Ecotoxicology and Environmental Safety 272 (2024) 116031

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Aging of nanosized titanium dioxide modulates the effects of dietary copper exposure on *Daphnia magna* – an assessment over two generations

Rajdeep Roy [a,*], Lucas Kempter [a], Allan Philippe [a], Eric Bollinger [a], Lea Grünling [a], Mugilvannan Sivagnanam [a], Frederik Meyer [a], Alexander Feckler [a], Frank Seitz [a], Ralf Schulz [a], Mirco Bundschuh [a,b,*]

[a] *iES Landau, Institute for Environmental Sciences, RPTU Kaiserslautern-Landau, Germany*
[b] *Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden*

ARTICLE INFO

Edited by: Dr. Caterina Faggio

Keywords:
Nanoparticles
Aging
Natural organic matter
Multigeneration
Dietary metal exposure
Adaptation

ABSTRACT

Nanosized titanium dioxide ($nTiO_2$) is widely used in products, warranting its discharge from various sources into surface water bodies. However, $nTiO_2$ co-occurs in surface waters with other contaminants, such as metals. Studies with $nTiO_2$ and metals have indicated that the presence of natural organic matter (NOM) can mitigate their toxicity to aquatic organisms. In addition, “aging” of $nTiO_2$ can affect toxicity. However, it is a research challenge, particularly when addressing sublethal responses from dietary exposure over multiple generations. We, therefore exposed the alga *Desmodesmus subspicatus* to $nTiO_2$ (at concentrations of 0.0, 0.6 and 3.0 mg $nTiO_2$/L) in nutrient medium aged for 0 or 3 days with copper (Cu) at concentrations of 0 and 116 µg Cu/L and with NOM at concentrations equivalent to 0 and 8 mg total organic carbon (TOC) per litre. Subsequently, the exposed alga was fed to *Daphnia magna* for 23 days over two generations and survival, reproduction and body length were assessed as endpoints of toxicity. In parallel, Cu accumulation and depuration from *D. magna* were measured. The results indicate that the reproduction of *D. magna* was the most sensitive parameter in this study, being reduced by 30% (at both parental (F_0) and filial (F_1) generations) and 50% (at F_0 but not F_1) due to the dietary Cu exposure in combination with $nTiO_2$ for 0 and 3 days aging, respectively. There was no relationship between the effects observed on reproduction and Cu body burden in *D. magna*. Moreover, *D. magna* from the F_1 generation showed an adaptive response to Cu in the treatment with 3.0 mg $nTiO_2$/L aged for 3 days, potentially due to epigenetic inheritance. Unexpectedly, the presence of NOM hardly changed the observed effects, pointing towards the function of algal exopolymeric substances or intracellular organic matter, rendering the NOM irrelevant. Ultimately, the results indicate that the transferability of the impacts observed during the F_0 to the responses in the F_1 generation is challenging due to opposite effect directions. Additional mechanistic studies are needed to unravel this inconsistency in the responses between generations and to support the development of reliable effect models.

1. Introduction

The rapid expansion of nanotechnology has enabled the extensive usage of engineered nanoparticles (ENPs) in a wide variety of industrial and commercial products (Abdel-Latif et al., 2020), including paints, cosmetics, catalysts and personal care products (Chen and Mao, 2007; Luo et al., 2020; Mueller and Nowack, 2008). Consequently, ENPs are discharged in increasing quantities into the environment through, for example, wastewater treatment plant effluents (Jacobasch et al., 2014). Once in aquatic systems, ENPs interact with other substances, such as NOM or metals, with consequences on ENP and metal toxicity, respectively (Roy et al., 2022). Changes in metal toxicity due to the presence of ENP have attracted substantial attention with a strong focus on waterborne exposure (Geffard et al., 2008; Hartmann et al., 2012; Kim et al., 2016; Li et al., 2017; Rosenfeldt et al., 2014; Roy et al., 2022, 2021; Tan et al., 2012; Wang et al., 2023). However, growing evidence suggests that dietary exposure, including food chain transfer, is crucial when assessing the environmental risks of metals or ENPs (Awoyemi et al., 2020; Li and Slaveykova, 2022; Shi et al., 2020).

Studies have shown that ENPs can alter bioaccumulation behaviour

---

* Corresponding authors at: iES Landau, Institute for Environmental Sciences, RPTU Kaiserslautern-Landau, Germany.
E-mail addresses: rajdeep@uni-landau.de (R. Roy), mirco.bundschuh@rptu.de (M. Bundschuh).

https://doi.org/10.1016/j.ecoenv.2024.116031
Received 21 August 2023; Received in revised form 18 January 2024; Accepted 25 January 2024

and trophic transfer of metals in aquatic organisms. For example, Chen et al. (2019) found that fullerene (C_{60}) NPs can mitigate cadmium (Cd) uptake in an aquatic food chain model using *D. magna* and zebrafish (*Danio rerio*). The presence of C_{60} NPs assisted Cd bioaccumulation in *D. magna* from water but reduced dietary Cd bioaccumulation in *D. rerio*. This observation was attributed to a reduced gut residence time and thus stimulated excretion due to C_{60} (Chen et al., 2019). In contrast, $nTiO_2$ increased lead (Pb) bioaccumulation from copepods to African catfish (Matouke and Mustapha, 2020) leading to neurotoxicity and reduction of antioxidant enzyme levels relative to the absence of $nTiO_2$. Similarly, $nTiO_2$ attached to marine microalgae promotes arsenic (As) sorption, stimulating As transfer to *Artemia salina* but reducing As bioaccumulation in *A. salina* (Yang et al., 2018a). In contrast, $nTiO_2$ diminished intracellular inorganic mercury (Hg) uptake by algae, which was mirrored at the trophic level of its consumer, *D. magna* (Li and Slaveykova, 2022). These insights highlight the relevance of ENPs in metal accumulation, trophic transfer (i.e., dietary exposure) and effects. At the same time, these studies are limited to short-term exposures or chronic exposure over one generation, questioning their relevance for longer (i.e., multi-generational) exposure durations that are to be expected in surface water. It remains, moreover, unclear whether and how environmental factors, such as the presence of NOM, the time ENPs undergo a modification of their surface characteristics or interactions between ENP and metal (i.e., aging) affect bioaccumulation, trophic transfer and effects on higher trophic levels.

Against this background, the present study assessed the impact of $nTiO_2$ aging (0 vs 3 days) and NOM (0 vs 8 mg TOC/L) on Cu toxicity through dietary exposure to *D. magna* over two generations. As model ENP and metal, $nTiO_2$ and Cu were selected driven by their wide application (Ahmed et al., 2021; Akakuru et al., 2020; Richardson, 1997) and, thus, their environmental relevance (Donnachie et al., 2014; Fan et al., 2020, 2019; Luo et al., 2020). Moreover, as an essential element, Cu, is of physiological relevance for organisms (Bossuyt and Janssen, 2005) but becomes detrimental when it exceeds those physiologically required levels (Nordberg, 2007). Since $nTiO_2$ may act as a carrier for metals (Matouke and Mustapha, 2020; Yang et al., 2018a), it was hypothesized that (1) Cu in the presence of this ENP impairs the fitness of *D. magna* when exposed through their diet being related to (2) an elevated Cu accumulation compared to the absence of ENP. Moreover, due to parental exposure, (3) *D. magna* can adapt to this situation already with the subsequent generation (Bossuyt and Janssen, 2004, 2003), reflected by less severe impacts in the filial relative to the parental generation. Finally, it was hypothesised that (4) the presence of NOM reduces any negative impact through the formation of an eco-corona (Liu et al., 2023) and complexation of metals that may cause the metal to be less bioavailable, as documented in earlier studies (Adusei-Gyamfi et al., 2019; Rosenfeldt et al., 2015; Roy et al., 2021).

