc., it is an important aspect which needs to be considered while assessing the environmental implication of Cu-based NPs. Similarly, the translocation and biotransformation of NPs is a plant-specific phenomenon which requires adequate attention.

The nano-phytotoxicity studies on accumulation and uptake of NPs have generated important data for understanding the fate of Cu-based NPs in plants (Ingle et al. 2014; Ma et al. 2010). Once NPs infiltrate the plant system, they may traverse to different organs (leaves, stem, and fruits) or may get compartmentalized at different locations *viz.* vacuoles, walls, stellar system, cytoplasmic matrix, lipid envelopes, and nucleus (Ahmed et al. 2018b; Rajput et al. 2017b, 2018a; Rastogi et al. 2017). The translocation efficiency varies greatly in different plant species, for instance, alfalfa translocates 3-5% of Cu from root to shoot on exposure to 0-20 mg L^{-1} Cu-NPs, whereas only 0.5-0.6% translocation was observed in lettuce (Hong et al. 2016). Before the plant uptake, the dissolution of Cu-NPs increases the likelihood that Cu is internalized as Cu^{2+} ions or in the form of organic complexes (Keller et al. 2017). A recent study revealed the adsorption and accumulation of Cu-based NPs in tomato plants leads to the adsorption of nano-CuO on the roots (Ahmed et al. 2018b). Similarly, maize roots showed 3.6 fold greater Cu content under CuO-NPs treatments (Wang et al. 2012). Also, the Cu content was 7 times higher in shoots of maize treated with 100 mg L^{-1} CuO-NPs. In this context, Zuverza-Mena et al. (2015) also reported the translocation of Cu-based NPs in cilantro and their significant accumulation in shoots. Differential accumulation profile of CuO-NPs has been reported in ryegrass and radish (Atha et al. 2012). Wheat and bean seedlings grown on dual agar media have been adequately discussed pertaining to the bioavailability of Cu-NPs and their relationship between accumulation and uptake (Woo-Mi et al. 2008). Cu-NPs were toxic to both plants and also bioavailable. A Cu ion released from Cu-NPs has negligible effects in the studied concentration range, and the apparent toxicity is clearly due to Cu-NPs. Bioaccumulation increased with increasing concentration of Cu-NPs and agglomeration of particles was observed in the plant cells by using transmission-electron microscopy-energy-dispersive spectroscopy (TEM-EDX). In shoots of wheat grown in the sand matrix, the bioaccumulated Cu was detected as Cu(I)S complex and CuO (Dimkpa et al. 2012). The level of Cu accumulation in wheat shoots under CuO-NPs exposure was almost equal to the concentrations quantitated in bulk (Dimkpa et al. 2012).

