Effects of copper in *Daphnia* are modulated by nanosized titanium dioxide and natural organic matter: what is the impact of aging duration?

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Abstract
During its aquatic life cycle, nanosized titanium dioxide (nTiO₂) may interact with natural organic matter (NOM) ultimately altering the ecotoxicity of co-occurring chemical stressors such as heavy metals (e.g. copper (Cu)). In this context, the following hypotheses were tested: (1) aging of nTiO₂ along with Cu reduces Cu toxicity, (2) nTiO₂ agglomerates have a lower potential to reduce Cu toxicity and (3) aging of nTiO₂ in presence of NOM reduces Cu toxicity further. A multifactorial test design crossing three nTiO₂ levels (0.0, 0.6 and 3.0 mg/L) with two levels of NOM (0 versus 8 mg total organic carbon (TOC)/L) and seven nominal Cu concentrations (ranging from 0 to 1536 μg/L) aged in ASTM medium for 0, 1, 3 and 6 days was realised, while two aging scenarios were applied (type 1: nTiO₂ jointly aged with Cu; type 2: Cu added after nTiO₂ aging). Subsequently, Cu toxicity was assessed using the immobility of *Daphnia magna* after 48 h of exposure as response variable. The experiments revealed that neither aging duration nor the extent of agglomeration (type 1 vs. type 2 aging) has a substantial impact on Cu induced toxicity. Moreover, it was confirmed that the presence of NOM substantially reduced Cu induced toxicity, independent of the aging scenario and duration. More importantly, the data suggest the ingestion of Cu loaded nTiO₂ as additional exposure pathway contributing to Cu toxicity. In conclusion, it seems unlikely that nTiO₂ concentrations currently detected in or predicted for aquatic ecosystems, which are at least one order of magnitude below the concentration tested here, influence Cu toxicity meaningfully.

Keywords Aging · Nanomaterials · Natural organic matter · Metal ions · Combined effect · Mixture toxicity

Introduction
As a consequence of their small size, nanoparticles (NPs) possess physical and chemical features that are fundamentally different from their water-soluble or bulk counterparts. Those features include a high specific surface area, reactivity in liquid or gas phase and rapid diffusion (Nowack and Bucheli 2007; Fan et al. 2011) making nanotechnology a multi-trillion dollars business involving approximately 6 million employees worldwide (He et al. 2019). Furthermore, the Nanotechnology Consumer Products Inventory (CPI) listed 1814 consumer products from 6222 companies of 32 countries in 2015 (Mohajerani et al. 2019). This success goes along with an estimated annual production of NPs of approximately 300,000 metric tons in 2010. From this amount, 21%, 17% and 2.5% are assumed to be released at different stages of the nanoparticle’s life cycle into water, soils and air, respectively (Keller et al. 2013; Keller and Lazareva 2014). In the environment, NPs interact with a range of natural (such as natural organic matter (NOM)) and anthropogenic substances including dissolved heavy metals that may represent a hazard for the integrity of freshwater ecosystems (Millennium Ecosystem
Assessment 2005) raising concerns about their joint environmental health risk.

Indeed, some publications have reported increased metal toxicity in the presence of NPs (e.g., Fan et al. 2011; Wang et al. 2011; Rosenfeldt et al. 2014), while others highlight a reduction (Chen et al. 2015; Canesi et al. 2015). This remediation like effect may be explained by the adsorption of heavy metal ions onto the NPs surface, followed by an intraparticle diffusion, ultimately reducing heavy metal toxicity (Qu et al. 2013). The discrepancy of NP-induced changes in metal toxicity may depend on the NP concentration (Wang et al. 2011) as well as the metal identity or more specifically the metal ion’s charge (Rosenfeldt et al. 2014). It was, moreover, highlighted that the combined toxicity of metals and NP depends on the environmental conditions triggering their fate and interaction over time (Rosenfeldt et al. 2016). Indeed, Rosenfeldt et al. (2016) highlighted that Cu toxicity was reduced with increasing aging duration in presence of titanium dioxide (nTiO2) relative to NP’s absence. While this pattern was observed in medium with ionic strength (9.25 mmol/L), distilled water (ionic strength ~ 0 mmol/L) did not influence Cu toxicity. The authors also documented that the presence of NOM could contribute to mitigation of Cu toxicity through complexation, an effect that is elevated in presence of nTiO2. At the same time, Seitz et al. (2015) documented that the presence of NOM during nTiO2 aging can slightly and transiently increase NP toxicity. These studies jointly suggest a complex interplay of NOM, NP and metal ions over time warranting further research targeting the temporal dynamics of this interaction and ultimately their consequence for aquatic biota.