2. Materials and methods

2.1. Test substances

An additive-free, stable $nTiO_2$ stock dispersion (in Milli-Q water) was obtained from the Institute for Particle Technology (TU Braunschweig, Germany). The stock dispersion was produced by stirred media milling (PML 2; Bühler AG, Switzerland) of P25 $nTiO_2$ powder (AEROXIDE® TiO_2 P25; anatase:rutile:amorphous ratio 78:14:8; Evonik) with the advertised primary particle size and surface area of 21 nm and 50 ± 15 m^2/g, respectively in deionized water with a low pH (~3). The $nTiO_2$ stock dispersion (3000 mg $nTiO_2$/L) was ultra-sonicated (Bandelin Sonorex Digitec DT 514 H ultrasonic bath, frequency 35 kHz) for 10 min before application ensuring a homogeneous particle distribution (with an average particle hydrodynamic diameter of ~100 nm; Table S1). The Cu stock solution (26 mg Cu/L) was prepared freshly before application using $Cu(NO_3)_2 \times 3\ H_2O$ (Carl Roth; purity ≥ 99%, p.a., ACS). The salt was mixed with deionized water (until complete dissolution) in a polypropylene volumetric flask, followed by serial dilution to obtain the desired nominal Cu concentration (refer to the section *"Aging process, analytical measurements and algal exposure"* for further details).

2.2. Test organisms

The primary producer, *D. subspicatus* was obtained from the Culture Collection of Algae (SAG 86.81; Göttingen University, Germany) and maintained in an environmental test chamber (SANYO, Japan) at 20 ± 1 °C and a 16:8 h light:dark cycle (5000 − 5500 lux; cool-white illumination). The alga was cultured in a nutrient medium (Table S2; pH adjusted to ~8.2; Kuhl and Lorenzen, 1964), facilitated with continuous stirring (250 rpm; Magnetic stirrer, VARIOMAG POLY 15, Thermo Fisher, USA) and constant aeration (OECD 2011).

The primary consumer, *D. magna* (clone V) was originally obtained from Eurofins-GAB laboratories (Germany) and maintained in a climate-controlled chamber (Weiss Environmental Technology Inc., Germany) at 20 ± 1 °C with a 16:8 h light:dark cycle (800 − 1000 lux; OSRAM L 58 W/21 −840 ECO, Germany). ASTM reconstituted hard water (pH ~8.2) supplemented with selenium, vitamins (biotin, thiamine, cyanocobalamin) and 8 mg TOC/L seaweed extract (Marinure®, Glenside, Scotland) served as culture medium (ASTM, 2007). The medium was renewed thrice per week and feeding with *D. subspicatus* was done daily at ~ 66, 100, 150 and 200 µg C/animal for *D. magna* aged 0–3, 4–7, 8 and > 8 days, respectively.

2.3. Aging process, analytical measurements and algal exposure

First, Cu was aged in a nutrient medium (Table S2; Kuhl and Lorenzen, 1964) in the presence or absence of NOM, $nTiO_2$ or both, following a 2 × 2 × 2 × 3 fractional design (Fig. 1A). In detail, the fractional design comprised two Cu concentrations (0 and 116 µg Cu/L; representing the absence of Cu as well as the 96 h EC_{50} of Cu for *D. subspicatus* (Fig. S1)), two aging durations (i.e., 0 and 3 days), two NOM levels (0 and 8 mg TOC/L) and three $nTiO_2$ concentrations (0.0, 0.6 and 3.0 mg $nTiO_2$/L; representing the absence of $nTiO_2$ as well as the 96 h EC_{10} and EC_{50} of $nTiO_2$ for *D. magna* (Roy et al., 2021)). The selection of these parameters was motivated by a previous study (Roy et al., 2021), easing a direct comparison of the results between studies. The aging process was carried out at 16 ± 1 °C in darkness on a shaker (150 rpm; VKS-B-50, Edmund Bühler GmbH, Germany) following Roy et al. (2021). The darkness during the aging process prevented photo-activation of $nTiO_2$ and thus the oxidation of NOM (Seitz et al., 2015) The continuous shaking kept $nTiO_2$ in suspension (no sedimentation was visible during shaking). The mean hydrodynamic diameter, measured by dynamic light scattering (DelsaNano C, Beckman Coulter, Germany) of $nTiO_2$ (in the presence and absence of Cu and NOM) was evaluated after aging and immediately before exposure of *D. subspicatus* (Table S4).

Additionally, spiked Cu and $nTiO_2$ concentrations in the aged medium were quantified (Table 1) using inductively coupled plasma mass spectrometry (ICP-MS; NexION 1000, Perkin Elmer). For this purpose, 2 mL of 0-day-aged (i.e., unaged) medium was acid digested using 5 mL of H_2O_2 (30% Rotipuran® Carl Roth GmbH) and 10 mL H_2SO_4 (95% Rotipuran® Carl Roth GmbH) for 2.5 h at 250 °C before being diluted and injected to ICP-MS (Philippe et al., 2022). The detection limits for Cu and Ti were 0.4 and 0.5 µg/L, respectively. The 3-day-aged medium was centrifuged (Heraeus Multifuge 4KR, Thermo Scientific) at 8600g for 20 min and 10 mL of the supernatant was acidification with 2% HNO_3 (Suprapur® 65%) prior to ICP-MS analysis. As centrifugation separates $nTiO_2$ from dissolved Cu, the chemical analyses of the supernatant focused on the dissolved Cu. The concentration of Cu adsorbed by $nTiO_2$ was not analytically determined. Although we refer to Cu throughout the document, the concentration of each possible Cu species present in the aging medium at equilibrium has been determined theoretically using visual MINTEQ version 3.1 (Gustafsson, 2013) as detailed

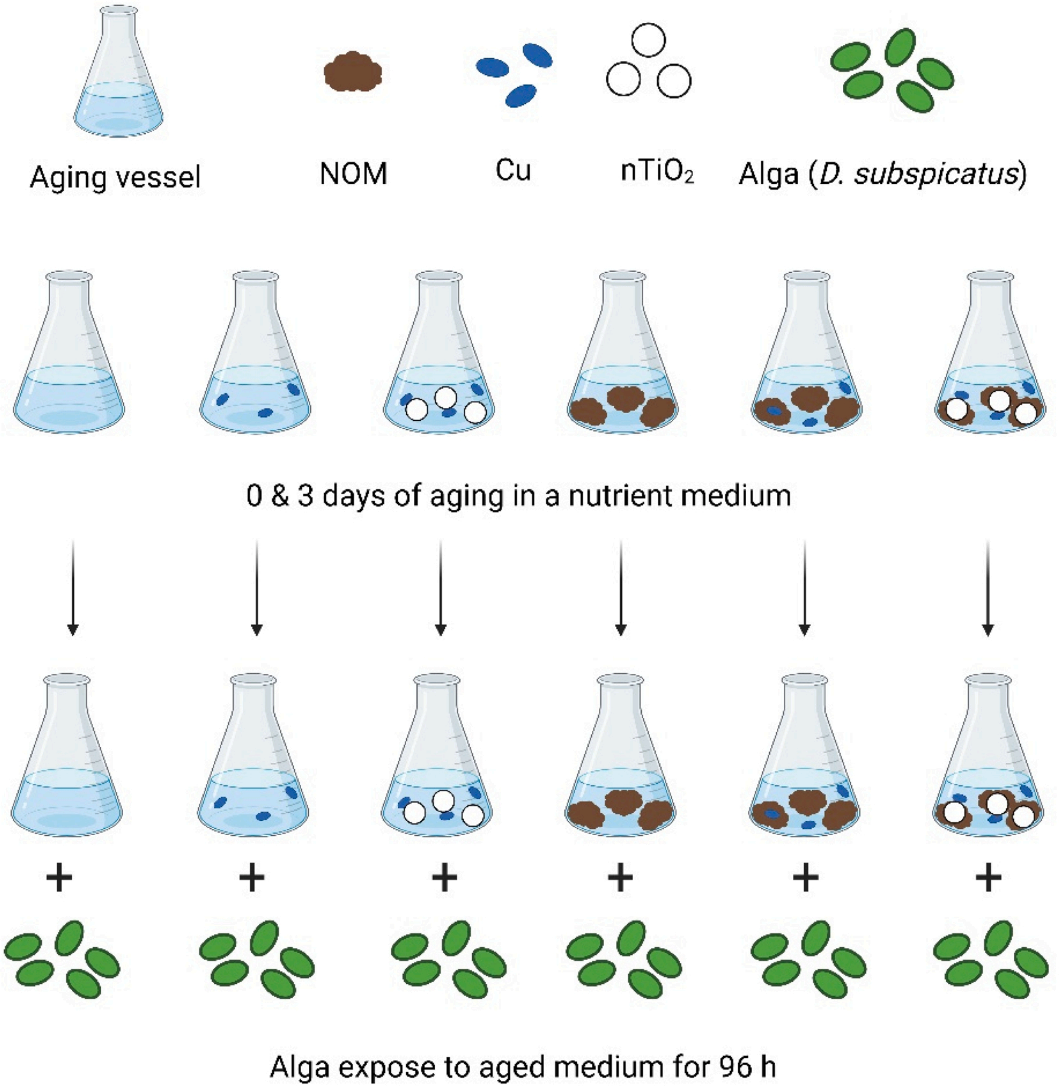

Fig. 1. Graphical representation of the experimental setup for (A) aging of Cu (0 and 116 µg Cu/L) in combination with NOM (0 and 8 mg TOC/L) and $nTiO_2$ (0.0, 0.6 and 3.0 mg $nTiO_2$/L) for 0 and 3 days followed by (B) exposure of alga to the aged medium for 96 h. The exposed alga served as food for *D. magna* during a two-generational experiment and for assessing Cu accumulation via dietary exposure.

