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Photocatalytic properties of titanium dioxide nanoparticles affect habitat selection of and food quality for a key species in the leaf litter decomposition process

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ABSTRACT

Interactions with environmental parameters may alter the ecotoxicity of nanoparticles. The present study therefore assessed the (in)direct effects of nanoparticulate titanium dioxide (nano-TiO_2) towards *Gammarus fossarum*, considering nano-TiO_2's photocatalytic properties at ambient UV-intensities. Gammarids' habitat selection was investigated using its feeding preference on leaf discs either exposed to or protected from UV-irradiation in presence of nano-TiO_2 as proxy ($n = 49$). UV-irradiation alone induced a significant preference for UV-protected habitats, which was more pronounced in simultaneous presence of nano-TiO_2. This behaviour may be mainly explained by the UV-induced formation of reactive oxygen species (ROS) by nano-TiO_2. Besides their direct toxicity, ROS may have lowered the leaf-quality in UV-exposed areas contributing (approximately 30%) to the observed behavioural pattern. Since the predicted no effect concentration of nano-TiO_2 in combination with UV-irradiation falls below the predicted environmental concentration this study underpins the importance of considering environmental parameters during the risk assessment of nanoparticles.

1. Introduction

The nanotechnological industry is growing exponentially and its annual contribution to global economy is expected to reach $2.4 trillion by 2015 (cf. Pearce, 2012). The growing importance of this industry sector together with the widespread application of nanoparticles in various products (Buzea et al., 2007) inevitably results in their unintended release into aquatic ecosystems e.g. *via* wastewater treatment plant effluents with predicted concentrations of up to 70 μg/L (Gottschalk et al., 2013; Keller and Lazareva, 2014; Kiser et al., 2009). However, the risks of nanoparticles for aquatic ecosystems as well as the underlying modes of action are still largely unknown (Scown et al., 2010). This knowledge gap is particularly noticeable for benthic organisms, which are, due to the rapid agglomeration (Velzeboer et al., 2008) and sedimentation (Dabrunz et al., 2011) of nanoparticles, potentially subjected to continuously increasing nanoparticle exposure in future.

Particularly, titanium dioxide nanoparticles (nano-TiO_2) are frequently used in a wide range of products such as food, sun screens, cosmetics and façade paints (Weir et al., 2012) but also for advanced oxidation processes to purify air and (waste)water (Herrmann, 2005). The latter takes advantage of nano-TiO_2's photocatalytic properties, which may vary among its crystalline phases, capable of degrading organic compounds (Prado et al., 2013): By illuminating nano-TiO_2 with photons, e.g. UV-irradiation ($\lambda < 365$ nm; Ahmed et al., 2010), they form reactive oxygen species (ROS) – mainly hydroxyl radicals (•OH) and superoxide radicals (O_2^-; Kim et al., 2013; Nowotny, 2008). Even though useful for disinfection purposes, ROS may simultaneously pose risks for the integrity of ecosystems. However, adverse effects caused by a combination of nano-TiO_2 and UV-irradiation are not satisfactorily assessed yet (but see e.g. Bar-Ilan et al., 2013; Bundschuh et al., 2011a; Kalčíková et al., 2014; Marcone et al., 2012; Li et al., 2014a, 2014b), which potentially underestimates risks associated with the application of nano-TiO_2 for the ecosystems' integrity.

By assuming a substantial increase in the ecotoxicological potential of nano-TiO_2 under environmentally relevant intensities of UV-A and UV-B irradiation (e.g. Bar-Ilan et al., 2013; Bundschuh et al., 2011a; Kalčíková et al., 2014; Marcone et al., 2012; Li et al.,

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2014a, 2014b), the present study investigated direct and indirect behavioural effects on a key species in the detrital food web in central Europe, i.e. *Gammarus fossarum* KOCH (Crustacea: Amphipoda) (Dangles et al., 2004). On the one hand, it was assessed whether gammarids are capable to actively avoid habitats characterised by an increased level of ROS formed by nano-TiO_2 in presence of ambient UV-irradiation (=habitat selection). For this purpose, a two-factorial design was employed assessing the individual and combined effects of both stressors, namely nano-TiO_2 and UV-irradiation: gammarids were exposed to several concentrations of nano-TiO_2 (including a nano-TiO_2 free control) in the presence and absence of UV-irradiation, whereas half of each test vessels' surface was covered by a transparent UV-absorbing protection film providing the test organisms habitats with distinct characteristics in terms of UV-irradiation. By measuring the preference of gammarids for leaf discs either exposed to or protected from UV-irradiation, the habitat selection was assessed. On the other hand, potential implications of ROS-induced shifts in the food quality on the observed pattern of habitat selection were evaluated (=indirect effects). Thus, half of the leaf discs were pre-treated by UV-irradiation in presence and absence of nano-TiO_2 prior to offering them to gammarids in habitat selection experiments performed in total darkness. Finally, the experiments of the present study were supplemented by the quantification of ROS, using •OH as a proxy, which allows – although ignoring O_2^- – for a quantitative assessment of nano-TiO_2's photocatalytic activity in general.