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

The bioaccumulated nano-Cu or CuO is also subject to transportation and transformation in plants (Ahmed et al. 2018c). For instance, the treatment of hydroponically cultured lettuce plant with CuO/Cu-based NPs caused a greater accumulation of Cu than cupric ions (Trujillo-Reyes et al. 2014). Additionally, the xylem and phloem based transport system to shoots and back to roots were proposed for CuO-NPs accumulation in root cells, cytoplasm, intracellular space, and nuclei of xylem and cortical cells. However, the CuO-NPs was reduced from Cu (II) → Cu (I) in due course of translocation (Wang et al. 2012). A similar transformation of CuO-NPs has been reported with an elevation in the degree of saturation of fatty acids (Yuan et al. 2016). In another study, when *Zea mays* were exposed to CuO-NPs, ionic, and bulk CuO, the Cu content in root and shoot of the plant was found enhanced under CuO-NPs (Wang et al. 2012). A micro X-ray fluorescence (µXRF) study revealed that Cu-NPs may get accumulated in outer parts of the root (Servin et al. 2017a). The translocation of Cu-NPs also varies depending upon the growth media. For instance, alfalfa, lettuce and cilantro exposed to CuO, Cu and $Cu(OH)_2$ NPs based pesticide in soil showed >87-99% Cu accumulation mostly in roots with very little transportation to shoots and negligible in leaves (Hong et al. 2015; Zuverza-Mena et al. 2015). In some recent studies, Cu-NPs were also detected in leaves, stems, and fruits of cucumber and tomato when grown in soil system (Zhao et al. 2016a). The uptake of CuO-NPs in tomato, alfalfa, cucumber, and radish seedlings was also noticed in the range of 4-1748 $\mu g\ g^{-1}$ dry biomass when grown on semi-solid agar media (Ahmed et al. 2019). In a comparative study between soil and hydroponically grown tomato plants, the organ wise distribution of CuO-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 having enough physicochemical features to exert toxicity, plants trigger their antioxidant defence mechanism to prevent oxidative damage, as well as enhance their resistance towards NPs toxicity. For instance, cucumber plants grown hydroponically in the presence of CuO-NPs (50 nm) were found with augmented anti-oxidative enzymes *viz.* catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Kim et al. 2012). However, *C. sativus* when grown hydroponically in the presence of Cu NPs (10-30 nm) experienced significant phytotoxic effects which were not ameliorated by antioxidant enzymes adequately (Mosa et al. 2018). The NPs arbitrated phytotoxicity is predominantly related to their physicochemical properties. The Cu-based NPs cause phytotoxicity via the dissolution and release of higher concentration of ions such as Cu^{2+} or the production of excess reactive oxygen species (ROS) (Ahmed et al. 2019; Letelier et al. 2010). ROS can affect mitochondrial respiration, apoptosis, lipid peroxidation in the cell membrane, and induce a range of antioxidant responses (Dimkpa et al. 2012; Shaw and Hossain 2013). Recent studies of CuO-NPs phytotoxicity showed negative impacts on seed germination and overall plant growth of various crops such as *Lactuca sativa* (100-300 mg L^{-1}), *Medicago sativa* (0-20 mg L^{-1}), *Triticum aestivum* (200 mg L^{-1}), *Vigna radiate* (500 mg L^{-1}), *Zea mays* (2-100 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 *Glycine max* (50-500 mg L^{-1}) (Rajput et al. 2017b).