Table 1
The measured Cu and $nTiO_2$ concentrations (mean ± SD; n = 3) in the aged medium as a function of aging duration, NOM concentration and their respective nominal concentrations. The $nTiO_2$ concentrations were not determined for 3-day-aged medium, as deviations between nominal and measured concentrations were expected to be the same as reported for treatments without aging (0 days). Additionally, Cu concentration (mean ± SD; n = 3) in *D. subspicatus* after 96 h of exposure to the respective aged medium is reported. Cu concentration in *D. subspicatus* was below the detection limit (< LOD) in absence of Cu and $nTiO_2$ and is hence not reported here. Significant differences in the Cu concentration in *D. subspicatus* in presence of lower (0.6 mg $nTiO_2$/L) or higher (3.0 mg $nTiO_2$/L) $nTiO_2$ level relative to its absence for the same combinations of factors are denoted by asterisk (*). The ‘a′ denotes significant differences ($p < 0.05$) relative to the same combinations of factors but without aging (0 day). Not assessed = n.a.

Aging duration (days)	NOM (mg TOC/L)	$nTiO_2$ concentration in water		Cu concentration in water		Cu concentration in *D. subspicatus*
		Nominal (mg/L)	Measured (mg/L)	Nominal (µg/L)	Measured (µg/L)	(µg/g dry weight)
0	0	0.0	< LOD	116	96.91 ± 0.60	57.43 ± 2.42
	8		< LOD		104.74 ± 14.64	61.52 ± 3.40
	0	0.6	0.7 ± 0.01		96.91 ± 0.98	53.79 ± 3.24
	8		0.6 ± 0.04		95.81 ± 0.76	68.73 ± 3.78
	0	3.0	3.0 ± 0.11		96.56 ± 0.10	86.89 ± 3.89 * **
	8		2.8 ± 0.08		97.17 ± 0.67	104.04 ± 6.38 * *
3	0	0.0	n.a.	116	95.17 ± 1.13	82.99 ± 3.58^{a}
	8		n.a.		93.61 ± 0.18	69.68 ± 3.56^{a}
	0	0.6	n.a.		93.72 ± 0.88	74.15 ± 0.37 *^{a}
	8		n.a.		93.04 ± 0.19	58.36 ± 12.63
	0	3.0	n.a.		94.92 ± 0.70	98.52 ± 5.13 *^{a}
	8		n.a.		93.08 ± 0.61	81.69 ± 5.62 *^{a}

and provided in the Supporting information (Table S5).

Second, exponentially grown *D. subspicatus* (5×10^3 cells/mL; obtained from a four days old inoculum culture (OECD, 2006)) were exposed to the aged medium (Fig. 1B) at 20 ± 1 °C in an environmental test chamber (SANYO, Japan), facilitated by stirring (250 rpm) and uniform cool-white illumination (5000 – 5500 lux). After 96 h of exposure, the algal suspensions were centrifuged (Heraeus Multifuge 4KR, Thermo Scientific) at 2700 rpm (20 °C) for 4 min, followed by four successive centrifugal cleaning steps with phosphate-buffered saline (as per Shi et al., 2020). The centrifugal cleaning steps ensure the separation of algal cells from the exposure medium (Shi et al., 2020). The algal pellets were re-suspended in the nutrient medium (Table S2; Kuhl and Lorenzen, 1964) and fed to *D. magna* during the two-generational experiment (Fig. S2). Moreover, an aliquot of algal pellets, stored at − 20 °C, served to quantify Cu in alga (Table 1). Before chemical analysis, the algal pellets were oven-dried for ~48 h at 60 °C, weighed and acid digested (Microwave MarsXpress; CEM GmbH) with aqua regia at 175 °C for 1 h. After cooling down, the digested samples were diluted (1:20; v:v) with Milli-Q water and analysed using ICP-MS (NexION 1000, Perkin Elmer).

2.4. *Two-generational dietary Cu exposure of Daphnia*

The two-generational experiment of *D. magna* was established (Fig. S2) following Völker et al. (2013) and Hartmann et al. (2019) with minor modifications. Briefly, neonates (age < 17 h) originating from the lab culture were raised individually (n = 1) in glass microcosms (n = 10) containing 50 mL of ASTM medium. These animals were fed *D. subspicatus* from the different aging treatments (Fig. 1 and S2; Table 1) daily in an age-dependent manner (see above *"Test organisms"*) for 23 days (i.e., F_0 generation). The third brood from the F_0 generation of each treatment was used to initiate the F_1 generation. More specifically, a random neonate was taken from each replicate of the third brood in the F_0 generation to initiate one replicate of the F_1 generation. To ensure a constant replication (n = 10) between generations, replicates without a third brood (i.e., due to mortality) were replaced by a randomly selected neonate from the remaining replicates of the same treatment. The number of offspring was counted and removed daily. As endpoints, survival, time to first brood, inter-brood duration (i.e., the average time between broods), cumulative mean number of offspring produced per adult *D. magna*, and body length (i.e., distance from nauplius eye to the base of the apical spine) of adult *D. magna* were determined. To measure body length, a digital image was taken from each replicate at test termination and analysed using ImageJ (version 1.53k; National Institutes of Health, USA). Additionally, environmental parameters were set at the conditions used during culturing. The ASTM medium was renewed three times per week with the physico-chemical parameters (pH, temperature and dissolved oxygen) of the fresh and old ASTM medium being recorded (using WTW Multi 340i; Table S6).

2.5. *Dietary Cu accumulation in Daphnia*

Five-day-old unexposed *D. magna* (originating from the lab culture) were allowed to evacuate their guts for 3 h as suggested by Zhao et al. (2009). Afterwards, dietary Cu accumulation was assessed by transferring 700 *D. magna* to each of the nine replicates set up per treatment. Each replicate consisted of a glass microcosm filled with 1.5 L fresh ASTM medium, while daphnids were fed 100 µg C of *D. subspicatus* per animal (Table 1) for 48 h. Medium and food were renewed after 24 h. Due to the lack of effects caused during the two-generational experiment, NOM treatments were excluded. We employed 700 five-day-old animals per replicate to ensure sufficient biomass for Cu quantification and to avoid reproduction during the study duration. To quantify internal Cu concentrations in *D. magna*, three replicates, each containing 700 animals, per treatment were removed from the experiment after 48 h of exposure and 50 min as well as 12 h after transfer to fresh ASTM medium (depuration). Animals were rinsed with 10 µM Na_2EDTA for 5 min (Hartmann et al., 2012), followed by MilliQ water for 1 min to remove any contaminated debris attached to the surface. After that, the animals are stored at − 20 °C until chemical analysis, following the procedure outlined for alga.

2.6. *Data analysis*

The statistical analyses and visualization of data were performed using R version 2022.12.0 for Windows (R Core Team, 2022) and the extension packages "DescTools" (Signorell et al., 2019), "permuco" (Frossard and Renaud, 2021), "ggplot2″ (Wickham, 2016) and "ggpubr" (Kassambara, 2020). The data were analysed for normality using Shapiro–Wilk tests and visual inspection. Due to normality but unequal variances of the data, Welch t-tests (Welch, 1947) were performed to compare two treatments (with a similar combination of factors) for statistical significance of Cu concentrations in alga (Table 1) or in *D. magna* (Table 2) in terms of absence vs. presence of $nTiO_2$ or dietary accumulation vs. depuration and between aging durations. In contrast, the data from the two-generational experiment (for instance, time to first brood, inter-brood duration, cumulative mean offspring/adults and body length) were analysed with a permutational analysis of variance (Frossard and Renaud, 2021) due to the lack of normality and variance homogeneity. Additionally, Benjamini-Hochberg post hoc testing was used for comparison of the effects as a function of Cu, NOM, $nTiO_2$, aging and their interaction (Fig. 2, Table S7 – 10). Statistically significant differences of the response variable among the treatments in the two-generational experiment relative to a control ("Ctrl"; in absence of Cu and $nTiO_2$) or in absence of $nTiO_2$, but the presence of Cu of the respective aging duration and generation are reported in Figs. 3, 4, S4 and S5. In the comparisons, statistically significant differences are represented at levels of $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*) and $p < 0.1$ (°).