2. Materials and methods

2.1. Preparation and characterization of nano-TiO_2

The nano-TiO_2-composite P25, used as test item in the present study, was purchased as powder containing ~70% anatase and ~30% rutile (Bhatkhande et al., 2002) with an advertised primary particle size of approximately 21 nm from Evonik (Germany). A dispersant- and additive-free, size-homogenized stable suspension was prepared by stirred media milling (PML2, Bühler AG, Switzerland) and subsequent centrifugation for removal of residually coarse material. Prior to each experiment, the undiluted stock suspension was ultrasonicated for 10 min to homogenize particle distribution (35 kHz; Bandelin Sonorex DT 514H, Germany), which was subsequently determined *via* dynamic light scattering (Delsa™ Nano C, Beckman Coulter, Germany). These measurements revealed a mean (±95% confidence interval (CI); $n = 3$) measured particle diameter in the stock solution of 59 (±2; 10^{th} and 90^{th} percentile of 38.0 and 97.7 nm, respectively) nm with a polydispersity index of approximately 0.1 (for more detailed information on particle characteristics see Seitz et al., 2014). Moreover, mean particle sizes were measured in the test medium after 24 h and thus at the time of bioassays' termination revealing an approximately 16-fold increase compared to the initial measurements (950 ± 155 nm; polydispersity index of approximately 0.294).

Measurements of TiO_2 levels were performed for the control as well as the lowest and the highest test concentration at the start and the end of the experiments following the method described in detail in Rosenfeldt et al. (2014). Briefly, triplicate 10 mL water samples were taken approximately 1 cm below the water surface (=middle of the water column) and acidified with HNO_3 to ensure a better storability until further processing. Using a quadrupole ICP-MS (XSeries 2, Thermo Fisher Scientific, Germany) equipped with a FAST auto sampler (ESI, Thermo Fisher Scientific, Germany), a peek spray chamber (Thermo Fisher Scientific, Germany) and a robust Mira Mist peak nebulizer (Burgener, England), the titanium concentrations were finally quantified for the mass ^{49}Ti. Time-weighted average concentrations (OECD, 2008) were calculated for the entire test duration of 24 h (Table 1), accounting for potentially reduced exposure concentrations in the water phase driven by nanoparticles' agglomeration and sedimentation (Dabrunz et al., 2011; Velzeboer et al., 2008). As the time-weighted measured mean concentrations deviated by less than 10% from the nominal concentrations, the latter will be referred to in the following.

2.2. Collection and maintenance of G. fossarum

Test organisms were collected by kick sampling from a near-natural stream (Hainbach) near Frankweiler, Germany (N 49°14′19″; E 08°02′59″), and acclimated to laboratory conditions and the test medium (=SAM-5S; Borgmann, 1996) for one week prior to each set of experiments. Animals were stepwise acclimated to SAM-5S in a temperature-controlled chamber set at 20 ± 1 °C in total darkness while fed *ad libitum* on preconditioned black alder leaves (*Alnus glutinosa* (L.), Gaertn.). A sufficient number of test organisms ($n \geq 49$), however, were starved for 96 h prior to each assay (Bundschuh et al., 2009).

The Hainbach population, which is sampled upstream of any settlement, treatment plant effluents and agricultural activity (cf. Feckler et al., 2012), has been routinely used in several previous studies (e.g. Bundschuh et al., 2011a; Feckler et al., 2012). Organisms were assorted into size classes immediately after sampling using a passive underwater separation technique (Franke, 1977). Only adult male gammarids – identified by their position in precopula pairs – with a cephalothorax length of approximately 1.2–1.6 mm were assessed in the present study, avoiding sex (Sornom et al., 2010) and life stage (McCahon and Pascoe, 1988) as potentially confounding factors. Likewise, parasitized specimens were excluded because of an anticipated altered behaviour (Pascoe et al., 1995).

2.3. Conditioning of leaf discs

Leaf discs were prepared as described in detail in Bundschuh et al. (2011b) with slight alterations. Briefly, senescent but undecomposed *A. glutinosa* leaves were collected in autumn 2012 shortly before leaf fall from trees near Landau, Germany (N 49°12′39″; E 8°13′15″). Subsequently, leaves were stored at −20 °C until further processing. After thawing, leaf discs of 20 mm were cut from leaves with a cork borer (avoiding the midrip) and subjected to a conditioning process: leaf discs were placed in aerated nutrient medium (Dang et al., 2005) for 10 days and inoculated using *A. glutinosa* leaves previously exposed in the Rodenbach, Germany (N 49°33′59″; E 8°02′33″), as *inoculum*. Hence, a near-natural microbial community *inter alia* consisting of fungi and bacteria was allowed to establish that increases leaves' nutritional value and palatability for leaf shredding organisms (Bärlocher, 1985). Afterwards, leaf discs were dried to constant weight at 60 °C, weighed to the nearest 0.01 mg and re-soaked in pure SAM-5S for 48 h prior to the start of each experiment avoiding fragmentation of leaf discs during handling.

Table 1
Nominal and time-weighted average (±95% CIs; $n = 4$) TiO_2 concentrations calculated for the entire test duration of 24 h. Limit of detection (LOD) = 1.4 µg/L.

Nominal concentration (µg/L)	Time-weighted average concentration (µg/L)
0	< LOD
7.5	6.8 ± 0.4
1500	1393 ± 126.1