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Interactive effects of copper oxide nanoparticles and light to green alga *Chlamydomonas reinhardtii*

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ABSTRACT

The present study explores the effect of light with different spectral composition on the stability of CuO-nanoparticle (CuO-NP) dispersions and their effects to green alga *Chlamydomonas reinhardtii*. The results showed that simulated natural light (SNL) and light with enhanced UVB radiation (UVR*) do not affect the dissolution of CuO-NPs as compared to light irradiation conditions typically used in laboratory incubator (INC). Comparable values of ζ-potential and hydrodynamic size during 24 h were found under all studied conditions. Concentrations of CuO-NPs below 1 mg L^{-1} do not attenuate the light penetration in the algal suspensions in comparison with NP-free system. Exposure to a combination of 8 μg L^{-1} or 0.8 mg L^{-1} CuO-NPs and INC or SNL has no significant effect on the algal growth inhibition, algal fluorescence and membrane integrity under short-term exposure. However, an enhancement of the percentage of cells experiencing oxidative stress was observed upon exposure to 0.8 mg L^{-1} CuO-NPs and SNL for 4 and 8 h. Combination of UVR* and 0.8 mg L^{-1} CuO-NPs resulted in synergistic effects for all biological end-points. Despite the photocatalytic properties of CuO-NPs no significant increase in abiotic reactive oxygen species (ROS) production under simulated solar radiation was observed suggesting that the synergistic effect observed might be correlated to other factors than CuO-NP-mediated ROS photoproduction. Tests performed with $CuSO_4$ confirmed the important role of dissolution as toxicity driving force for lower CuO-NP concentration. However, they failed to clarify the contribution of dissolved Cu on the combined effects at 0.8 mg L^{-1} CuO-NPs. The results point out the necessity of taking into account the possible interactions between ENPs and changing light conditions when evaluating the potential effects of ENPs to phytoplankton in natural waters.

1. Introduction

Understanding the environmental impact of engineered nanomaterials (ENMs) has become an important issue of concern over the past years due to their industrial-scale production and unavoidable discharge to ecosystems. Although much effort has been put in this emerging field, a complete and predictive environmental risk assessment is still lacking particularly under environmentally relevant conditions (Batley et al., 2012; Grieger et al., 2012; Kahru and Ivask, 2013; von Moos et al., 2014). Copper oxide nanoparticles (CuO-NPs) are largely used as biocide, especially in antifouling paints, inks, plastics, antimicrobial coatings, and in many other products including ceramics, electronics, and textiles (Ingle et al., 2014; Shi et al., 2012). Based on the multispecies battery tests, CuO-NPs were classified as toxic to the aquatic organisms with algae and crustaceans being most sensitive species (Bondarenko et al., 2012; Ivask et al., 2014). The mechanisms of toxic action of CuO-NPs to different aquatic organisms and mammalian cells in vitro are extensively reviewed (Ivask et al., 2014). In addition to the intrinsic properties of the material, the release of Cu ions, CuO-NP uptake and their potential to induce oxidative stress are considered as key drivers of the toxicity (Ivask et al., 2010, 2014; von Moos and Slaveykova, 2014). What is more, the toxic potential of CuO-NPs can be modulated by basic water quality parameters, such as water hardness, pH, dissolved organic matter and salinity, test medium composition (von Moos et al., 2015), as well as the interactions with other contaminants and varying environmental factors, including light of different intensity and spectral composition. Although recognized that the NP photoreactivity might significantly increase hazard and risk of the ENMs in the environment, this aspect has not yet received enough attention and few studies deal with the combined effect of the ENMs and light of different intensity and spectral

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characteristics on the toxicity to aquatic phytoplankton (Hong and Otaki, 2006; Lee and An, 2013; Miller et al., 2012). Relatively low levels of ultraviolet radiation (UVR), consistent with those found in the environment were shown to induce toxicity of TiO_2-NPs to marine phytoplankton, ROS in seawater increased with increasing TiO_2-NP concentrations (Miller et al., 2012). The photocatalytic inhibition of *Chroococcus* sp. growth by TiO_2-NPs was shown to be larger under ultraviolet and fluorescent light irradiations (Hong and Otaki, 2006). On the other hand, no enhancement of the effects of TiO_2 or ZnO-NPs on growth inhibition of green alga *Pseudokirchneriella subcapitata* was observed under UV radiation with respect to visible light (Lee and An, 2013). However, in these studies the NP concentrations were in the mg L^{-1} ranges, several orders of magnitude higher than those expected in the aquatic environment. To our knowledge no studies exploring the combined effect of light and CuO-NPs to green microalgae have been yet published.

The objectives of the present study are to explore the effect of light with different spectral characteristics on the stability of CuO-NP suspensions and the toxicity responses of green microalga *Chlamydomonas reinhardtii*. Biological responses measured by flow cytometry (FCM) included growth, chlorophyll fluorescence, membrane damage and intracellular oxidative stress. *C. reinhardtii* was chosen as a model organism since algae are one of the most sensitive aquatic species to CuO-NPs (Kahru and Dubourguier, 2010), as well as potentially vulnerable to high light intensity and UVR (Cheloni et al., 2014). The effect of the different light treatments on CuO-NP dissolution, agglomeration and surface charge, and abiotic ROS generation were studied in parallel.