3. Results

3.1. *Effects on Daphnia over two generations*

Survival of *D. magna* was virtually unaffected by dietary Cu exposure over two generations (Fig. S3). In fact, mortality never exceeded 10% and randomly occurred between generations and treatments. Similarly, the time to first brood of *D. magna* was not affected among generations if

Table 2
Cu body burden of *D. magna* after dietary exposure. Daphnids were fed for 48 h with *D. subspicatus* exposed for 96 h to Cu aged for 0 and 3 days in presence of $nTiO_2$ (Table 1). Exposure of *D. magna* was followed by 50 min and 12 h depuration in uncontaminated ASTM medium. The data are represented as the mean ± SD (n = 3). Asterisk (*) indicates a significant difference in Cu concentration in *D. magna* after 50 min or 12 h of depuration relative to the end of the exposure duration. The ‘a′ denotes a significant difference ($p < 0.05$) in Cu concentration within the organism relative to the same combinations of factors but without aging (0 days).

		Cu concentration in *D. magna* (µg Cu/g dry body weight)		
Aging (days)	$nTiO_2$ (mg/L)	At termination of exposure	Depuration: 50 min	Depuration: 12 h
0	0	19.36 ± 3.60	17.44 ± 3.71	12.45 ± 1.15
	0.6	21.01 ± 0.72	14.24 ± 0.73 * **	10.55 ± 0.75 * **
	3.0	22.29 ± 0.27	13.64 ± 3.02 *	11.47 ± 0.42 * **
3	0	20.89 ± 2.55	14.40 ± 1.51 *	5.62 ± 1.35 * *[a]
	0.6	20.15 ± 1.13	13.70 ± 2.41 *	8.12 ± 0.58 * **
	3.0	21.64 ± 1.24	15.18 ± 1.31 * *	6.51 ± 3.01 * *[a]

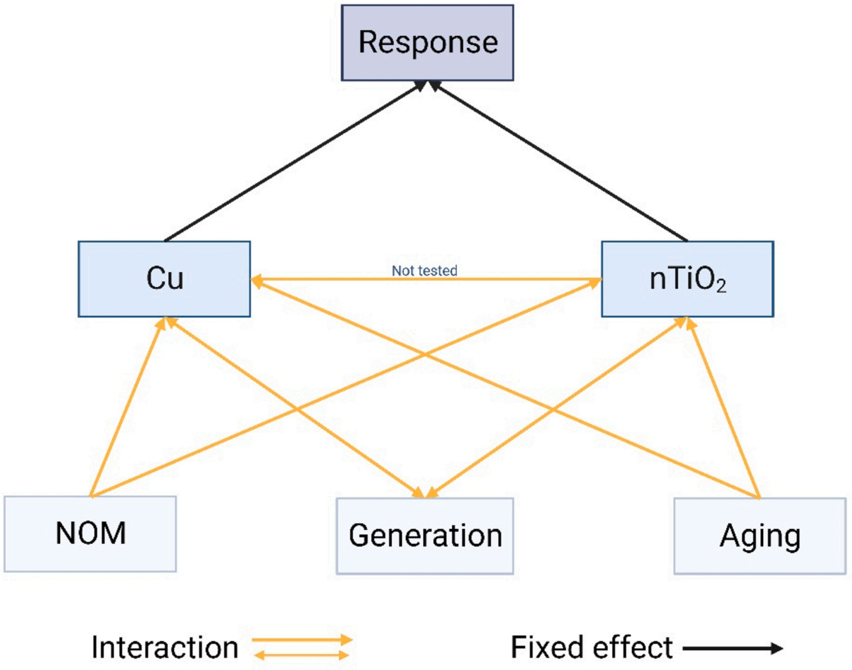

Fig. 2. Directed acyclic graph for the analyses of the response variables (e.g., time to first, inter-brood duration) of *D. magna* in the two-generational experiment as a function of Cu, NOM, $nTiO_2$, aging and their interactions. Please note that it was not intended by design (i.e., lack of treatments assigned to $nTiO_2$ in absence of Cu) to analyse the interactions between $nTiO_2$ and Cu.

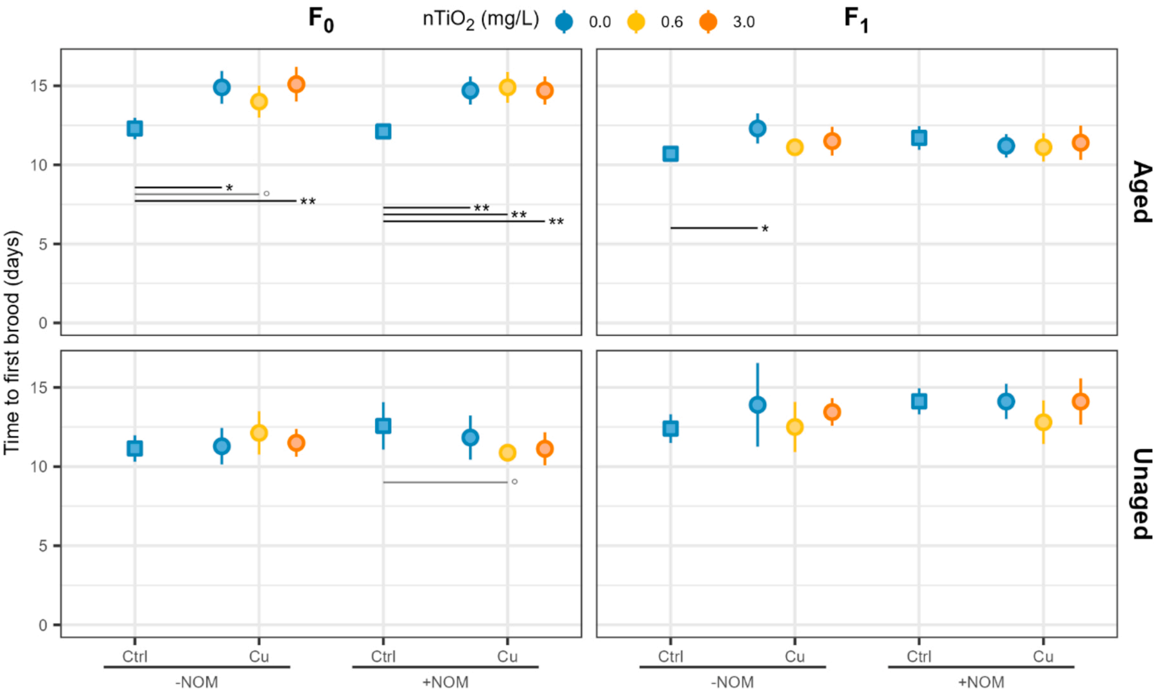

Fig. 3. Time to first brood for *D. magna* over two generations fed with *D. subspicatus* exposed for 96 h to Cu (0 or 116 µg Cu/L) aged for 0 (i.e., unaged) and 3 days in presence of NOM (0 or 8 mg TOC/L) and $nTiO_2$ (0.0, 0.6 or 3.0 mg $nTiO_2$/L). The data are represented as the mean ± 95% confidence interval (n = 10). Statistically significant differences of the response variable among the treatments relative to a control ("Ctrl"; in absence of Cu and $nTiO_2$) of the respective aging duration and generation are denoted by 'asterisk' (*) and 'opened circle' (°) which represent $p < 0.05$ and < 0.1, respectively. Statistical analyses of the data using permutational analysis of variance are displayed in Table S7.

alga was exposed to an unaged mixture of factors relative to the control (Fig. 3). Contrary, this parameter was delayed in the F_0 but not the F_1 generation by approximately 30% through dietary Cu exposure at all factor combinations when aged for 3 days. Additionally, the inter-brood duration remained unaffected (Fig. S4).