In the present study, the consequences of nTiO2 aging (0, 1, 3 and 6 days) at three concentrations (i.e. reflecting the 96 h EC10 and EC50 of nTiO2 (Fig. 1S) for Daphnia as well as the absence of nTiO2) in the presence and absence of NOM (seaweed extract as used in many earlier papers, namely Rosenfeldt et al. 2015a, 2016) on Cu toxicity (seven concentrations from the range 0–1536 μg/L; using a spacing factor of 2) were assessed for the model species Daphnia magna. Thereby, two aging scenarios in ASTM reconstituted hard water (ASTM 2007) were realised prior to the toxicity assessment: the first scenario ensured the interaction of nTiO2 with Cu in presence and absence of NOM during aging. The second scenario focused on the aging of nTiO2 and NOM (presence vs. absence) with Cu being spiked to the test medium just before the introduction of the test species. These aging scenarios simulate the interaction of Cu with fresh nTiO2 (often in their nanoform) or with aged and thus agglomerated particles, whose average particle size is often in the μm-range (Cupi et al. 2015; Seitz et al. 2015; Rosenfeldt et al. 2016), allowing to assess for the importance of the particle or agglomerate size. The selection of nTiO2 as model NP is motivated by its high production volume (Jovanović 2015) and widespread application (Baqi et al. 2006; Chen and Mao 2007; Mueller and Nowack 2008). This NP has additionally only limited acute toxicity for Daphnia, which is even further reduced by NOM (Seitz et al. 2015). Copper (Cu) served as model heavy metal interfering with the sodium (Na+) regulation and metabolism in freshwater animals (Grosell and Wood 2002; Grosell et al. 2002), whose toxicity can also be reduced in presence of NOM (Al-Reasi et al. 2012) and potentially nTiO2 (Liu et al. 2015). Additionally, Cu has a broad application range, including agriculture leading to surface water levels in the high micrograms per litre range (Süß et al. 2006).

In the present study, it was hypothesised that (1) aging of nTiO2 along with Cu reduces Cu toxicity with increasing aging duration as a consequence of a reduced Cu bioavailability at test initiation (Rashidi et al. 2010). It was, moreover, assumed that (2) the aging of nTiO2 in the absence of Cu limits the NPs possibility to reduce effects of Cu when freshly added to the test medium just before toxicity testing. This is likely triggered by a lower surface area of NP agglomerates and the short interaction time before the test organisms experience Cu exposure (Aydin et al. 2008). Finally, it was presumed that (3) aging of nTiO2 in the presence of NOM reduces Cu toxicity with increasing aging duration. This pattern is triggered by NOM colloidally stabilising nTiO2 extending its interaction time with Cu, ultimately reducing Cu effects (Lee et al. 2011). Moreover, NOM forms complexes with metal ions contributing to reduced Cu toxicity (Rosenfeldt et al. 2015a).

Materials and methods

Chemicals

A stable nTiO2 dispersion, produced by stirred media milling (PML 2; Bühler AG, Switzerland) of P25 nTiO2 powder (AEROXIDE® TiO2 P25; advertised primary particle size of 21 nm and surface area of 50 ± 15 m2/g; Evonik) in deionised water, was provided by the Institute for Particle Technology (TU Braunschweig, Germany). The stock dispersion was stabilised at low pH (~3), exhibiting a monodisperse size distribution and an average particle diameter of approximately 80 nm (Electronic supplementary material, Table 1S). The nTiO2 stock dispersion (2000 mg/L) was ultra-sonicated for 10 min prior use, to ensure a homogeneous particle distribution. The dissolved Cu stock solution was prepared separately for each experimental run using Cu(NO3)2 × 3H2O (Carl Roth; purity ≥ 99%, p.a., ACS). The salt was mixed with deionised water in a volumetric flask (polypropylene), followed by serial dilution to obtain the seven nominal test Cu concentrations ranging from 0 to 1536 μg/L (Tables 2S and 3S). The exact concentration range depended on the aging scenario and was selected to obtain a full dose-response curve. Dissolved Cu species including Cu2+, CuOH+ and
Cu$_2$(OH)$_2$$^{2+}$ (Rosenfeldt et al. 2016) are hereafter summarised as Cu.