2. Materials and methods

2.1. Nanoparticles, algal culture and exposure medium

Spherical copper oxide nanoparticles (CuO-NPs) of 99% purity with a primary size of 30–50 nm was obtained from Nanostructured & Amorphous Materials, Inc. (Houston, TX). CuO-NPs specific surface and density provided by the manufacturer were 13.1 m^2 g^{-1} and 0.79 g cm^3, respectively. The powder was dispersed in MilliQ water at a concentration of 2 g L^{-1} by 1-min sonication at 130 W and 20 kHz (Sonics Vibra Cell, Sonic & Materials INC., Newtown, USA) as previously described (von Moos et al., 2015).

Green microalga *C. reinhardtii* (CPCC 11) was purchased from the Canadian Phycological Culture Center (CPCC, Department of Biology, University of Waterloo, Canada). Axenic cultures were grown in 4× diluted Tris-Acetate-Phosphate medium (Harris, 2009) at 114.2 μmol phot m^{-2} s^{-1} illumination regime and under rotary shaking (100 rpm) at 20 °C in a specialized incubator (Infors, Bottmingen, Switzerland). At the mid-exponential phase algae were harvested by gentle centrifugation at 2083 × *g* (Sigma 3–16 K) at 20 °C for 5 min. Cell pellets were washed twice with the CuO-NPs free medium and re-suspended in it to a final cell density of 10^6 cells m L^{-1}. The experimental medium contained 10^{-3} M 2-(*N*-morpholino) ethanesulfonic acid (MES, Sigma–Alrdich, Buchs, Switzerland) at pH 6.8 enriched with the macronutrients in the same concentration as the growth medium (Cheloni and Slaveykova, 2013) and CuO-NPs.

2.2. Incubator and simulated solar light conditions

C. reinhardtii exposures to CuO-NPs were performed under artificial light in a laboratory incubator and simulated solar light. The latter was obtained by a solar simulator (Sun 2000, Abet Technologies, Milford, CT) equipped with a xenon arc lamp (type UXL-553, Ushio inc. Tokyo, Japan). Spectral irradiance curves of the adopted light conditions are provided in Fig. S1. Two set-ups were used to produce light with different proportion of photosynthetically active radiation (PAR) and UVR components. In the first one an atmospheric edge filter and air mass 1.5G atmospheric absorption filter were combined to mimic solar radiation distribution on Earth at middle latitudes. Light produced with these filters had a PAR/UVR ratio comparable to natural sun light and an intensity similar to that measured in a clear sky day at noon at mid latitudes (Fig. S1). PAR, UVAR and UVBR corresponded to 230 W m^{-2}, 9.75 W m^{-2} and 0.73 W m^{-2}, respectively. Further in the text we will refer to this light condition as simulated natural light (SNL). In the second set up the air mass 1.5G atmospheric absorption filter was used alone. The light was characterized by altered PAR/UVR ratio due to an enhancement of the UVR intensity and the PAR, UVAR and UVBR corresponded to 232.2 W m^{-2}, 10.01 W m^{-2} and 2.01 W m^{-2}, respectively. In the text we will refer to this condition as light with enhanced UVB radiation (UVR*). PAR 13.3 W m^{-2}, UVAR 0.29 W m^{-2} and UVBR 0.08 W m^{-2} were measured in the incubator (INC). The last irradiation condition was chosen to represent the standard light level recommended in OECD guidelines for algal toxicity testing. The above irradiance values were measured at the surface of algal suspensions by using a spectrophotoradiometer (ILT950 Spectrilight, International Light Technologies, USA). Average UVA and UVB light doses received by the suspended cells (Table 1) were calculated as previously described (Navarro et al., 2014). Potential attenuation of the different light spectrum components due to the presence of CuO-NPs in the algal suspensions was determined by measuring the optical densities at 350 nm for UVA, 300 nm for UVB and 750 nm for PAR.

2.3. CuO-NP characterization in the exposure medium

Measurements of hydrodynamic size and electrophoretic mobility (EPM) of CuO-NPs in the exposure medium were carried out by a Malvern Zetasizer Nano-ZS (Malvern, Renens, Switzerland) as detailed in our previous work (Regier et al., 2015; von Moos et al., 2015). The results are the means of three replicate runs performed with freshly prepared samples on separate days. Each run is the mean of 7 measurements.

To determine the amount of the dissolved Cu in CuO-NP dispersions with a short-time lapse resolution a method based on ultrafiltration was applied (Ma et al., 2014). After 30 min and 2, 4, 8 and 24 h of exposure to different light conditions 10 m L of CuO-NP dispersion were transferred in ultrafiltration tubes (Amicon Ultra-15 Centrifugal Filter Units, cut-off 3000 Da, Merck Millipore, Darmstadt, Germany) and centrifuged at 3000 × *g* for 20 min. To reduce adsorption of the dissolved copper ions to the ultrafiltration membrane 1 mM EDTA was added to the suspension before centrifugation. The amount of Cu in the fraction recovered after centrifugation was measured by inductively coupled plasma mass spectrometry (Agilent 7700, Morges, Switzerland) following acidification with HNO_3 (suprapur Merck, Darmstadt, Germany). The percentage of Cu^{2+} loss due to adsorption to the membrane was estimated via ultrafiltration of $CuSO_4$ solutions at concentrations ranging from 10^{-8} to 10^{-5} M. The amount of dissolved Cu in the CuO-NP dispersion was calculated by using the linear equation (Fig. S3).

Abiotic ROS generation by CuO-NPs induced under different light regimes was determined using the fluorescent dye 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA, Sigma–Aldrich, Buchs, Switzerland). H_2DCF-DA deacetylation and sample incubation were performed as previously described (Aruoja et al., 2015). In the present work CuO-NPs suspensions were first exposed to INC, SNL and UVR* and then incubated with H_2DCF for 30 min in the dark due to the dye photosensitivity to the high simulated solar light irradiations (Fig. S4).