The cumulative reproduction of *D. magna* at F_0 generation was significantly stimulated by around 30% through dietary Cu exposure when combined with 0.0 and 0.6 mg $nTiO_2$/L and irrespective of the NOM level without aging (Fig. 4). Inversely, this parameter was approximately 30% significantly reduced in both generations when

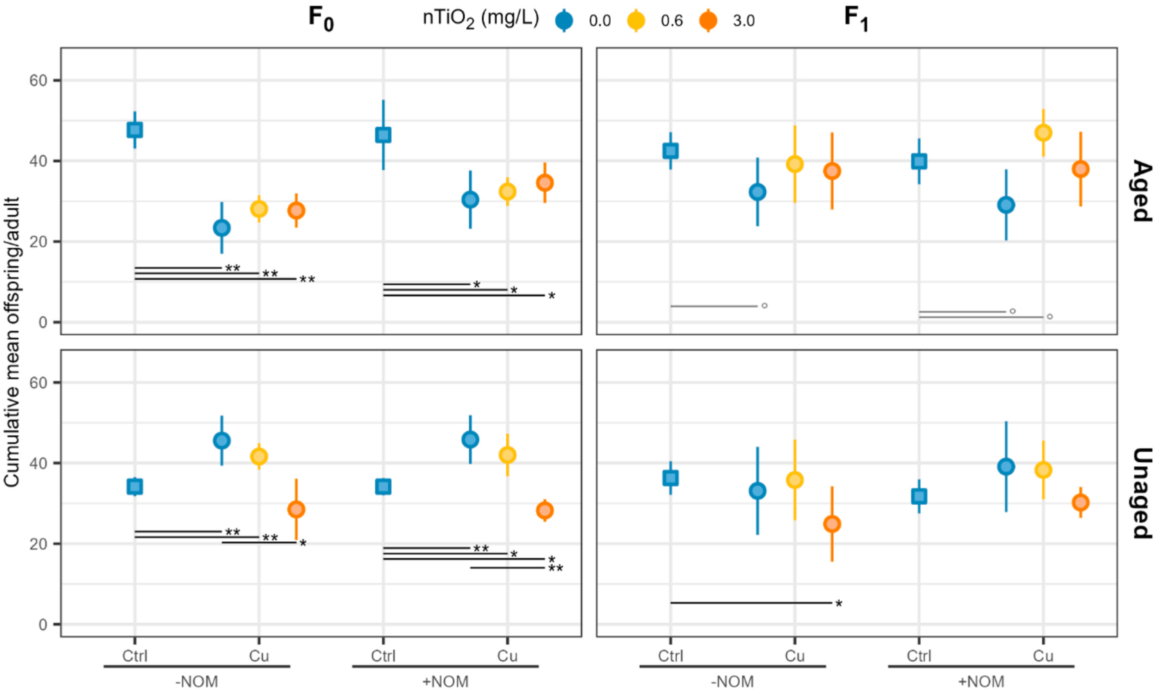

Fig. 4. The cumulative mean number of offspring produced per adult *D. magna* over two generations fed with *D. subspicatus* exposed for 96 h to Cu (0 or 116 µg Cu/L) aged for 0 (i.e., unaged) and 3 days in presence of NOM (0 or 8 mg TOC/L) and $nTiO_2$ (0.0, 0.6 or 3.0 mg $nTiO_2$/L). The data are represented as the mean ± 95% confidence interval (n = 10). Statistically significant differences of the response variable among the treatments relative to a control ("Ctrl"; in absence of Cu and $nTiO_2$) or in absence of $nTiO_2$ but the presence of Cu of the respective aging duration and generation are denoted by 'asterisk' (*) or 'opened circle' (°) which represent $p < 0.05$ and < 0.1, respectively. Statistical analyses of the data using permutational analysis of variance are displayed in Table S9.

combined with 3.0 mg $nTiO_2$/L without aging. In addition, cumulative reproduction of the F_0 but not the F_1 generation was significantly reduced by up to a factor of two after 3 days of aging and irrespective of $nTiO_2$ levels. In contrast to reproduction, the body length of *D. magna* was in most cases, except for a 30% reduction when Cu was combined with 3.0 mg $nTiO_2$/L without aging and NOM in the F_0 generation, unaffected across treatments and generations (Fig. S5).

3.2. Dietary Cu accumulation in Daphnia

The Cu concentration in *D. magna* reached approximately 20 µg Cu/g dry weight after 48 h of exposure (Table 2), an observation that is independent of $nTiO_2$ concentrations and aging duration. Similarly, after a 50 min depuration, the Cu body burden was significantly reduced by 30–35% independent of $nTiO_2$ concentrations and aging duration. In contrast, 0 and 3 days of aging between Cu and $nTiO_2$ reduced the Cu body burden after 12 h of depuration by 2- and 4-fold, respectively (Table 2).

4. Discussion

4.1. Impact of $nTiO_2$ on the toxicity of dietary Cu exposure in absence of NOM

Dietary Cu increased the overall offspring production per adult in the F_0 generation if exposure was realised without aging as well as in absence or presence of NOM and $nTiO_2$ at low (0.6 mg $nTiO_2$/L) levels. This observation is in line with an earlier study documenting a stimulatory impact on the reproduction of *D. magna* at low Cu levels (Dave, 1984). In contrast, the presence of 3.0 mg $nTiO_2$/L reduced the overall offspring production. Earlier reports emphasize a negative impact on *D. magna* due to the higher Cu bioaccumulation with increasing $nTiO_2$ level (mainly via waterborne exposure (Fan et al., 2011; Liu et al., 2019; Wang et al., 2023)). This observation could not be confirmed in the present study. In fact, dietary Cu accumulation in *D. magna* did not significantly vary among $nTiO_2$ levels even though Cu concentration in food (i.e., *D. subspicatus*) was enhanced in the presence of 3.0 mg $nTiO_2$/L relative to its absence (Table 1). These insights point to other mechanisms, such as the combined effect of $nTiO_2$ and Cu, driving the observed impacts in *D. magna* reproduction. In addition to the negative impact on *D. magna* reproduction at high $nTiO_2$ levels (without aging and in the absence of NOM), the body length of adults was reduced, which may point to a reallocation of energy towards survival and detoxification that would otherwise be used for reproduction and somatic growth (McNab, 2002; Martínez-Jerónimo, 2012). According to DuRant et al. (2007), energy assimilated by organisms is mainly assigned to maintenance, i.e., survival through basal metabolism, activities (such as locomotion, foraging and digestion) and detoxification. Substantially less energy is allocated towards production, i.e., reproduction and somatic growth. Therefore, contaminant-induced increases in the amount of energy allocated to maintenance may lead to proportionately higher reductions in production, which in turn may cause variations in life history traits, including maturity age, reproductive output, growth rate and size (Lucas, 1996). This logic is represented by the net-production model (Peeters et al., 2010) where maintenance is prioritised using assimilated energy over somatic growth and reproduction.

In contrast to the effects observed without aging, the onset of first brood was delayed and overall offspring production per adult *D. magna* was reduced in presence of Cu across all $nTiO_2$ levels in the F_0 generation when fed with the alga exposed for 96 h to 3 days aged medium. Although Cu concentrations in alga of these treatments are significantly higher relative to the unaged treatments (Table 1), our bioaccumulation study suggests that Cu body burdens were comparable after a 48 h exposure and accumulated Cu was more efficiently depurated (Table 2). Thus, the reduction in reproduction is not explained by an elevated dietary metal accumulation in *D. magna* (Yang et al., 2018a). One possible explanation for these observations is the feeding preference of *D. magna*

(De Schamphelaere et al., 2007; DeMott, 1982), leading to a potential avoidance of alga exposed to medium aged for 3 days. This, in turn, may reduce food consumption, as alternative food sources have been lacking. Moreover, the nutritional quality of alga may have been affected (i.e., limited elemental C, N, P; essential fatty acids and amino acids), contributing to the observed changes in reproduction (Kilham et al., 1997). As both mechanisms lack empirical support from the present study, enhanced metabolic cost due to detoxification mechanisms may serve as an additional explanation (De Schamphelaere et al., 2007; Zhao et al., 2009) as proposed by the dynamic energy budget theory (Kooijman, 2000). While the underlying mechanisms remain unclear, we can conditionally confirm the first hypothesis, namely that the presence of $nTiO_2$ impairs the fitness of *Daphnia* through dietary Cu exposure, with the caveat that this is a function of aging duration. This observation was, however, not supported by our studies targeting Cu body burdens. Therefore, the mechanistic link covered by our second hypothesis is rejected.

