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In situ effects of titanium dioxide nanoparticles on community structure of freshwater benthic macroinvertebrates☆

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ABSTRACT

For the first time in the current literature, the effect of titanium dioxide (TiO_2) nanoparticles on the community structure of macroinvertebrates has been investigated *in situ*. Macroinvertebrates were exposed for 100 days to an environmentally relevant concentration of TiO_2 nanoparticles, 25 mg kg^{-1} in sediment. Czekanowski's index was 0.61, meaning 39% of the macroinvertebrate community structure was affected by the TiO_2 treatment. Non-metric multidimensional scaling (NMDS) visualized the qualitative and quantitative variability of macroinvertebrates at the community level among all samples. A distance-based permutational multivariate analysis of variance (PERMANOVA) revealed the significant effect of TiO_2 on the macroinvertebrate community structure. The indicator value analysis showed that the relative frequency and abundance of *Planorbarius corneus* and *Radix labiata* were significantly lower in the TiO_2 treatment than in the control. Meanwhile, Ceratopogonidae, showed a significantly higher relative frequency and abundance in the TiO_2 treatment than in the control.

1. Introduction

Titanium dioxide (TiO_2) nanoparticles, microparticles, and bulk material present ecotoxicological concerns due to the rapid increase of anthropogenic input into the aquatic environment. Between 1916 and 2011, an estimated 165,050,000 metric tonnes of TiO_2 were produced worldwide, including all particle sizes and forms (Jovanović, 2015). TiO_2, among many industrial and commercial applications, is commonly used as a human food colorant ingredient labeled as E171 (other synonym labels for E171 includes CI77891 or Pigment White 6). A recent review article is however calling for the immediate safety reassessment on the use of TiO_2 as an inactive human food ingredient due to the potential TiO_2 toxic effect on human health (Jovanović, 2015). Recently, the daily human excretory contribution of E171 TiO_2 food color to municipal wastewater in large metropolis (with approximate population of 15 millions) was estimated to be roughly 1 tonne (Jovanović et al., 2016). Although majority of TiO_2 would be retained by wastewater treatment plants (96%) it was assumed that up to 15 tonnes per year will be released into aquatic environment in this metropolis example (Jovanović et al., 2016). This essentially means that approximately up to one tonne of human food grade TiO_2 is released annually into the aquatic environment per million inhabitants. Release of TiO_2 into aquatic environment from other sources (eg. textile washing, facade leaching, leaching from solid waste, release from sunscreens, etc) should be taken into account as well (Dulger et al., 2016; Gondikas et al., 2014; Kaegi et al., 2008; Windler et al., 2012).

The aggregation and sedimentation rate of TiO_2 in water is very high (Keller et al., 2010; Li et al., 2014a; Sharma, 2009; Velzeboer et al., 2014), and high sedimentation of engineered particles translates directly into potential risk for benthic communities (Koelmans et al., 2015; Li et al., 2014b). Benthic macroinvertebrates may be exposed to TiO_2 nano/micro particles through mechanical contact with their body parts, but TiO_2 nano/micro particles are also of suitable size for ingestion by various macroinvertebrates. One attribute used in macroinvertebrate classifications is the size of organic particles that can be ingested: coarse particulate organic matter feeders (particles > 1 mm) or fine particulate organic matter feeders (particles 0.45 μm < x > 1 mm). TiO_2 nano and microparticles are easily adsorbed to dissolved organic matter (DOM) or

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natural organic matter (NOM) (Keller et al., 2010; Domingos et al., 2009; Yang et al., 2009, 2013; Zhang et al., 2009). Such adsorption of TiO_2 can alter the bioavailability of NOM to macroinvertebrates or expose macroinvertebrates to TiO_2 by ingestion. In either case where TiO_2 expresses toxicity toward benthic invertebrates, their community structure and abundance are significantly altered.

The community structure and abundance of aquatic biota provide valuable information regarding water quality status. Aquatic communities reflect overall ecological integrity (chemical, physical, and biological) when responding to environmental changes via the loss of taxa, with opportunistic (tolerant) species becoming more numerically dominant (Gray, 1989). Therefore, changes in the multivariate structure of communities integrate the effects of different stressors. Also, macroinvertebrate assemblages are good indicators of localized condition, since many benthic macroinvertebrates have limited migration patterns. Thus, this group is appropriate for assessing site specific impacts. In all macroinvertebrate groups, the taxonomic and functional diversity of aquatic ecosystems seems to be crucially affected by the level of environmental degradation (Milošević et al., 2012). Therefore, various indices have been used to assess biodiversity variation of pristine versus impacted systems (Magurran, 2004), providing additional information about changes in community structure.

The aim of the present research was to investigate the effects of TiO_2 on the community structure of freshwater benthic macroinvertebrates. Since benthic macroinvertebrates are an intermediate and crucial link in the trophic food pyramid between primary producers and consumers in aquatic ecosystems, such assessment is of utmost importance.