**Test species**

The test species *D. magna* (clone V, Eurofins-GAB laboratories, Germany) was cultured in ASTM reconstituted hard water modified with selenium and vitamins (biotin, thiamine, cyanocobalamin) according to OECD 202 (OECD 2004) and 8 mg TOC/L seaweed extract (Marinure®, Glenside, Scotland) (ASTM 2007). The culture medium was changed three times per week, and the organisms were fed with the green algae *Desmodesmus* sp. at an equivalent of 200 μg C per organism and day. The culture was maintained in a climate-controlled chamber (Weiss Environmental Technology Inc., Germany) at 20 ± 1 °C and a 16:8 h light:dark rhythm (800–1000 lx; OSRAM L 58 W/21–840 ECO, Germany). At the initiation of the bioassays, newly hatched juveniles (age < 24 h) were collected and randomly introduced into the respective replicates using pasture pipette.

**Experimental setup**

The impact of nTiO$_2$ on the toxicity of Cu was assessed under two aging scenarios involving a 3-factorial (2 × 3 × 7) experimental design each (Fig. 1). The aging procedures were performed due to space limitations at 16 ± 1 °C and thus at a lower temperature relative to the toxicity bioassays (as the behaviour of nTiO$_2$ was not impacted at the lower temperature (Table 4S), this was considered acceptable), in darkness on a horizontal shaker (50 rpm; VKS-B-50, Edmund Bühler GmbH, Germany). At the initiation of the bioassays, newly hatched juveniles (age < 24 h) were collected and randomly introduced into the respective replicates using pasture pipette.

![Fig. 1 Schematic diagram visualizing the two aging scenarios (type 1 and type 2) assessing the impact of variable aging durations (i.e. 0, 1, 3 and 6 days) of nTiO$_2$ in combination with NOM on the toxicity of co-aged or freshly added Cu towards *D. magna*. The test assessing for the sensitivity baseline of *D. magna* is also visualised (unaged Cu)](image-url)
bioassays for the first aging scenario (type 1). As Cu was spiked to the aged medium just before the bioassay initiation for all experiments of the second scenario (type 2), accurate Cu spiking was anticipated based on the analytical results of the first aging scenario following 0 days of aging. Similarly, we quantified nTiO₂ concentrations (Table 7S) only for one of the exposure scenarios (0 day aged samples) assuming a similar deviation between nominal and measured concentrations for other factor combinations. Quantification of nTiO₂ and Cu at a later stage of the bioassays was not realised as the present study indicated that relevant interactions between nTiO₂ and Cu occurred during the first hours (see Table 8S, “Results” and “Discussion”).

The high number of acute toxicity tests required a temporal separation of the experiments, called experimental runs. To allow for a direct comparison of Daphnia responses among experimental runs, an additional dose-response experiment was performed during each experimental run quantifying the sensitivity of D. magna to unaged Cu (without nTiO₂ and NOM). The EC₅₀ values obtained from these experiments were ultimately used as a sensitivity baseline allowing the quantification of changes in Cu toxicity depending on the aging conditions.

Bioassays

Per replicate, five juvenile daphnids (<24 h age) were introduced into the aged ASTM medium containing respective concentrations of nTiO₂, Cu and NOM and exposed for a duration of up to 96 h. The immobilisation of Daphnia was recorded every 24 h (i.e. 24 h, 48 h, 72 h and 96 h). The acute toxicity tests were performed in polystyrene beakers (filled with 50 mL of aged test medium) under controlled laboratory conditions (20 ± 1 °C with a 16:8 h light:dark rhythm) with four independent replicates per treatment, largely following OECD 202 (OECD 2004).

Quantification of nTiO₂ and Cu

The concentrations of nTiO₂ or Cu in the aged medium were quantified according to the method described by Rosenfeldt et al. (2015b) with minor modification. Briefly, after aging (1, 3 and 6 days), 10 mL supernatant was taken from the water phase after centrifugation (4000 rpm for 10 min) of the aged medium. Centrifugation forced nTiO₂ to sediment, ensuring the separation of Cu from NPs. The concentration of Cu adsorbed on the surface of nTiO₂ was not analytically confirmed. After that, the samples were acidified with HNO₃ (65%) and stored at 4 °C. However, in the case of 0 day aging, samples with nTiO₂ were acidified with HCl (35%) to avoid agglomeration and analysed without centrifugation. The samples were analysed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; Agilent 720, Germany) using the wavelengths of 336.12 and 327.39 nm for Ti and Cu quantification, respectively. The limit of detection was 2 μg/L for both Ti and Cu. The measured concentration of nTiO₂ (recalculated based on Ti quantification) deviated from their nominal levels by less than 20% justifying the use of the latter throughout the study (see Table 7S).