As a consequence of parental exposure to dietary Cu, *D. magna* of the subsequent generation (i.e., F_1) showed a reduced reproduction when fed over two generations with the alga exposed for 96 h to Cu and 3.0 mg $nTiO_2$/L without aging. As such an effect was not observed in the F_0 generation, it may be assumed that the released offspring may have suffered from a lower transfer of energy by birth, which in combination with the investment for detoxification, diverted less energy to reproduction of the F_1 generation (Castro et al., 2018). Additionally, the higher (at least relative to 3 days aging; Table 2) Cu body burden directly after birth due to a potential maternal transfer (Wu et al., 2017) and malfunction of metallothionein-like proteins (Tsui and Wang, 2007) may cause additional stress mirrored in the reproductive output.

In contrast to the increasing effects over generations when no aging was realised, an aging duration of 3 days led to a disappearance of negative effects in reproduction observed at 3.0 mg $nTiO_2$/L relative to the control during F_1, while during the F_0 generation a negative effect was observed (see above). Though the underlying mechanisms for such adaptive responses are still obscure, the observation may partially be explained by a physiological process (Guan and Wang, 2006; Klerks and Weis, 1987) in the filial generation during the early stages of development, including oogenesis and early embryogenesis (Clubbs and Brooks, 2007). Cu may have promoted epigenetic inheritance to the filial generation via changes in DNA methylation, which further triggers genes responsible for dynein light chain, ribosomal kinase, metallothionein and glutathione-S-transferase to counteract the direct and indirect effects of Cu and oxidative stress, respectively (Chain et al., 2019; Jeremias et al., 2022). Moreover, Cu as an essential trace element may have been actively regulated (i.e., adjusting excretion rates) or stored (as detoxified forms, such as in inorganic cell inclusions) or transported and metabolised involving low molecular-weight proteins (Bossuyt and Janssen, 2005, 2004). Although the underlying mechanisms remain unclear, we can postulate that the third hypothesis, which suggests adaptation in the filial generation reflected in higher tolerance, can be partially accepted.

4.2. *Role of NOM on the toxicity of dietary Cu exposure*

NOM, which is ubiquitously present in the aquatic environment, is known to form complexes with co-existing metals, ultimately reducing metal bioavailability (Rosenfeldt et al., 2015) and, thus, toxicity. Moreover, it forms an eco-corona on $nTiO_2$ with increasing aging duration (please see Table S4), which stabilizes their dispersion via electrostatic repulsion (Liu et al., 2023; Loosli et al., 2014). Consequently, the surface area of $nTiO_2$ available for metal ions adsorption increases (Chen et al., 2012), modulating the metal's impact on organisms. Additionally, the eco-corona restricts metal remobilization during gut passage (Levard et al., 2012), reducing adverse effects. NOM also serves as a depurating ligand, facilitating the rapid elimination of metal from aquatic organisms (Glover and Wood, 2005), and reducing metal toxicity. However, in the present study, NOM did not substantially change Cu toxicity through dietary pathways on *D. magna* irrespective of aging duration and generation. This observation may be attributed to algal exopolymeric substances (Mahana et al., 2021; Nogueira et al., 2005) or intracellular organic matter (Fang et al., 2010; Yang et al., 2018b) as the contaminants (here Cu and $nTiO_2$ irrespective of the treatments) may be entrapped within or around alga, which may make the impact of NOM redundant. Consequently, the fourth hypothesis suggesting NOM for reducing any negative impact via the formation of eco-corona and complexation of metal over two generations cannot be confirmed.

In conclusion, this study shows that the aging of $nTiO_2$ plays a crucial role in determining the effects induced through the dietary exposure pathways for Cu. More importantly, the data suggest that it is impossible to extrapolate from the effects observed during the parental generation to the responses in the filial generation. These discrepancies may be triggered by an altered maternal transfer of energy and potentially chemical stressors (here Cu) to the filial generation as well as adaption. However, both mechanisms need further attention, particularly as our assumption that the effects may be linked to the Cu body burden of *D. magna* was not supported. Consequently, basing an assessment of potential chronic risks associated with chemicals on the responses of *Daphnia* over just two generations may not be sufficient.

CRediT authorship contribution statement

Sivagnanam Mugilvannan: Investigation, Writing – review & editing. **Grünling Lea:** Investigation, Writing – review & editing. **Feckler Alexander:** Methodology, Writing – review & editing. **Meyer Frederik:** Methodology, Writing – review & editing. **Kempter Lucas:** Investigation, Methodology, Writing – review & editing. **Bollinger Eric:** Formal analysis, Methodology, Visualization, Writing – review & editing. **Philippe Allan:** Investigation, Methodology, Writing – review & editing. **Schulz Ralf:** Conceptualization, Resources, Supervision, Writing – review & editing. **Seitz Frank:** Methodology, Writing – review & editing. **Roy Rajdeep:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Bundschuh Mirco:** Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available on the Mendeley data repository at doi: 10.17632/wy4vx8bc83.1.

Acknowledgments

The authors thank Anna Baskal and Fintan Hahn for the ICP-MS analysis and Carsten Schilde for providing the $nTiO_2$ dispersion. We are grateful to Erin Bennett for proofreading and checking the language. We are also thankful to Aditya Narang, Maxime Bonin, Makhtar Bashir and Sven Korz for their support in various aspects. We also thank the German Academic Exchange Service (Grant No. 57299294) for the financial support. Furthermore, the illustrations (Figs. 1, 2 and S2) are created with BioRender.com.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116031.

References

Abdel-Latif, H.M.R., Dawood, M.A.O., Menanteau-Ledouble, S., El-Matbouli, M., 2020. Environmental transformation of n-TiO_2 in the aquatic systems and their ecotoxicity in bivalve mollusks: a systematic review. Ecotoxicol. Environ. Saf. 200, 110776 https://doi.org/10.1016/j.ecoenv.2020.110776.

Adusei-Gyamfi, J., Ouddane, B., Rietveld, L., Cornard, J.-P., Criquet, J., 2019. Natural organic matter-cations complexation and its impact on water treatment: a critical review. Water Res. 160, 130–147. https://doi.org/10.1016/j.watres.2019.05.064.

Ahmed, I., Zhang, B., Muneeb-Ur-Rehman, M., Fan, W., 2021. Effect of different shapes of Nano-Cu_2O and humic acid on two-generations of *Daphnia magna*. Ecotoxicol. Environ. Saf. 207, 111274 https://doi.org/10.1016/j.ecoenv.2020.111274.

Akakuru, O.U., Iqbal, Z.M., Wu, A., 2020. TiO_2 nanoparticles: properties and applications. In: Wu, A., Ren, W. (Eds.), TiO_2 nanoparticles. Wiley, pp. 1–66. https://doi.org/10.1002/9783527825431.ch1.

ASTM, 2007. E729-96 Standard Guide For Conducting Acute Toxicity Tests On Test Materials With Fishes, Macroinvertebrates And Amphibians. ASTM International.

Awoyemi, O.M., Subbiah, S., Thompson, K.N., Velazquez, A., Peace, A., Mayer, G.D., 2020. Trophic-level interactive effects of phosphorus availability on the toxicities of cadmium, arsenic, and their binary mixture in media-exposed *Scenedesmus acutus* and media and dietary-exposed *Daphnia pulex*. Environ. Sci. Technol. 54, 5651–5666. https://doi.org/10.1021/acs.est.9b07657.

Bossuyt, B.T.A., Janssen, C.R., 2003. Acclimation of *Daphnia magna* to environmentally realistic copper concentrations. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 136, 253–264. https://doi.org/10.1016/j.cca.2003.09.007.

Bossuyt, B.T.A., Janssen, C.R., 2004. Influence of multigeneration acclimation to copper on tolerance, energy reserves, and homeostasis of *Daphnia magna Straus*. Environ. Toxicol. Chem. 23, 2029. https://doi.org/10.1897/03-377.