2. Methodology

The upper sediment layer (5–10 cm deep) of a pristine pond in Vlasi county (42,999N 22,638E), Republic of Serbia, was collected and processed through a 5 mm mesh sieve to remove rocks, pebbles, and other large debris. The total organic carbon in the sediment was measured according to standard methodology (Walkley and Black, 1934). The percent of organic carbon in the sediment was 3.57 ± 0.52 [mean ± standard error of the mean (SEM)] of dry weight (N = 10). The sieved sediment was also autoclaved to eliminate all macroinvertebrates. Such measures prevented “false” colonization during the experiment. The autoclaved sediment was mixed with commercial human food grade E171 TiO_2 particles of 99% purity (Pharmorgana GmbH, Eppstein, Germany) suspended in water.

Previously, we performed extensive characterization of the E171 TiO_2 powder (Jovanović et al., 2016), and the exact jar of E171 used in that study was also used in the present study. The specific Brunauer–Emmett–Teller surface area of E171 is 6.137 $m^2\ g^{-1}$, its pore diameter is 2.968 nm, and its pore volume is 0.123 cc g^{-1}. X-ray diffraction peaks are well defined and assigned to the anatase crystal structure, while the surface is partially covered with OH groups. The geometry of the particles is amorphous and spherical-like with sharp and well-defined edges. According to atomic force microscopy, the estimated mean particle size ±SEM is 167 ± 50 nm.

Two sets of sediments were prepared: the control and a 25 mg kg^{-1} sediment treatment. This concentration was based on the upper quantile for estimated annual increase of nano-TiO_2 ($\Delta\mu g\ kg^{-1}\ y^{-1}$) in European water sediments using the Switzerland model ($\approx$2.5 mg kg^{-1}) (Gottschalk et al., 2009) and multiplying the safety factor by 10. The 25 mg kg^{-1} sediment was prepared using a stirring device (AEG Elektrowerkzeuge SB2E 1200 RST) operating continuously for 60 min per day for three consecutive days. After the stirring, TiO_2-spiked sediments were placed in forty plastic trays 25 × 20 × 6 cm (L × W × H). Trays were arranged in 8 parallel lines/transects. Each transect consisted of 5 trays in the same treatment. These 5 trays were aligned and attached to a rope using metal rings (see Fig. 1). The spacing between the trays was 5 cm. Four ropes (4 × 5 trays) were prepared for each TiO_2 treatment group and each control group. After attaching the trays to the ropes, the trays were carefully returned to the pond and submerged to align the walls with the sediment. Ropes with trays were placed as parallel transects alternating between treatment and control groups. Spacing between each transect was 1 m.

Trays were left in the pond for 100 days (July 1–November 10) and then collected and analyzed for macroinvertebrate content. Recorded specimens were identified at the lowest possible taxonomic level (genus, subgenus, species level) using relevant identification keys (Andersen et al., 2013; Moller Pillot, 1984a; Moller Pillot, 1984b; Schmid, 1993; Vallenduuk and Moller Pillot, 2007).

Shannon's diversity index was calculated for each tray as $H' = -\sum_{i=1}^{n}(p_i \ln p_i)$, where p_i is the relative abundance of a taxon i, calculated as the proportion of individuals in a given taxon to the total number of individuals in the community, and n is the number of taxa in the community. H′ indices for the control and treatment groups (H'_c and H'_t respectively) were then statistically compared with the paired two tail t-test. Relative community abundance (N) was calculated for each tray as the total sum of the individual species present in each experimental unit, expressed as # individuals per cm, and statistically compared with the paired two tail t-test.

Species richness and evenness were measured for each tray using Simpson's indices. Simpson's Index (D) measures the probability that two individuals randomly selected from a sample will belong to the same species. $D = (\sum n_i(n_i-1))/(N(N-1))$, where n is the total number of specimens of a particular species and N is the total number of specimens of all species. Simpson's Index of Diversity (the probability that two individuals randomly selected from a sample will belong to different species) was calculated as 1-D, while Simpson's Reciprocal Index was calculated as 1/D.

The Jaccard similarity index was calculated for all trays combined as $S_j = c/(A + B - c)$, where A is the total number of species in the control, B is the total number of species in the treatment, and c is the number of shared species. Similarly, the Sørensen–Dice qualitative similarity index was calculated for all trays combined as $S_s = 2c/(A + B)$, where A and B are the number of species in the control and treatment, respectively, and c is the number of shared species.

Czekanowski's quantitative similarity index was calculated for all trays combined as $S_c = 2\sum c/(A + B)$, where A and B are the community absolute abundance for treatment and control, respectively, while c is the lowest abundance of all species common to both groups. Since the S_c similarity index itself is calculated as a ratio of the common abundances between the two communities being compared, it can also be used to represent the level of impact of the toxicant on the community (Sánchez-Bayo and Goka, 2012). For example, if an S_c ratio is 0.3, then 30% of the community remains unaffected by a given chemical, while 70% is affected either positively or negatively.

To visualize the qualitative and quantitative variability of macroinvertebrates at the community level among all samples, non-metric multidimensional scaling (NMDS) was applied. NMDS is an iterative ordinational method in which the distance between points (sampling sites) correspond to pair-wise similarities in a resemblance matrix. A distance-based permutational multivariate analysis of variance (PERMANOVA) was conducted to test the significant effect of TiO_2 (fixed factor) on the macroinvertebrate community structure. Both NMDS and PERMANOVA were based on Bray–Curtis dissimilarities of previously transformed data (fourth-