Statistical analysis

The statistical analyses of this study were performed with R version 3.0.1 (for windows) and the extension package drc (Ritz and Streibig 2005; R Core Team 2013). EC₅₀ values, the half median concentration causing immobilisation of 50% of daphnids, together with their 95% confidence interval were abstracted from dose-response models, while the model fitting the data best was selected by means of Akaike’s information criterion (Table 9S and 10S) and expert judgement. Model building was in a first-place based on nominal Cu concentrations (Figs. 2S–8S; Table 9S) and subsequently normalised to the effective (= measured) Cu concentration quantified in the water phase at bioassay initiation (Figs. 9S–15S; Tables 8S and 10S). The latter step should aid data interpretation as to inform about negative effects on Daphnia, which may not be directly explainable by Cu water phase concentrations. All comparisons discussed in this paper are based on 48 h Cu EC₅₀ values, as effects caused by Cu after 24 h, 72 h and 96 h of exposure were either low (24 h) or already disguised by nTiO₂ toxicity (72 h and 96 h) (Dabrunz et al. 2011). Models and model parameters are reported in the electronic supplementary material (Figs. 2S–15S; Tables 9S and 10S). The EC₅₀ values of each aging situation were evaluated for statistically significant differences as compared with the respective EC₅₀ value of unaged Cu solution, as well as relative to the respective EC₅₀ value of unaged (0 day aging) situation using 95% confidence interval testing (Wheeler et al. 2006). If 95% CIs of the difference between two EC₅₀ values did not include zero, the difference was considered statistically significant (Rosenfeldt et al. 2014).

Results

Type 1 aging scenario

Based on nominal Cu concentrations (Fig. 2), 3.0 mg nTiO₂/L reduced Cu toxicity approx. 2-fold, relative to its absence, whereas 0.6 mg nTiO₂/L did not change Cu toxicity after 0, 1, 3 and 6 days of aging. The presence of NOM significantly increased EC₅₀ values independent of aging duration up to 2-fold in the absence of nTiO₂ relative to the unaged Cu treatment. Adjusting the Cu EC₅₀ values to the Cu concentration, confirmed analytically in the water phase (Fig. 3), uncovered
that in the absence of NOM at 0.0 and 0.6 mg nTiO$_2$/L, hardly any alteration occurred relative to the unaged Cu treatment. Contrary, the presence of 3.0 mg nTiO$_2$/L initially (0 days of aging) led to a 2-fold reduction, but after 1, 3 and 6 days of aging, it turned into a 2-fold increase in Cu toxicity. The amendment of NOM to the aging medium caused a reduction in Cu toxicity by 1.5–2-fold at 0.0 and 0.6 mg nTiO$_2$/L, which was independent of the aging duration. In the presence of 3.0 mg nTiO$_2$/L in combination with 8 mg NOM/L, in contrast, a reduction in Cu toxicity was observed reaching a factor
of up to three. This mitigation like effect continuously decreased to a factor of two, approaching 48 h EC\textsubscript{50} values comparable with 6 days of aging at 0.0 and 0.6 mg nTiO\textsubscript{2}/L.

**Type 2 aging scenario**

Aging of nTiO\textsubscript{2} with or without NOM before adding Cu and the test organisms (Fig. 4; see based on measured concentration in Fig. 16S) resulted in a similar pattern as discussed for the nominal concentrations when applying the type 1 aging scenario (Fig. 2). In general, while NOM reduced Cu toxicity, the low nTiO\textsubscript{2} concentration had no additional mitigating effect neither in presence nor in the absence of NOM. Only at 3.0 mg nTiO\textsubscript{2}/L, a further reduction in Cu toxicity was observed. These patterns were independent of the aging duration.