Bossuyt, B.T.A., Janssen, C.R., 2005. Copper regulation and homeostasis of *Daphnia magna* and *Pseudokirchneriella subcapitata*: influence of acclimation. Environ. Pollut. 136, 135–144. https://doi.org/10.1016/j.envpol.2004.11.024.

Castro, B.B., Freches, A.R., Rodrigues, M., Nunes, B., Antunes, S.C., 2018. Transgenerational effects of toxicants: an extension of the *Daphnia* 21-day chronic assay? Arch. Environ. Contam. Toxicol. 74, 616–626. https://doi.org/10.1007/s00244-018-0507-0.

Chain, F.J.J., Finlayson, S., Crease, T., Cristescu, M., 2019. Variation in transcriptional responses to copper exposure across *Daphnia pulex* lineages. Aquat. Toxicol. 210, 85–97. https://doi.org/10.1016/j.aquatox.2019.02.016.

Chen, Q., Yin, D., Zhu, S., Hu, X., 2012. Adsorption of cadmium(II) on humic acid coated titanium dioxide. J. Colloid Interface Sci. 367, 241–248. https://doi.org/10.1016/j.jcis.2011.10.005.

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

Chen, Z., Zhu, X., Lv, X., Huang, Y., Qian, W., Wang, P., Li, B., Wang, Z., Cai, Z., 2019. Alleviative effects of C_{60} on the trophic transfer of cadmium along the food chain in aquatic environment. Environ. Sci. Technol. 53, 8381–8388. https://doi.org/10.1021/acs.est.9b01636.

Clubbs, R.L., Brooks, B.W., 2007. *Daphnia magna* responses to a vertebrate estrogen receptor agonist and an antagonist: a multigenerational study. Ecotoxicol. Environ. Saf. 67, 385–398. https://doi.org/10.1016/j.ecoenv.2007.01.009.

Dave, G., 1984. Effects of copper on growth, reproduction, survival and haemoglobin in *Daphnia magna*. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 78, 439–443. https://doi.org/10.1016/0742-8413(84)90112-9.

De Schamphelaere, K.A.C., Forrez, I., Dierckens, K., Sorgeloos, P., Janssen, C.R., 2007. Chronic toxicity of dietary copper to *Daphnia magna*. Aquat. Toxicol. 81, 409–418. https://doi.org/10.1016/j.aquatox.2007.01.002.

DeMott, W.R., 1982. Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina* 1: Cladoceran grazing. Limnol. Oceanogr. 27, 518–527. https://doi.org/10.4319/lo.1982.27.3.0518.

Donnachie, R.L., Johnson, A.C., Moeckel, C., Pereira, M.G., Sumpter, J.P., 2014. Using risk-ranking of metals to identify which poses the greatest threat to freshwater organisms in the UK. Environ. Pollut. 194, 17–23. https://doi.org/10.1016/j.envpol.2014.07.008.

DuRant, S.E., Hopkins, W.A., Talent, L.G., 2007. Energy acquisition and allocation in an ectothermic predator exposed to a common environmental stressor. Comp. Biochem. Physiol. C145, 442–448.

Fan, W., Zhang, Y., Liu, S., Li, X., Li, J., 2020. Alleviation of copper toxicity in *Daphnia magna* by hydrogen nanobubble water. J. Hazard. Mater. 389, 122155 https://doi.org/10.1016/j.jhazmat.2020.122155.

Fan, W., Liang, D., Wang, X., Ren, J., Xiao, S., Zhou, T., 2019. Two-generational effects and recovery of arsenic and arsenate on *Daphnia magna* in the presence of nano-TiO_2. Ecotoxicol. Environ. Saf. 172, 136–143. https://doi.org/10.1016/j.ecoenv.2019.01.072.

Fan, W., Cui, M., Liu, H., Wang, C., Shi, Z., Tan, C., Yang, X., 2011. Nano-TiO_2 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.

Fang, J., Yang, X., Ma, J., Shang, C., Zhao, Q., 2010. Characterization of algal organic matter and formation of DBPs from chlor(am)ination. Water Res 44, 5897–5906. https://doi.org/10.1016/j.watres.2010.07.009.

Frossard, J., Renaud, O., 2021. Permutation tests for regression, ANOVA, and comparison of signals: The permuco Package. J. Stat. Soft. 99 https://doi.org/10.18637/jss.v099.i15.

Geffard, O., Geffard, A., Chaumot, A., Vollat, B., Alvarez, C., Tusseau-Vuillemin, M.-H., Garric, J., 2008. Effects of chronic dietary and waterborne cadmium exposures on the contamination level and reproduction of *Daphnia magna*. Environ. Toxicol. Chem. 27, 1128. https://doi.org/10.1897/07-431.1.

Glover, C.N., Wood, C.M., 2005. Accumulation and elimination of silver in *Daphnia magna* and the effect of natural organic matter. Aquat. Toxicol. 73, 406–417. https://doi.org/10.1016/j.aquatox.2005.05.002.

Guan, R., Wang, W.-X., 2006. Multigenerational cadmium acclimation and biokinetics in *Daphnia magna*. Environ. Pollut. 141, 343–352. https://doi.org/10.1016/j.envpol.2005.08.036.

Gustafsson, J.P., 2013. Visual MINTEQ, 3.1. KTH. Department of sustainable development, Environmental Science and Engineering, Stockholm, Sweden.

Hartmann, N.B., Legros, S., Von Der Kammer, F., Hofmann, T., Baun, A., 2012. The potential of TiO_2 nanoparticles as carriers for cadmium uptake in *Lumbriculus variegatus* and *Daphnia magna*. Aquat. Toxicol. 118–119, 1–8. https://doi.org/10.1016/j.aquatox.2012.03.008.

Hartmann, S., Louch, R., Zeumer, R., Steinhoff, B., Mozhayeva, D., Engelhard, C., Schönherr, H., Schlechtriem, C., Witte, K., 2019. Comparative multi-generation study on long-term effects of pristine and wastewater-borne silver and titanium dioxide nanoparticles on key lifecycle parameters in *Daphnia magna*. NanoImpact 14, 100163. https://doi.org/10.1016/j.impact.2019.100163.

Jacobasch, C., Völker, C., Giebner, S., Völker, J., Alsenz, H., Potouridis, T., Heidenreich, H., Kayser, G., Oehlmann, J., Oetken, M., 2014. Long-term effects of nanoscaled titanium dioxide on the cladoceran *Daphnia magna* over six generations. Environ. Pollut. 186, 180–186. https://doi.org/10.1016/j.envpol.2013.12.008.

Jeremias, G., Veloso, T., Gonçalves, F.J.M., Van Nieuwerburgh, F., Pereira, J.L., Asselman, J., 2022. Multigenerational DNA methylation responses to copper exposure in *Daphnia*: Potential targets for epigenetic biomarkers? Chemosphere 308, 136231. https://doi.org/10.1016/j.chemosphere.2022.136231.

Kassambara, A., 2020. ggpubr:"ggplot2" based publication ready plots. R package version 0.4. 0, 438.

Kilham, S., Kreeger, D., Goulden, C., Lynn, S., 1997. Effects of algal food quality on fecundity and population growth rates of Daphnia. Freshw. Biol. 38 (3), 639–647.

Kim, I., Lee, B.-T., Kim, H.-A., Kim, K.-W., Kim, S.D., Hwang, Y.-S., 2016. Citrate coated silver nanoparticles change heavy metal toxicities and bioaccumulation of *Daphnia magna*. Chemosphere 143, 99–105. https://doi.org/10.1016/j.chemosphere.2015.06.046.

Klerks, P.L., Weis, J.S., 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. Environ. Pollut. 45, 173–205. https://doi.org/10.1016/0269-7491(87)90057-1.

Kooijman, S.A.L.M., 2000. Dynamic energy and mass budgets in biological systems. Cambridge university press.

Kuhl, A. and Lorenzen, H., 1964. Handling and culturing of *Chlorella*. D.M. Prescott (ed.) Methods Cell Physiol. 1, N.Y. and London: 152–187.

Levard, C., Hotze, E.M., Lowry, G.V., Brown, G.E., 2012. Environmental transformations of silver nanoparticles: impact on stability and toxicity. Environ. Sci. Technol. 46, 6900–6914. https://doi.org/10.1021/es2037405.

Li, L., Sillanpää, M., Schultz, E., 2017. Influence of titanium dioxide nanoparticles on cadmium and lead bioaccumulations and toxicities to *Daphnia magna*. J. Nanopart. Res. 19, 223 https://doi.org/10.1007/s11051-017-3916-5.