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

6.1 Effects on seed germination, morphometry and plant growth

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

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

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

6.2 Effects on cellular ultrastructure

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

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

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

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

6.3 Effects on plant physiology and photosynthetic systems

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

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

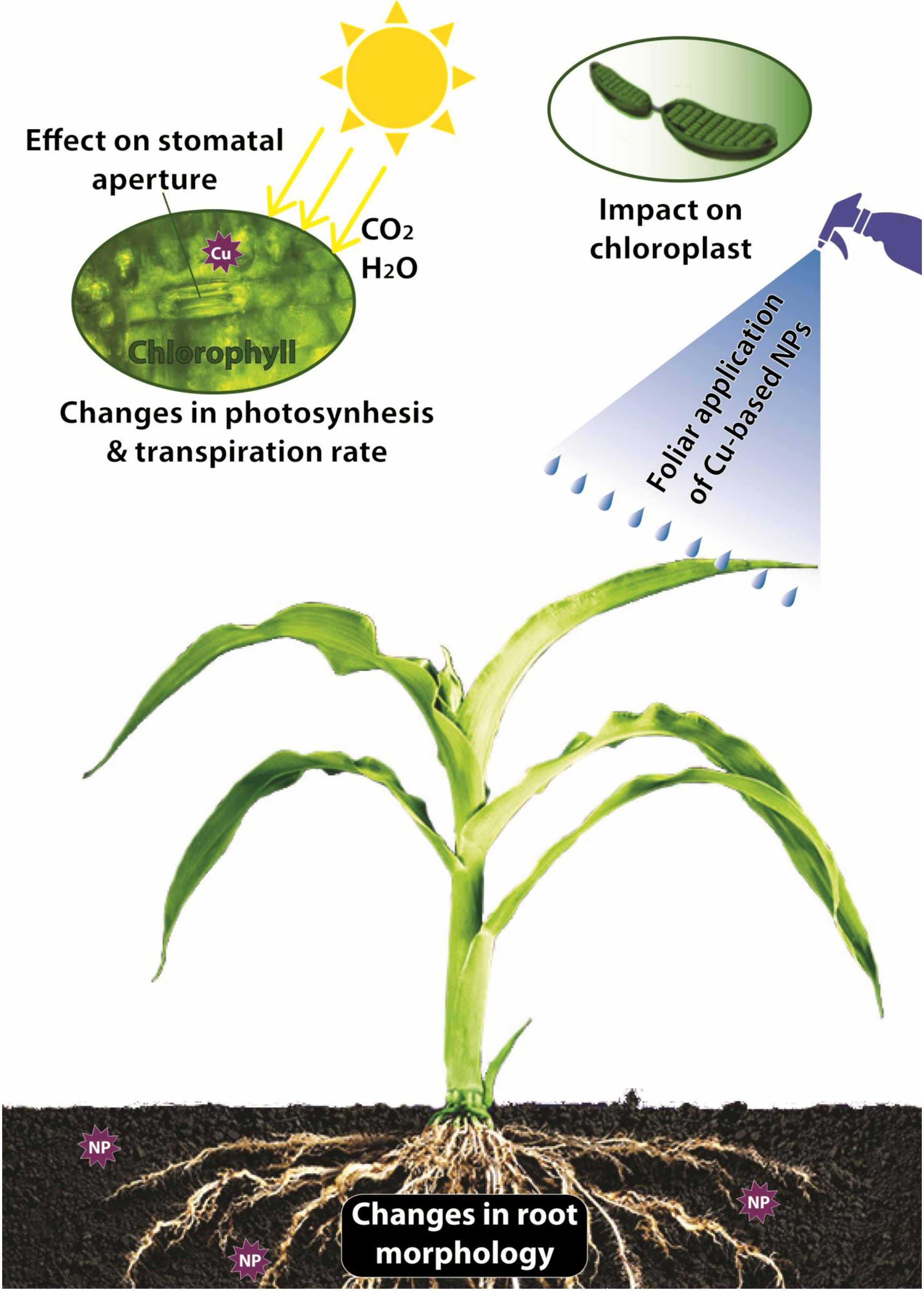

Fig 2. Schematic presentation of effects of Cu-based NPs on photosynthesis

6.4 Effects on plant metabolism and nutrient content

Several studies have demonstrated that Cu-based NPs also significantly affect the metabolism and nutrient content of plants. For example, foliar application of $Cu(OH)_2$ nano pesticide (50-1000 nm) at 1050-2100 mg L^{-1} alters metabolite level of *L. sativa* leaves (Zhao et al. 2016b). Gas Chromatography-Time-of-Flight Mass Spectrometry (GCTOF-MS) based analysis combined with Partial Least Squares-Discriminant Analysis (PLS-DA) multivariate analysis shows disturbance in tricarboxylic acid (TCA) cycle and amino acid related pathways (Zhao et al. 2016b; 2017b). An increased level of potassium, putrescine, and spermidine in $Cu(OH)_2$ nano-pesticide treated plants has been suggested to reduce the oxidative stress and enhance the tolerance (Zhao et al. 2016b). Similarly, in cucumber grown with Cu-NPs (40 nm) in soil (200-800 mg kg^{-1}) and hydroponics (10 and 20 mg L^{-1}) exhibited perturbation in iron, sodium, phosphorus, zinc, sulphur, and molybdenum uptake and alterations in cucumber fruit metabolite profile (Zhao et al. 2016a). Additionally, TCA cycle and galactose metabolism also get compromised (Zhao et al. 2016b). CuO and $Cu(OH)_2$ nano pesticides also decrease the level of shoot phosphorus and iron in lettuce (Hong et al. 2015). Moreover, CuO- NPs (<50 nm) at 500 mg kg^{-1} soil has shown to reduce iron, manganese, zinc, and calcium in common bean (Dimkpa et al. 2015). Moreover, micro- and macronutrients elemental composition in cilantro has been found to be suppressed when grown with CuO-NPs (10^1-10^2 nm) and Cu-NPs (10^2-10^3 nm) at 0-80 mg kg^{-1} soil (Zuverza-Mena et al. 2015). The Cu-based NPs have also been documented to bring down the agronomically important characteristics of plants. The CuO-NPs (<50 nm) reduces carotenoids level in rice at 1 and 1.5 mM (Shaw and Hossain 2013). Similarly, the decrease in the firmness of cucumber fruits has been reported upon treatment with CuO-NPs of <50 nm (Hong et al. 2016). Also, the grain yield of rice has been shown to reduce significantly by CuO-NPs (~ 43 nm) at 500 and 1000 mg kg^{-1} (Peng et al. 2017).