**Discussion**

**Influence of nTiO\textsubscript{2} on Cu toxicity in the absence of NOM**

The changes in Cu-induced toxicity in presence of nTiO\textsubscript{2} is likely triggered by the NP’s negative surface charge (approx. \(-9 \text{ mV}\) in absence of NOM as documented by (Rosenfeldt et al. 2015a) attracting positively charged Cu species (e.g. Cu\textsuperscript{2+}, CuOH\textsuperscript{+}, Cu\textsubscript{2}(OH)\textsubscript{2}\textsuperscript{2+}) (Rosenfeldt et al. 2016). Although the present study does not address the underlying chemical mechanisms of the observations reported here, it clearly displays that the concentration of nTiO\textsubscript{2} is an important driver influencing Cu toxicity. At 0.6 mg nTiO\textsubscript{2}/L, there was hardly any difference in Cu toxicity relative to the NP’s absence. The presence of 3.0 mg nTiO\textsubscript{2}/L, in contrast, caused substantial changes in Cu-induced toxicity. Besides the NP concentration, also the particle size, an indicator for the available surface area, might trigger the magnitude of changes in Cu toxicity (Rosenfeldt et al. 2015a). As the particle size was comparable at the start as well as at the termination of the aging procedure among the two applied NP concentrations (Table 6S), the latter determines the surface area available for interactions with Cu and thus not the particle size. Consequently, the lower nTiO\textsubscript{2} concentration provided insufficient surface area to reduce Cu concentrations in the water phase (Table 8S) and, thus, its effects in *Daphnia*. The type 2 aging scenario supports this interpretation, namely that the concentration of nTiO\textsubscript{2} is the main driver in the present study for the observed effects: We aged nTiO\textsubscript{2} in the absence of Cu, which increased its particle size (Table 6S), before interacting with Cu. The mitigation potential of nTiO\textsubscript{2} for Cu-induced toxicity did not substantially change with increasing aging duration and thus particles size, while the observed effects were comparable among aging scenarios (Figs. 2 and 4). Moreover, the interaction between Cu and nTiO\textsubscript{2} seems to be a rapid process highlighted by the reduction in Cu toxicity independent of the aging scenario and aging duration. However, as the Cu concentration was only quantified at the start of the *Daphnia* bioassay but not during the actual aging duration.
bioassay, this requires further evaluation. Conjointly, the first and second hypotheses suggesting an impact of aging time and agglomeration size of nTiO₂ for Cu mitigation need to be rejected.

As indicated above, the direction of Cu toxicity changed (i.e. increased) when based on its analytically verified concentration at the start of the experiment with D. magna, relative to its nominal concentration in the presence of 3.0 mg nTiO₂/L under the type 1 aging scenario. As the nTiO₂ concentration tested here did not induce any mortality after comparable exposure durations when tested individually (Fig. 1S), these differences point to additional exposure pathways relative to the waterborne exposure alone. In other words, if the Cu water phase concentration would explain the observed toxicity, the Cu EC₅₀ value should be comparable with those obtained in the absence of NOM and nTiO₂ and without aging (baseline Cu toxicity experiments). This pattern would have remained unnoticed when the assessment is based exclusively on Cu concentrations confirmed before aging—in fact, the Cu concentration measured in the water phase would lead to an underestimation of acute effects. As additional exposure pathway, Daphnia may have ingested NPs together with Cu. During gut passage (at pH = neutral), Cu may have been remodelised as a consequence of enzyme (including protease, amylase, lipase and cellulase) activity (Weltens et al. 2000; Rosenfeldt et al. 2014). Ultimately, this pathway interacts with the waterborne Cu exposure explaining the increased toxicity when based on measured concentrations (Fig. 3).

Influence of NOM on Cu toxicity

The reduction in Cu induced toxicity in the presence of NOM and independent of the aging scenario or duration can mainly be explained by the formation of Cu chelates. These Cu chelates are formed through the interaction with carboxylic and phenolic groups of NOM, ultimately reducing the bioavailability of Cu (Lorenzo et al. 2002; Rosenfeldt et al. 2015a; Yu et al. 2018). In the presence of nTiO₂, NOM further decreased the toxicity triggered by Cu (Figs. 2 and 4). This pattern, which is in line with our third hypothesis, may be driven by a coating of the NP’s surface (Fig. 17S) with hydrophobic or aromatic components of the NOM (Lee et al. 2011; Pakarinen et al. 2013). This process is, on the one hand, known to reduce agglomeration and thus stabilises nTiO₂ (see Table 6S) through electrostatic repulsion in the water phase (Loosli et al. 2014). Thereby, the available surface area for Cu adsorption is increased relative to the absence of NOM. Moreover, the presence of hydrophobic or aromatic organic material on nTiO₂ surfaces further enhances the surface charge (from approx. −9 mV in absence to −20 mV in presence of NOM, respectively, as documented by Rosenfeldt et al. 2015a), which may have stimulated Cu adsorption and complexation. Those processes, however, in combination with the direct interaction of Cu and NOM in the water phase, might have contributed to the observed decrease in Cu toxicity. This assumption is also supported when comparing the EC₅₀ values based on measured and nominal Cu concentrations during the type 1 aging scenario (Figs. 2 and 3). The fact that the EC₅₀ values—particularly after aging in the presence of the high nTiO₂ concentration—are substantially reduced when based on measured relative to nominal concentrations (Figs. 2 and 3) points towards the significant Cu adsorption onto NOM coated nTiO₂. Furthermore, the EC₅₀ values based on measured Cu concentrations in the presence of NOM were higher relative to the absence of both NOM and nTiO₂. This outcome suggests that Cu complexation is reducing its toxicity by roughly 50% (Fig. 3).