Li, M., Slaveykova, V.I., 2022. Dual role of titanium dioxide nanoparticles in the accumulation of inorganic and methyl mercury by crustacean *Daphnia magna* through waterborne and dietary exposure. Environ. Pollut. 295, 118619 https://doi.org/10.1016/j.envpol.2021.118619.

Liu, S., Cui, M., Li, X., Thuyet, D.Q., Fan, W., 2019. Effects of hydrophobicity of titanium dioxide nanoparticles and exposure scenarios on copper uptake and toxicity in *Daphnia magna*. Water Res. 154, 162–170. https://doi.org/10.1016/j.watres.2019.01.055.

Liu, S., Zhang, X., Zeng, K., He, C., Huang, Y., Xin, G., Huang, X., 2023. Insights into eco-corona formation and its role in the biological effects of nanomaterials from a molecular mechanisms perspective. Sci. Total Environ. 858, 159867 https://doi.org/10.1016/j.scitotenv.2022.159867.

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.

Lucas, A., 1996. Bioenergetics of aquatic animals. Taylor and Francis Ltd, London.

Luo, Z., Li, Z., Xie, Z., Sokolova, I.M., Song, L., Peijnenburg, W.J.G.M., Hu, M., Wang, Y., 2020. Rethinking Nano-TiO_2 safety: overview of toxic effects in humans and aquatic animals. Small 16, 2002019. https://doi.org/10.1002/smll.202002019.

Mahana, A., Guliy, O.I., Mehta, S.K., 2021. Accumulation and cellular toxicity of engineered metallic nanoparticle in freshwater microalgae: current status and future challenges. Ecotoxicol. Environ. Saf. 208, 111662 https://doi.org/10.1016/j.ecoenv.2020.111662.

Martínez-Jerónimo, F., 2012. Description of the individual growth of *Daphnia magna* (Crustacea: Cladocera) through the von Bertalanffy growth equation. Effect of photoperiod and temperature. Limnology 13, 65–71. https://doi.org/10.1007/s10201-011-0356-2.

Matouke, M., Mustapha, M., 2020. Impact of co-exposure to titanium dioxide nanoparticles (TiO_2 NPs) and lead (Pb) on African catfish *Clarias gariepinus* (Burchell, 1922) fed contaminated copepods (*Eucyclop* sp.). Environ. Sci. Pollut. Res. 27, 16876–16885. https://doi.org/10.1007/s11356-020-08234-0.

McNab, B.K., 2002. The physiological ecology of vertebrates: A view from energetics. Cornell University Press.

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

Nogueira, P.F.M., Melao, M.D.G.G., Lombardi, A.T., Vieira, A.A.H., 2005. The effects of *Anabaena spiroides* (Cyanophyceae) exopolysaccharide on copper toxicity to

Simocephalus serrulatus (Cladocera, Daphnidae). Freshw. Biol. 50, 1560–1567. https://doi.org/10.1111/j.1365-2427.2005.01406.x.

Nordberg, G. (Ed.), 2007. Handbook on the toxicology of metals, 3rd ed. Academic Press, Amsterdam; Boston.

OECD, 2006. Test No. 201: Alga, growth Inhibition test, OECD Guidelines for the testing of chemicals, Section 2: Effects on Biotic Systems. OECD Publishing. https://doi.org/10.1787/9789264069923-en.

Peeters, F., Li, J., Straile, D., Rothhaupt, K.O., Vijverberg, J., 2010. Influence of low and decreasing food levels on Daphnia-algal interactions: Numerical experiments with a new dynamic energy budget model. Ecol. Modell. 221 (22), 2642–2655.

Philippe, A., Bazoobandi, A., Goeppert, N., 2022. Quantification of anthropogenic TiO_2 nanoparticles in soils and sediments combining size fractionation and trace element ratio. J. Anal. . Spectrom. 37, 338–350. https://doi.org/10.1039/D1JA00304F.

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Richardson, H.W. (Ed.), 1997. Handbook of copper compounds and applications. Marcel Dekker, New York.

Rosenfeldt, R.R., 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, R.R., Seitz, F., Senn, L., Schilde, C., Schulz, R., Bundschuh, M., 2015. 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.

Roy, R., Kandrapu, V.K., Kempter, L., Islam, R., Kalčíková, G., Schulz, R., Bundschuh, M., 2022. Nanosized titanium dioxide elevates toxicity of cationic metals species for *Daphnia* – have aging and natural organic matter an unexpected impact? Nanotoxicology 16, 16–28. https://doi.org/10.1080/17435390.2022.2027538.

Roy, R., Lüderwald, S., Alawi Ahmed Maknoon, A., Metreveli, G., Schulz, R., Bundschuh, M., 2021. Effects of copper in *Daphnia* are modulated by nanosized titanium dioxide and natural organic matter: what is the impact of aging duration? Environ. Sci. Pollut. Res. 28, 13991–13999. https://doi.org/10.1007/s11356-020-11578-2.

Seitz, F., Lüderwald, S., Rosenfeldt, R.R., 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.

Shi, Q., Wang, C.L., Zhang, H., Chen, C., Zhang, X., Chang, X.-L., 2020. Trophic transfer and biomagnification of fullerenol nanoparticles in an aquatic food chain. Environ. Sci.: Nano 7, 1240–1251. https://doi.org/10.1039/C9EN01277J.

Signorell, A., Aho, K., Alfons, A., Anderegg, N., Aragon, T., & Arppe, A., 2019. DescTools: Tools for descriptive statistics. R package version 0.99, 28, 17.

Tan, C., Fan, W.-H., Wang, W.-X., 2012. Role of titanium dioxide nanoparticles in the elevated uptake and retention of cadmium and zinc in *Daphnia magna*. Environ. Sci. Technol. 46, 469–476. https://doi.org/10.1021/es202110d.

Tsui, M.T.-K., Wang, W.-X., 2007. Biokinetics and tolerance development of toxic metals in *Daphnia magna*. Environ. Toxicol. Chem. 26, 1023. https://doi.org/10.1897/06-430R.1.

Völker, C., Boedicker, C., Daubenthaler, J., Oetken, M., Oehlmann, J., 2013. Comparative toxicity assessment of nanosilver on three *Daphnia* species in acute, chronic and multi-generation experiments. PLoS ONE 8, e75026. https://doi.org/10.1371/journal.pone.0075026.

Wang, Y., Gao, X., Cheng, Y., Peijnenburg, W.J.G.M., Dong, Z., Fan, W., 2023. Nano-TiO_2 modifies heavy metal bioaccumulation in *Daphnia magna*: a model study. Chemosphere 312, 137263. https://doi.org/10.1016/j.chemosphere.2022.137263.

Welch, B.L., 1947. The generalization of 'Student's' problem when several different population variances are involved. Biometrika 34, 28. https://doi.org/10.2307/2332510.

Wickham, H., 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag New York.

Wu, F., Bortvedt, A., Harper, B.J., Crandon, L.E., Harper, S.L., 2017. Uptake and toxicity of CuO nanoparticles to *Daphnia magna* varies between indirect dietary and direct waterborne exposures. Aquat. Toxicol. 190, 78–86. https://doi.org/10.1016/j.aquatox.2017.06.021.

Yang, F., Zeng, L., Luo, Z., Wang, Z., Huang, F., Wang, Q., Drobne, D., Yan, C., 2018a. Complex role of titanium dioxide nanoparticles in the trophic transfer of arsenic from *Nannochloropsis maritima* to *Artemia salina* nauplii. Aquat. Toxicol. 198, 231–239. https://doi.org/10.1016/j.aquatox.2018.03.009.

Yang, X., Zheng, X., Wu, L., Cao, X., Li, Y., Niu, J., Meng, F., 2018b. Interactions between algal (AOM) and natural organic matter (NOM): Impacts on their photodegradation in surface waters. Environ. Pollut. 242, 1185–1197. https://doi.org/10.1016/j.envpol.2018.07.099.

Zhao, C.-M., Fan, W.-H., Wang, W.-X., 2009. Aqueous and dietary copper uptake and elimination in *Daphnia magna* determined by the ^{67}Cu radiotracer. Environ. Toxicol. Chem. 28, 2360. https://doi.org/10.1897/09-069.1.