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

6.5 Genotoxic and cytotoxic effects

Genotoxicity is one of the most devastating effects exerted by NPs on plants. A variety of toxic effects have been reported for NPs which may interact with biological systems via five main modes: (i) chemical effects as metal ions in solution upon dissolution; (ii) mechanical effects owing to hard spheres and defined interfaces; (iii) catalytic effects on surfaces; (iv) surface effects owing to binding of proteins to the surface, either by non-covalent or covalent mechanisms or oxidative effects; and (v) changes in the chemical environment (pH). Metal and metal oxide NPs have been shown to act as mediators of DNA damage in mammalian cells, organisms, and even in bacteria, but the molecular mechanisms through which this occurs are poorly understood. For the first time, it was reported that CuO-NPs induce DNA damage in crops and grassland plants (Atha et al. 2012). The Cu–NPs, up to 20 μg ml^{-1} increased the mitotic index of actively dividing cells in *A. cepa* with a gradual decline in the mitotic index as the concentration increased (Nagaonkar et al. 2015). Smaller sized NPs, increasing concentrations, and exposure duration of NPs have been related to greater genotoxic responses, leading to mito-depressive effects in the cell cycle. Micronuclei formation, disturbed chromosomes, chromosome fragments, stickiness, bridge, laggards' chromosomes and decrease in mitotic index are the most obvious anomalies in plants exposure to silver, copper, titanium dioxide, zinc, zinc oxide, selenium oxide, multi-wall carbon nanotube, tetramethylammonium hydroxide and Bismuth (III) oxide NPs. The severity of abnormalities depending on the concentration, duration time and particle size are different. Finally, if the DNA repair mechanisms are not enough to restore these alterations, it can lead to loss of genetic material and mutation in DNA (Karami and Lima 2016). The plant DNA is also affected by cellular oxidative stress generated by Cu-based NPs. Atha et al. (2012) reported oxidative-stress induced DNA lesions in *R. sativus*, *Lolium perenne*, and *L. rigidum* by CuO-NPs (10-1000 mg L^{-1}) that include 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 8-OH-dG, the 2′-deoxynucleoside form of 8-OH-G, and 4,6-diamino-5-formamidopyrimidine (Atha et al. 2012). Cu-based NPs exposure has been attributed to induce genotoxic effects and affect the normal cell cycle. Chromosomal aberrations such as sticky and disturbed chromosomes in metaphase/anaphase, c-metaphase, bridges, laggard chromosomes, disturbed telophase, and vacuolated nucleus resulted after exposure to Cu/CuO-NPs in onion and black cumin (Deng et al. 2016; Kumbhakar et al. 2016; Nagaonkar et al. 2015). These aberrations are very similar to those induced by ethyl methanesulphonate (EMS) and gamma radiation. With the use of random amplified polymorphic DNA (RAPD), the genotoxicity of CuO-NPs (~50 nm) has been demonstrated in buckwheat (Lee et al. 2013). The authors demonstrated changes in DNA bands in 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 genetic pattern induced by Cu-NPs toxicity could be attributed to changes in genomic DNA template stability due to mutations homologous recombination, deletion of large DNA segments and might be due to the strong binding of NPs with plant DNA (Ahmed et al. 2018b; Lee et al. 2013). The DNA isolated from young tomato leaves upon interaction with various concentrations of CuO-NPs exhibited concentration-dependent fluorescence quenching of acridine orange-DNA complex and ethidium bromide-DNA complex (Ahmed et al. 2018b). The CuO-NPs are able to interact with plant DNA in both intercalative and non-intercalative mode with perceptible changes in other macromolecules like amide I and II of proteins and carbohydrates (Ahmed et al. 2018b). The transfer of CuO-NPs to progeny (harvested seeds) of *Arabidopsis thaliana* has been studied by XANES in the form of CuO (88.8%), moreover, Cu in seeds has been detected as Cu-acetic acid (3.2%), $Cu_2(OH)PO_4$ (2%), and Cu_2O (6%) (Wang et al. 2016). Recently, the change in the gene expression pattern of plants exposed to CuO-NPs has been reported. Wang et al. (2016) documented differential expression of gene Fe-SOD and gene Aux/IAA in the regulation of *A. thaliana* root growth when exposed to 20 and 50 mg L^{-1} CuO-NPs. Similarly, altered gene expression has been observed by surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF) in cucumber seeds after treatment with nano-CuO at 600 mg L^{-1} (Moon et al. 2014). In this study, among 34 differentially expressed proteins about 9 differed from those exposed to control and bulk-CuO treated plants. A protein (5977-m/z) has been found as the most distinguished biomarker for the determination of CuO-NPs induced phytotoxicity (Moon et al. 2014).

