Environmental Pollution 234 (2018) 692–698

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Dissolved organic matter reduces CuO nanoparticle toxicity to duckweed in simulated natural systems☆

Devin A. Rippner [a], Peter G. Green [a, b], Thomas M. Young [b], Sanjai J. Parikh [a, *]

[a] University of California, Davis, Department of Land, Air and Water Resources, One Shields Avenue, Davis, CA, 95616, USA
[b] University of California, Davis, Department of Civil and Environmental Engineering, One Shields Avenue, Davis, CA, 95616, USA

ARTICLE INFO

Article history:
Received 10 July 2017
Received in revised form
1 December 2017
Accepted 4 December 2017

Keywords:
Nanotoxicology
Metal toxicology
Copper
Dissolved organic matter

ABSTRACT

With increasing demand for recycled wastewater for irrigation purposes, there is a need to evaluate the potential for manufactured nanomaterials in waste water to impact crop production and agroecosystems. Copper oxide nanoparticles (CuO NPs) have previously been shown to negatively impact the growth of duckweed (*Landoltia punctata*) a model aquatic plant consumed by water fowl and widely found in agricultural runoff ditches in temperate climates. However, prior studies involving CuO NP toxicity to duckweed have focused on systems without the presence of dissolved organic matter (DOM). In the current study, duckweed growth inhibition was shown to be a function of aqueous Cu^{2+} concentration. Growth inhibition was greatest from aqueous $CuCl_2$ and, for particles, increased with decreasing CuO particle size. The dissolution of CuO NPs in ½ Hoagland's solution was measured to increase with decreasing particle size and in the presence of Suwannee river humic and fulvic acids (HA; FA). However, the current results suggest that HA, and to a lesser extent, FA, decrease the toxicity of both CuO NPs and free ionized Cu to duckweed, likely by inhibiting Cu availability through Cu-DOM complex formation. Such results are consistent with changes to Cu speciation as predicted by speciation modeling software and suggest that DOM changes Cu speciation and therefore toxicity in natural systems.

1. Introduction

The market for manufactured nanomaterials (MNMs) is rapidly expanding, with a global predicted value of 3 trillion dollars by 2020 (Roco, 2011). These materials are used in a wide range of products including paints, personal care products, high definition TVs, and electronic cooling fluids (Keller et al., 2013; Khaleduzzaman et al., 2014; Lin et al., 2010; Luo et al., 2006). One class of MNMs, copper oxide nanoparticles (CuO NPs), exhibit significant toxicity to plants in terrestrial and aquatic ecosystems (Dimkpa et al., 2013; Perreault et al., 2014b; Shi et al., 2011). Estimates of CuO NP toxicity to plants of the sub-family Lemnoideae, a sub-family frequently used for aquatic toxicity assays, vary widely (Lalau et al., 2015; Perreault et al., 2014b; Shi et al., 2011; Song et al., 2015). Specifically, for *Landoltia punctata* (duckweed), a member of the Lemnoideae sub-family, measured concentrations of CuO NPs that inhibited duckweed growth by at least 20% range from 1 mg CuO NPs L^{-1} to 85 mg CuO NPs L^{-1} (Lalau et al., 2015; Perreault et al., 2014b; Shi et al., 2011; Song et al., 2015). Differences in measured toxicity reported in previous studies may be due to differences in the size of CuO NPs administered to the duckweeds and differing experimental endpoints (Song et al., 2015; Thwala et al., 2016).

Previous investigations into CuO NP toxicity to duckweed primarily focused on the effects of CuO NP concentration, particle size, and particle coating, on duckweed growth, with experiments conducted in nutrient media without dissolved organic matter (DOM). In systems without DOM, ionic strength and pH control the physical and chemical properties of CuO NPs, including dissolution and aggregation (Misra et al., 2012; Sousa and Teixeira, 2013). In high ionic strength solutions, such as nutrient media, CuO NPs are predicted to aggregate, reducing the reactive surface areas of the particles (Misra et al., 2012; Sousa and Teixeira, 2013). At low pH, CuO NPs are predicted to dissolve, increasing the concentration of free Cu (Misra et al., 2012).