Nonetheless, the uptake of Cu complexed by NOM on nTiO₂ surfaces is possible in Daphnia considering their particle size distribution (Table 6S) exceeds the mesh size (240–640 nm) of Daphnia’s filtering apparatus (Seitz et al. 2015). However, the Cu on the NP’s surface seems to be less bioavailable in presence relative to the absence of NOM (Figs. 2 and 3) as the EC₅₀ value based on measured Cu concentrations indicated a reduced toxicity in the presence of NOM relative to the elevated toxicity in the absence of NOM.

In conclusion, from the present study, it is evident that field relevant concentrations of nTiO₂, namely far below the lowest concentration of 0.6 mg/L as tested here, are unlikely to influence Cu toxicity significantly. Despite a trend to an increased Cu toxicity is observed in the presence of 0.6 mg nTiO₂/L but absence of NOM, this assumption seems reasonable particularly as in surface water bodies, NOM is ubiquitous, which triggered a reduction in Cu toxicity in our study. Moreover, we can see that the aging duration does not meaningfully affect the direction and magnitude of Cu induced effects pointing to a rapid interaction between NOM, Cu and nTiO₂. This rapid interaction also suggests that changes in Cu toxicity in the long run, are rather unlikely—at least under stable environmental conditions as tested here. Nonetheless, the present study highlights the possibility of NPs to increase toxicity if Cu is taken up during gut passage, a pathway of exposure largely overlooked in the assessment of combined effects of particulate stressors (nanoparticles or nanoplastics) and dissolved chemical stressors. Additionally, the direct effects of nTiO₂ seems of little relevance for the actual toxicity assessment as the toxicity of the NPs tested in the present study only appeared after 96 h of exposure. Finally, it is suggested to assess for the transferability of the results generated here to other heavy metals (with variable ionic charge) to allow for a general interpretation of the relevance of this process.
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-020-11578-2.

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Authors’ contributions MB and RR designed the experiments. RR, AAAM and SL performed the experiments. RR, AAAM, MB, SL, GM and RS analysed the data. MB and RR wrote the manuscript.

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Data availability All data generated or analysed during this study are included in this published research article and its electronic supplementary material.

Compliance with ethical standards

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Competing interests One of the authors (RS) is managing director of a small consultancy working in the field of ecotoxicology and environmental risk assessment, while another co-author (SL) is now working in the private sector, namely the chemical industry. The authors, however, do not feel a conflict of interest as a consequence of this situation.

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References

Al-Reasi HA, Scott Smith D, Wood CM (2012) Evaluating the ameliorative effect of natural dissolved organic matter (DOM) quality on copper toxicity to Daphnia magna: improving the BLM. Ecotoxicology 21:524–537. https://doi.org/10.1007/s10646-011-0813-z

ASTM (2007) E729-96 standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates and amphibians.

ASTM International, West Conshohocken, PA, 2007, www.astm.org

Aydin H, Bulut Y, Yerlikaya Ç (2008) Removal of copper (II) from aqueous solution by adsorption onto low-cost adsorbents. J Environ Manag 87:37–45. https://doi.org/10.1016/j.jenvman.2007.01.005

Baiqi W, Liqiang J, Yichun Q, Shudan L, Baojiang J, Libin Y, Baifu X, Honggang F (2006) Enhancement of the photocatalytic activity of TiO₂ nanoparticles by surface-capping DBS groups. Appl Surf Sci 252:2817–2825. https://doi.org/10.1016/j.apsusc.2005.04.025