Interaction of Cu-NPs with plant root exudates also influences the fate of Cu-NPs and magnitude of toxicity. Huang et al. (2017) determined the thermodynamic parameters for the interaction of Cu-NPs (40 nm) with a mixture of synthetic root exudates (SRE) and its components such as sugars, amino acids, organic acids, and phenolic acids by nano isothermal titration calorimetry. The data revealed a strong binding constant ($K_d = 5.645 \times 10^3\ M^{-1}$) for Cu-NPs SRE interaction, however, the binding of Cu^{2+} was found stronger but varied for individual SRE components (Huang et al. 2017).

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

6.6 Effects on plants ROS and anti-oxidative activities

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

The ROS generation reportedly induces damage to cellular membranes resulting in respiratory loss and lipid peroxidation leading to disruption of vital cellular functions (Gueraud et al. 2010; Maness et al. 1999). In the presence of high concentrations, Cu can promote the generation of ROS by Fenton reaction ($Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^\bullet + OH^-$) due to its high redox-active nature (Halliwell and Gutteridge 1985). ROS interaction with protein sulfhydryl (-SH) groups may cause enzyme inactivation which in all likelihood may lead to necrosis, chlorosis, and growth inhibition (Das and Roychoudhury 2014; Xiong and Wang 2005; Yruela 2009). Among ROS, hydroxyl radicals formed via Haber-Weiss reaction ($H_2O_2 + O_2^{\bullet-} \rightarrow OH^\bullet + OH^- + O_2$) are considered to be more toxic (Letelier et al. 2010). To mitigate the ROS stress induced by Cu-NPs, plants elevate the activity of antioxidant enzymes such as superoxide dismutase (SOD) (Wang et al. 2016), ascorbate peroxidase (APX) (Hong et al. 2015;