In natural aquatic systems, however, DOM may control these processes, influencing the toxicity of MNMs, especially CuO NPs (Aiken et al., 2011; Keller et al., 2017; Levard et al., 2012; Lin et al.,

☆ This paper has been recommended for acceptance by Baoshan Xing.
* Corresponding author.
E-mail address: sjparikh@ucdavis.edu (S.J. Parikh).

https://doi.org/10.1016/j.envpol.2017.12.014
0269-7491/

2010; Lowry et al., 2010; Odzak et al., 2014; Wang et al., 2016). Dissolution may be enhanced in the presence of DOM through ligand exchange processes whereby Cu is complexed and solubilized from CuO NPs (Adeleye et al., 2014; Odzak et al., 2014; Wang et al., 2015). The potential bioavailability of DOM-Cu complexes is complicated; previous investigations have reported contradictory results (Halim et al., 2003; Piccolo, 1989). Likely, DOM-Cu complex bioavailability is governed by DOM conformation and functional group chemistry, whereby Cu binding affinities for specific functional groups determine potential bioavailability (Aiken et al., 2011). Further, DOM adsorption on CuO NPs may change CuO NP surface properties, promoting or inhibiting aggregation through electrostatic repulsion/attraction and steric hindrance (Adeleye et al., 2014; Li et al., 2016; Wang et al., 2016). Small, monodispersed CuO NPs are more likely to penetrate plant cell walls than agglomerates or aggregates of CuO NPs, though intact NP internalization is dependent on numerous factors including plant species, NP surface charge, NP size, and NP composition (Davis, et al., 2017; Perreault et al., 2012, 2014a; Spielman-Sun et al., 2017; Thwala et al., 2013; Wang et al., 2012). Given the presence of DOM in natural aquatic systems, it is important to quantify the impact that DOM may have on the toxicity of CuO NPs to vascular aquatic plants such as duckweed.

The objectives of the current work were to 1) evaluate the effect of CuO particle size and concentration on duckweed growth, 2) quantify the impact of CuO particle size on CuO dissolution, measure the growth media. Further dissolution, 3) quantify changes in CuO NP toxicity to duckweed in the presence of DOM, measure changes in Cu uptake by duckweed in the presence of DOM, and 4) use existing Cu speciation models to evaluate changes in free Cu availability to duckweed after exposure to CuO NPs, with and without DOM.

2. Materials and methods

2.1. Copper oxide nanoparticle synthesis and characterization

The 22 nm CuO NPs were synthesized by wet precipitation as described previously (Siddiqui et al. (2013). Briefly, 300 mL of 0.2 M copper (II) acetate monohydrate [$Cu(CH_3COO)_2 \cdot H_2O$] and 1 mL glacial acetic acid were heated and vigorously mixed in an Erlenmeyer flask capped with a watch glass. Upon boiling, 15 mL of 6 M NaOH solution was added to the flask, inducing the rapid formation of 22 nm CuO. The resulting precipitate was concentrated by centrifugation, 30 min at 15,000× *g*, washed repeatedly on sterile 0.22 μm filter paper with 18.2 MΩ cm water (Barnstead NANOpure water, Thermo Fisher Scientific, USA) and 95% ethanol, and dried at 60 °C for 6 h. Bulk micron sized CuO (CuO MPs, 620 nm) and 50 nm CuO were procured from Acros Organics (USA) and Sigma Aldrich (USA), respectively.

Primary particle size and shape of CuO particles were characterized by transmission electron microscopy (TEM; Philips CM-12, The Netherlands) at 120 kV. Samples were prepared for TEM analysis by placing 10 μL of a stock 100 mg L^{-1} CuO suspension on formvar film coated Cu grids (Ted Pella, USA) and drying the grids over an incandescent bulb. Mean primary particle size ($n > 200$) for all 3 CuO samples was calculated from TEM images using Fiji (Schindelin et al., 2012). Particle mineralogy was confirmed by X-ray diffraction (Rigaku Ultima IV, Japan). Measurements were performed using a Cu X-ray source operating at a tube voltage of 40 kV, a tube current of 40 mA, at 25 °C, and a scan rate of 2° 2θ min^{-1}. Mineral identification was confirmed using Jade 9 (MDI, Livermore, USA). Hydrodynamic radius data was collected for 22 nm CuO and 50 nm CuO dispersed (100 mg L^{-1} CuO) in 1 mmol L^{-1} KCl and ½ Hoagland's solution by dynamic light scattering at a wavelength of 660 nm using a ZetaPlus (Brookhaven Instruments Corp., USA). Zeta potentials were determined for 22 nm CuO and 50 nm CuO dispersed (100 mg L^{-1} CuO) in 1 mmol L^{-1} KCl and ½ Hoagland's solution using ZetaPlus software. Hydrodynamic radius and zeta potential data were not determined for CuO MPs due to rapid sedimentation.