Canesi L, Ciacci C, Balbi T (2015) Interactive effects of nanoparticles with other contaminants in aquatic organisms: friend or foe? Mar Environ Res 111:128–134. https://doi.org/10.1016/j.marenvres.2015.03.010

Chen X, Mao SS (2007) Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. Chem Rev 107:2891–2959. https://doi.org/10.1021/cr0500535

Chen J, Qian Y, Li H, Cheng Y, Zhao M (2015) The reduced bioavailability of copper by nano-TiO₂ attenuates the toxicity to Microcystis aeruginosa. Environ Sci Pollut Res 22:12407–12414. https://doi.org/10.1007/s11356-015-4492-9

Cupi D, Hartmann NB, Baum A (2015) The influence of natural organic matter and aging on suspension stability in guideline toxicity testing of silver, zinc oxide, and titanium dioxide nanoparticles with Daphnia magna: natural organic matter and aging on nanoparticle. Environ Toxicol Chem 34:497–506. https://doi.org/10.1002/etc.2855

Dabrunz A, Duester L, Prasse C, Seitz F, Rosenfeldt R, Schilde C, Schaumann GE, Schulz R (2011) Biological surface coating and molting inhibition as mechanisms of TiO₂ nanoparticle toxicity in Daphnia magna. PLoS One 6:e20112. https://doi.org/10.1371/journal.pone.0020112

Fan W, Cui M, Liu H, Wang C, Shi Z, Tan C, Yang X (2011) Nano-TiO₂ enhances the toxicity of copper in natural water to Daphnia magna. Environ Pollut 159:729–734. https://doi.org/10.1016/j.envpol.2010.11.030

Fan W, Peng R, Li X, Ren J, Liu T, Wang X (2016) Effect of titanium dioxide nanoparticles on copper toxicity to Daphnia magna in water: role of organic matter. Water Res 105:129–137. https://doi.org/10.1016/j.watres.2016.08.060

Grosell M, Wood CM (2002) Copper uptake across rainbow trout gills. J Exp Biol 205:1179

Grosell M, Nielsen C, Bianchini A (2002) Sodium turnover rate determines sensitivity to acute copper and silver exposure in freshwater animals. Comp Biochem Physiol C Toxicol Pharmacol 133:281–287. https://doi.org/10.1007/s12271-003-0101-3

He X, Deng H, Hwang H (2019) The current application of nanotechnology in food and agriculture. J Food Drug Anal 27:1–21. https://doi.org/10.1016/j.jfda.2018.12.002

Jovanović B (2015) Review of titanium dioxide nanoparticle phototoxicity: developing a phototoxicity ratio to correct the endpoint values of toxicity tests: TiO₂ nanoparticles phototoxicity. Environ Toxicol Chem 34:1070–1077. https://doi.org/10.1002/etc.2891

Keller AA, Lazareva A (2014) Predicted releases of engineered nanomaterials: from global to regional to local. Environ Sci Technol Lett 1:65–70. https://doi.org/10.1021/ez400106d

Keller AA, McFerran S, Lazareva A, Suh S (2013) Global life cycle releases of engineered nanomaterials. J Nanopart Res 15:1692. https://doi.org/10.1007/s11051-013-1692-4

Lee S, Kim K, Shon HK, Kim SD, Cho J (2011) Biodiversity of nanoparticles: effect of natural organic matter. J Nanopart Res 13:3051–3061. https://doi.org/10.1007/s11051-010-0204-z

Liu L, Fan W, Lu H, Xiao W (2015) Effects of the interaction between TiO₂ with different percentages of exposed {001} facets and Cu²⁺ on biotoxicity in Daphnia magna. Sci Rep 5:11121. https://doi.org/10.1038/srep11121
Loosli F, Le Coustumer P, Stoll S (2014) Effect of natural organic matter on the disagglomeration of manufactured TiO$_2$ nanoparticles. Environ Sci Nano 1:154. https://doi.org/10.1039/c3en00061c

Lorenzo JI, Nieto O, Beiuus R (2002) Effect of humic acids on speciation and toxicity of copper to Paracutrotus lividus larvae in seawater. Aquat Toxicol 58:27–41. https://doi.org/10.1016/S0166-445X(01)00219-3

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: opportunities and challenges for business and industry. World Resources Institute, Washington, DC

Mohajerani, Burnett, Smith et al (2019) Nanoparticles in construction materials and other applications, and implications of nanoparticle use. Materials 12:3052. https://doi.org/10.3390/ma12193052