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

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

7. Toxicity on aquatic systems

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

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

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

7.1 Toxicity on aquatic plants

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

^{1}) for 14 days exhibited discolouration along with the visible signs of turgor loss in mesophilic cells. Morphological changes in the root system were more prominent. In comparison to the control plant, blackening of roots together with inhibition of new growth roots, and a decrease in plant weight, amino acids, and the content of ascorbic acid reduced by 63% was observed in exposed plants (Olkhovych et al. 2016). The morphological changes were also observed for plant *L. gibba* in the form of leaf reduction and detachment of fronds from the plant. The symptoms were detected after 24 h CuO-NPs exposure with 1.0 mg L^{-1} (Perreault et 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 comparison to Cu-free samples (Melegari et al. 2013, Song et al. 2016). The Cu-based NPs exposure on aquatic flora is mostly reflected in photosystem dysfunction. The chlorophyll content of *L. minor* decreased with the increase in concentration at 100 mg L^{-1} CuO-NPs (Song et al. 2016). In the algal culture of *C. reinhardti*, the decrease of total chlorophyll and carotenoids was observed at 1000 mg L^{-1} when exposure lasted for 72 h (Aruoja et al. 2009). For microalgae, *Pseudokirchneriella* 6.4 mg L^{-1} was sufficient to evoke abnormality in photosynthetic system performance (Melegari et al. 2013). In the study of Perreault et al. (2014), lower photosynthetic electron transport rate for *L. giba* was observed. The Cu-NPs at a concentration higher than 1 mg L^{-1} clearly suppresses photosynthesis on *Elodea densa* (waterweed) while low concentration (<0.25 mg L^{-1}) has a positive impact on photosynthesis effectiveness (Nekrasova et al. 2011). The main feature of Cu-based NPs is that they have the ability to cross the plasma membrane that results in alteration of subcellular organelles. This condition substantially may cause oxidative stress which is connected to increased enzymatic activity (i.e., POD, CAT, and SOD) (Melegari et al. 2013). The production of ROS may be the result of conditions when plants are subjected to harmful stress conditions. The chloroplasts and mitochondria of plant cells are important in intracellular generators of ROS. Internal O_2 concentration is high during photosynthesis, and chloroplasts are particularly prone to generate ROS; therefore, these cytotoxic ROS can remarkably disrupt normal metabolism through oxidative damage of lipids, nucleic acids, and proteins.

In general Cu-based pollutants induce various responses within the photosynthetic organism. The changes seem to be the most prominent for the CuO-NPs and Cu-NPs following by $CuSO_4$ and bulk CuO. The Cu-NPs toxicity heavily depends on dosage and further surface modification.

7.2 Toxicity on aquatic animals

There is currently a significant gap in our knowledge about CuO-NPs toxicity to aquatic animals. In general, the Cu(O) NPs toxicity may be a potential environmental concern for crustaceans, as LC50 values are within an order of magnitude of predicted wastewater concentrations, while chronic and developmental toxicity are a more relevant concern for fishes (Braz-Mota et al. 2018). A few studies have noted bioactivity in these animals at high concentrations (20 µg L^{-1}). The release of manufactured Cu-based NPs into the aquatic environment is rather rarely known (Moore 2006). Nevertheless, it was proven that NPs association with naturally occurring colloids may affect their bioavailability and uptake into cells and organisms. Uptake by endocytic routes was previously identified as probable major mechanisms of entry into cells; potentially leading to various types of toxic cell injury (Moore 2006). Griffitt et al. (2009) demonstrated that the effects of Cu-NPs were not solely due to the release of soluble metals into the water column. These studies highlight the need for further studies focused on understanding the mechanisms of NPs toxicity to aquatic organisms as dissolution and the presence of a generic NPs response are not sufficient to explain the observed effects.

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

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

Abdel-Khalek et al. (2015) compared the toxicity of CuO-NPs to Nile Tilapia (*Oreochromis niloticus*) with its bulk counterpart and reported that the LC50/96 h of CuO bulk particles (BPs) was higher than that of NPs indicating that CuO-NPs are more toxic. The CuO-NPs could exert more toxic effects despite the fact that they are smaller in size than the CuO-BPs, and they can form aggregates in suspensions. The authors demonstrated CuO (BPs & NPs) induced biochemical alterations and oxidative stress in *O. niloticus*, which suggest ecological implications of CuO-NPs released in aquatic ecosystems. The study conducted by Braz-Mota et al. (2018) aimed to understand the effects of CuO-NPs and Cu on two ornamental Amazon fish species: dwarf cichlid (*Apistogramma agassizii*) and cardinal tetra (*Paracheirodon axelrodi*). For fish exposed to 50% of the LC50 for CuO-NPs, aerobic metabolic rate (MO_2), gill osmoregulatory physiology and mitochondrial function, oxidative stress markers, and morphological damage were evaluated. The results revealed species specificity in metabolic stress responses. An increase of MO_2 was noted in cardinal tetra exposed to Cu, but not CuO-NPs, whereas MO_2 in dwarf cichlid showed little change with either treatment. In contrast, mitochondria from dwarf cichlid exhibited increased proton leak and a resulting decrease in respiratory control ratios in response to CuO-NPs and Cu exposure. This uncoupling was directly related to an increase in ROS levels. The authors revealed different metabolic responses between these two species in response to CuO-NPs and Cu, which are probably caused by the differences between species natural histories, indicating that different mechanisms of toxic action of the contaminants are associated to differential osmoregulatory strategies among species.