2.2. Characterization of Suwannee River humic acid and fulvic acid

Suwannee River humic acid (HA) and fulvic acid (FA) were purchased from the International Humic Substances Society (USA). Carbon contents of HA and FA powders as used were determined by dry combustion using a Costech CHN analyzer (Costech, USA). The degree of aromaticity of HA and FA was inferred by measuring specific ultraviolet absorbance at 254 nm ($SUVA_{254}$; SI Table 1). Briefly, solutions of HA and FA (40 mg C L^{-1}), made with 18.2 MΩ cm water, were filtered through a 0.45 μm filter membrane and absorbance was measured by a Genesys 10 S UV-Vis spectrophotometer (Thermo Fisher Scientific, USA) using a quartz cell with a 1 cm path length. Dissolved organic carbon (DOC) was determined by high temperature catalytic oxidation using a Shimadzu TOC-V (Shimadzu Corporation, Japan). Based on the results of the absorbance and DOC data, $SUVA_{254}$ was calculated according to EPA method 415.3 (Potter and Wimsatt, 2005).

2.3. Duckweed growth assays

Duckweed was surface sterilized with 0.025% NaClO and maintained in ½ Hoagland's solution buffered with 0.5 mmol L^{-1} MES at pH 6 (SI. Table 2). A preliminary growth study was conducted to assess the effect of Cu^{2+} concentration and CuO particle size on duckweed growth. Treatments were 0, 1.6, 3.2, 4.8, 6.4, and 8 mg Cu L^{-1} supplied by $CuCl_2$, 22 nm CuO, 50 nm CuO, and CuO MP. Prior to first use, solutions were sonicated for 10 min at 320 W in a FS 220 ultrasonic bath (Thermo Fisher Scientific, USA). In brief, 40 mL of treatment medium was pipetted into acid washed glass jars, replicated in quadruplicate. Four duckweed fronds were added to each jar on day zero and maintained on an orbital shaker set at 60 revolutions per minute (rpm) in a Conviron growth chamber maintained at 16 h days and 8 h nights at 25 °C until all replicates within any one treatment had at least 64 fronds, between 10 and 14 days (Shi et al., 2011). Duckweed frond area was quantified at the end of each experiment by pixel counting using Fiji (Schindelin et al., 2012; Song et al., 2015). The change in duckweed frond area over the course of the experiment (relative frond area) was calculated as:

$$Relative\ Frond\ Area = \frac{\ln Area_{final} - \ln Area_{initial}}{Days}$$

Based on the results of both the initial growth assay and the dissolution studies described in section 2.4, a second growth assay was conducted to evaluate the effect of HA and FA on the toxicity of $CuCl_2$ and 22 nm CuO to duckweed growth. Treatments were 0.8 mg Cu L^{-1} of $CuCl_2$, 1.6 mg Cu L^{-1} of $CuCl_2$ and 1.6 mg Cu L^{-1} of 22 nm CuO with and without 60 mg C L^{-1} FA or HA. The experimental conditions and frond area calculations were the same as previously described.

2.4. Dissolution curves for 22 nm CuO, 50 nm CuO, and CuO MPs

The dissolution of 1.6 mg Cu L^{-1} of 22 nm CuO (with and without 60 mg C L^{-1} of HA or FA), 50 nm CuO, and CuO MPs, repeated in quadruplicate, was measured over a 14-day period in ½ Hoagland's solution buffered with 0.5 mmol L^{-1} MES at pH 6.