Mueller NC, Nowack B (2008) Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol 42:4447–4453. https://doi.org/10.1021/es7029637

Nowack B, Bucheli TD (2007) Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut 150:5–22. https://doi.org/10.1016/j.envpol.2007.06.006

OECD (2004) Test No. 202: Daphnia sp. Acute Immobilisation Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

Pakarinen K, Petersen EJ, Alvila L, Waissi-Leinonen GC, Akkanen J, Leppänen MT, Kukkonen JVK (2013) A screening study on the fate of fullerenes (nC$_{60}$) and their toxic implications in natural freshwaters: Fate and toxicity of nC$_{60}$ by varying water characters. Environ Toxicol Chem 32:1224–1232. https://doi.org/10.1002/etc.2175

Qu X, Alvarez PJ, Li Q (2013) Applications of nanotechnology in water and wastewater treatment. Water Res 47:3931–3946. https://doi.org/10.1016/j.watres.2012.09.058

R Core Team (2013) R: a language and environment for statistical computing. 3.0.1 ed. R Foundation for Statistical Computing, Vienna

Rashidi F, Sarabi RS, Ghasemi Z, Seif A (2010) Kinetic, equilibrium and thermodynamic studies for the removal of lead (II) and copper (II) ions from aqueous solutions by nanocrystalline. Superlattice Microst 48:577–591. https://doi.org/10.1016/j.spmi.2010.09.011

Ritz C, Streibig JC (2005) Bioassay analysis using. R J Stat Softw 12. https://doi.org/10.18637/jss.v012.i05

Rosenfeldt RR, Seitz F, Schulz R, Bundschuh M (2014) Heavy metal uptake and toxicity in the presence of titanium dioxide nanoparticles: a factorial approach using Daphnia magna. Environ Sci Technol 48:6965–6972. https://doi.org/10.1021/es405396a

Rosenfeldt RR, Seitz F, Senn L, Schild C, Schulz R, Bundschuh M (2015a) Nanosized titanium dioxide reduces copper toxicity—the role of organic material and the crystalline phase. Environ Sci Technol 49:1815–1822. https://doi.org/10.1021/es506243d

Rosenfeldt RR, Seitz F, Zubrod JP, Feckler A, Merkel T, Lüderwald S, Bundschuh R, Schulz R, Bundschuh M (2015b) Does the presence of titanium dioxide nanoparticles reduce copper toxicity? A factorial approach with the benthic amphipod Gammarus fossarum. Aquat Toxicol 165:154–159. https://doi.org/10.1016/j.aquatox.2015.05.011

Rosenfeldt RR, Seitz F, Haigis A-C, Höger J, Zubrod JP, Schulz R, Bundschuh M (2016) Nanosized titanium dioxide influences copper-induced toxicity during aging as a function of environmental conditions: toxicity of copper and nanosized TiO$_2$ - the effect of aging. Environ Toxicol Chem 35:1766–1774. https://doi.org/10.1002/etc.3325

Seitz F, Lüderwald S, Rosenfeldt RR, Schulz R, Bundschuh M (2015) Aging of TiO$_2$ nanoparticles transiently increases their toxicity to the pelagic microcrustacean Daphnia magna. PLoS One 10: e0126021. https://doi.org/10.1371/journal.pone.0126021

Süß A, Bischoff G, Mueller ACW, Buhr L (2006) Chemisch-biologisches Monitoring zu Pflanzenschutzmittel-belastungen und Lebensgemeinschaften in Gräben des Alten Landes. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 58:28-42

Wang D, Hu J, Irons DR, Wang J (2011) Synergistic toxic effect of nano-TiO$_2$ and As(V) on Ceriodaphnia dubia. Sci Total Environ 409:1351–1356. https://doi.org/10.1016/j.scitotenv.2010.12.024

Welten R, Goossens R, Van Puymp S (2000) Ecotoxicity of contaminated suspended solids for filter feeders (Daphnia magna). Arch Environ Contam Toxicol 39:315–323. https://doi.org/10.1007/s002440010110

Wheeler MW, Park RM, Bailer AJ (2006) Comparing median lethal concentration values using confidence interval overlap or ratio tests. Environ Toxicol Chem 25:1441. https://doi.org/10.1897/05-320R.1

Yu S, Liu J, Yin Y, Shen M (2018) Interactions between engineered nanoparticles and dissolved organic matter: a review on mechanisms and environmental effects. J Environ Sci 63:198–217. https://doi.org/10.1016/j.jes.2017.06.021

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