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

The effect of Cu-NPs on the development of zebrafish embryos was depicted by Sun et al. (2016). The exposure to CuO-NPs at concentrations of 12.5 mg L^{-1} or higher leads to abnormal phenotypes and induces an inflammatory response in a dose-dependent pattern. Moreover, exposure to CuO-NPs at high doses results in an underdeveloped liver and a delay in retinal neurodifferentiation accompanied by reduced locomotor ability. The authors demonstrated that short-term exposure to CuO-NPs at high doses shows hepatotoxicity and neurotoxicity. On the other hand, cellular and molecular responses of adult zebrafish after exposure to CuO-NPs or ionic Cu were tested by Vicario-Pares et al. (2018). Another study performed by Bai et al. (2010) was undertaken to test the toxicity of nano-Cu suspension to zebrafish embryos. It was found that nano-Cu retarded the hatching of zebrafish embryos and caused morphological malformation of the larvae. The authors claimed that high concentrations (>0.1 mg L^{-1}) of nano-Cu can kill the gastrula-stage zebrafish embryos. Denluck et al. (2018) investigated the role of the chorion in nanomaterial toxicity. The authors found that the presence of the chorion inhibited Cu-NPs toxicity: while dechorionated embryonic zebrafish exposed to Cu-NPs had an LC50 of 2.5 ± 0.3 mg L^{-1}, a chorion-intact had LC50 of 13.7 ± 0.8 mg L^{-1}. In summary, embryo sensitivity increased by at least one order of magnitude when chorions were removed.

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

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

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

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

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

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

TEM remains one of the main tools to analyse Cu–based NPs distribution (Lee et al. 2008; Nhan Le et al. 2016) and composition in plants (Trujillo-Reyes et al. 2014; Wang et al. 2011). The XRF technique was applied to reveal the elemental composition of *C. sativus* shoot and root samples treated with Cu-NPs (Mosa et al. 2018). 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-XANES, and the most dominant form of Cu was from CuO-NPs (Peng et al. 2015). A combination of μ-XRF and μ-XANES was used to study bioaccumulation un-weathered (U) and weathered (W) CuO-NPs, bulk and ionic form by lettuce (Servin et al. 2017b). The μ-XRF analysis of W-NP-exposed roots showed a homogenous distribution of Cu in the tissues, while μ-XANES analysis of W-NP-exposed roots showed near complete transformation of CuO to Cu (I)-sulfur and oxide complexes in the tissues. Duran et al. (2017) showed that CuO-NPs did not affect seed germination of *Phaseolus vulgaris* L., but seedling weight gain was promoted by 100 mg Cu L^{-1} and inhibited by 1000 mg Cu L^{-1} of 25 nm CuO and $CuSO_4$. The μ-XRF analysis showed that most of the Cu taken up remained in the seed coat with Cu hotspots in the hilum. Moreover, μ-XANES unravelled that most of Cu remained in its pristine form. Zhao et al. (2017a) showed significant growth inhibition on both roots and shoots of *E. crassipes* after 8-day exposure of CuO-NPs (50 mg L^{-1}) which was much higher than that of the bulk CuO particles and dissolved Cu^{2+} ions of the same Cu concentration. The XANES was used to reveal the presence of CuO-NPs as well as Cu_2S and other Cu species in roots, submerged leaves, and emerged leaves of plants providing solid evidence of the transformation of CuO-NPs. Electron microscopy remains one of the most widely used tools to study distribution, morphology and composition of metal NPs in plants. The possibilities that such synchrotron radiation techniques as μ-XRF and μ-XANES open to environmental scientists could significantly change the situation in the sense of revealing precise information on its structure. Moreover, an sp-ICP-MS becomes one of the most promising technique to obtain the presence and size distribution of NPs at environmentally relevant concentrations.

9 Conclusion and future outlook

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

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

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

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Conflict of interest

The authors declare that they have no conflict of interest.

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