Science of the Total Environment 563–564 (2016) 81–88

Contents lists available at ScienceDirect

Science of the Total Environment

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

Toxicity of copper nanoparticles to *Daphnia magna* under different exposure conditions

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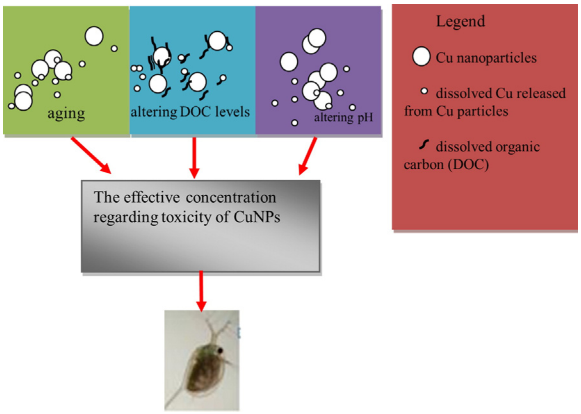
HIGHLIGHTS

- Toxicity of CuNP suspension to daphnids increased by about 30% during 7 days of aging.
- Toxicity of CuNPs increased about 3-fold with decreasing the pH of the exposure medium from 8.5 to 6.5, whereas it decreased >12-fold upon addition of DOC from 0 to 10 mg/L.
- Water chemistry parameters strongly affected the relative contribution of ions and particles in inducing toxicity.
- The role of ions shedding from CuNPs is very important in explaining the toxicity in natural waters.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form 14 April 2016
Accepted 14 April 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Copper nanoparticles
Water chemistry
Acute toxicity
Fate
Daphnia magna

ABSTRACT

Although the risks of metallic nanoparticles (NPs) to aquatic organisms have already been studied for >10 years, our understanding of the link between the fate of particles in exposure medium and their toxicity is still in its infancy. Moreover, most of the earlier studies did not distinguish the contribution of particles and soluble ions to the toxic effects caused by suspensions of metallic NPs. In this study, the toxicity of CuNPs to *Daphnia magna* upon modification of the exposure conditions, achieved by aging the suspensions of CuNPs and by altering water chemistry parameters like the pH and levels of dissolved organic carbon (DOC), was investigated. The LC50 values for CuNPs exposure decreased by about 30% after 7 days of aging. The LC50 values increased >12-fold upon addition of DOC at concentrations ranging from 0 to 10 mg/L to the exposure medium. Changing the pH from 6.5 to 8.5 resulted in a 3-fold higher LC50 value. Furthermore, it was found that during 7 days of aging of the exposure medium (without addition of DOC and at pH 7.8), the toxicity could be mostly ascribed to the particles present in the suspension (around 70%). However, adding DOC or decreasing the pH of the exposure medium reduced the contribution of the particles to the observed toxicity. We thus found that the effective concentration regarding the toxicity was mainly driven by the contribution of the soluble ions in the presence of DOC or at pH 6.5. Our results suggest that the toxicity results of CuNPs obtained from laboratory tests may overestimate the risk of the particles in polluted waters due to the common absence of DOC in laboratory test solutions. Moreover, the role of the ions shedding from CuNPs is very important in explaining the toxicity in natural waters.

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http://dx.doi.org/10.1016/j.scitotenv.2016.04.104
0048-9697/

1. Introduction

The toxicity of metallic nanoparticles (NPs) has been studied quite intensively during the last few years, as evidenced by a large number of publications on this topic (Chen et al., 2015). Most of the earlier studies focused on the influence of the physicochemical properties of metallic NPs, such as size, shape and surface coating, on their toxicity. Yet, conflicting and inconsistent results on the factors determining toxicity are commonly reported. For example, Hoheisel et al. (2012) observed that the toxicity of Ag NPs to *Daphnia magna* increased with decreasing particle size in Lake Superior (St, Louis County, MN, USA) water with low to moderate ionic strength and dissolved organic carbon (DOC) no > 2 mg C/L, whereas Li et al. (2010) found that there was no relevance between size of Ag NPs and toxicity to *D. magna* in standard synthetic freshwater prepared according to the EPA protocol. Once being released into the aquatic environment, NPs in fact are subject to environmentally driven modifications (e.g., dissolution, aggregation and sedimentation) over time (referred to as aging hereafter), which may affect the bioavailability of ions shedding from the metallic NPs (designed as NP$_{(ion)}$ hereafter) and particles themselves (designed as NP$_{(particle)}$ hereafter) and then their toxicity to specific organisms (Lin et al., 2012; Sørensen and Baun, 2015). It has been reported that exposure conditions including the aging time (e.g., freshly prepared or aged suspensions) and water chemistry parameters like the pH and DOC levels of the aqueous environment can affect the process of modifications of NPs (Gao et al., 2012; Li et al., 2013; Cupi et al., 2015). Thus, an emphasis on the effects of the exposure conditions on the fate and toxicity of NPs is needed to gain a better understanding of the risk of metallic NP in natural water systems.

To date, it is generally recognized that both NP$_{(ion)}$ as well as NP$_{(particle)}$ potentially contribute to the overall toxicity observed for organisms following exposure to suspensions of metallic NPs (Adam et al., 2014a; Hua et al., 2014a; Song et al., 2015). However, most of the existing studies have not separated the contributions of NP$_{(ion)}$ and NP$_{(particle)}$ to the toxic effects caused by suspensions of metallic NPs, let alone that they have investigated the influence of the exposure conditions on the relative contribution of NP$_{(ion)}$ and NP$_{(particle)}$ to the observed toxicity caused by metallic NP suspensions. Thus, the issue which form, NP$_{(ion)}$ or NP$_{(particle)}$, is the major source of the effective concentration that is actually causing the toxicity of metallic NPs is still under debate.

CuNPs were reported to be highly toxic to a wide range of aquatic organisms (Smith et al., 2014; Xiao et al., 2015), whereas the information concerning the toxic action of CuNPs in water systems under different exposure conditions (e.g., under different aging time as well as at different pH and DOC levels) is far less available compared to other types of metal-based NPs, such as Ag, TiO_2 and ZnO NPs. The aim of this study was to determine the fate and toxicity of CuNPs to *D. magna* upon modification of the exposure conditions. Furthermore, the relative contribution of ions released from CuNPs and of particles themselves in inducing toxic effects to *D. magna* upon modification of the exposure conditions was investigated. The modification of the exposure conditions in this study was achieved by storing the NP suspensions for different time periods, by changing the levels of DOC in the exposure solutions, and by changing the pH of the exposure medium in the range found in natural freshwaters.

2. Materials and methods

2.1. Test organisms

D. magna, originally obtained from the Dutch National Institute for Public Health and the Environment (RIVM), was selected as the test species. According to the OECD guideline 202, *D. magna* were cultured in plastic containers with Artificial ElendtM4 medium at a density of 1 individual/10 mL of ElendtM4 medium (OECD, 2004). The culture medium was refreshed three times a week. The test organisms were fed with *Pseudokirchneriella subcapitata* every two days and maintained inside a controlled-temperature chamber under a 16:8 light-dark cycle (20 ± 1 °C).

2.2. Preparation and characterization of the exposure CuNP suspensions

Spherical CuNPs (purity, 99.8%) were purchased from Iolitec GmbH with an advertised primary size of 50 nm and a specific surface area of 6–8 m^2/g. ISO standard testing medium (STM) recommended by OECD was used to prepare CuNP suspensions. The STM applied in this study (pH 7.8 ± 0.2) contained (mg/L MilliQ water): $CaCl_2 \cdot 2H_2O$: 294; $MgSO_4 \cdot 7H_2O$: 123.25; $NaHCO_3$: 64.75; KCl: 5.75. The morphology and size of CuNPs in STM were assessed by transmission electron microscopy (TEM, JEOL 1010, JEOL Ltd., Japan) and are shown in Fig. S1. To monitor the change of toxicity of CuNP suspensions towards *D. magna* within 7 days after preparation of suspensions of CuNPs in STM, a stock suspension (nominal 100 mg/L) was prepared after 30 min of sonication in a water bath sonicator. Thereafter, several identical sets of particle suspensions were prepared immediately by a series of dilution from the stock CuNP solution with STM and subsequently stored undisturbed in a climate chamber at 20 ± 1 °C under a 16 h light:8 h dark photoperiod cycle. Each set of exposure suspensions consisted of a series of actual exposure concentrations that ranged from 36 to 216 μg/L and a control. The application of the exposure concentration range was based on a preliminary range finding test. Meanwhile, 7 groups of suspension of 1 mg/L of CuNPs with three replicates were prepared, which were employed for determining the change of physicochemical properties of CuNP suspensions during storage process. Specifically, the size distribution and zeta potential of CuNPs at different sampling time points (i.e., immediately after preparation, which we will refer to as 0 d, and after 1, 2, 3, 4, 7 and 9 days of preparation) were determined by dynamic light scattering (DLS) on a Zetasizer Nano-ZS instrument (Malvern, Instruments Ltd., UK); meanwhile, to quantify the amount of Cu ions remaining in the water column released from CuNPs at 0.1 mg/L during 9 days of aging, a sample with three replicates was obtained by pipetting 15 mL of solution from the position around 3 cm below the surface (the volume of the solution in the beaker was 100 ml). The sample was subsequently centrifuged at 30,392 g for 30 min at 4 °C (Sorvall RC5Bplus centrifuge, Fiberlite F21–8 × 50y rotor) to obtain the supernatant. After centrifugation, the supernatant was filtered through a syringe filter with 0.02 μm pore diameter (Anotop 25, Whatman). 69% HNO_3 was then used to digest the supernatant and the concentration of Cu in the supernatant was determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

To investigate the influence of pH on the fate and toxicity of CuNPs in media, a series of exposure media were prepared by diluting the stock CuNP suspension (nominal 100 mg/L), after which the pH levels of the exposure medium were adjusted to 6.5 and 8.5, respectively, with 0.1 M NaOH or 0.1 M HCl. To maintain the pH value of the exposure medium constant during 48 h exposure, the pH of the exposure medium was adjusted every 24 h with 0.1 M NaOH or 0.1 M HCl. The application of the pH range from 6.5 to 8.5 (namely, 6.5, 7.8 and 8.5) was because they are commonly found in freshwaters (Vijver et al., 2008), which is suited for healthy, diverse, and productive fish and macroinvertebrates communities (Hirst et al., 2002). The physicochemical analysis and characterization of CuNPs in STM at pH 6.5 and 8.5, including the size distribution and zeta potential and dissolution results during 48 h, were also conducted by the above mentioned methods.

In addition, the effect of DOC on the fate and toxicity of CuNPs in the exposure solution was also explored. Suwannee River humic acid II (SRHA) was used as a model DOC in this study. A SRHA stock solution was prepared by dissolving SRHA in 0.002 N NaOH in deionized water. The SRHA solution was then stirred overnight and filtered through a 0.2 μm cellulose acetate membrane and stored at 4 °C. The total organic