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# Assessing the effect of different natural dissolved organic matters on the cytotoxicity of titanium dioxide nanoparticles with bacteria

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## ABSTRACT

Titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) are among the most widely manufactured nanomaterials on a global scale. However, prudent and vigilant surveillance, incumbent upon the scientific community with the advent of new technologies, has revealed potentially undesirable effects of TiO<sub>2</sub> NPs on biological systems and the natural environment during their application and discharge. Such effects are likely best evaluated by first assessing the fate of the TiO<sub>2</sub> NPs in natural environments. In this study, the effects of terrestrial humic acid (HA) and tannic acid (TA), two major members of the collective: dissolved organic matter (DOM), on the cytotoxicity of TiO<sub>2</sub> NPs to *Escherichia coli* were investigated in the presence and absence of natural sunlight. Qualitative (transmission electron microscopy (TEM)) and quantitative (LC<sub>50</sub>) analyses were employed in this study. In addition, the production of reactive oxygen species (ROS) in the form of .OH was further assessed—as HA or TA increased the production of ROS decreased. The inhibition of bacterial viability in the light treatment groups, with respective treatment organics at concentrations of 10 ppm, was less in TA than in terrestrial HA. SAS was used to analyze the treatment effect of individual factors of light irradiation, DOM, and concentration of TiO<sub>2</sub> NPs.

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## Introduction

Because of the diverse application of engineered nanoparticles in various personal and commercial products, they are produced in large quantities to support the growing global nanotechnology market. The rapid synthesis and use of these engineered nanoparticles in recent years have drawn the attention of the scientific community to their toxicology, health impacts, and their fate in the environment. Titanium

dioxide nanoparticles (TiO<sub>2</sub> NPs) are among the most broadly used nanomaterials with applicability in nanomedicine (He et al., 2015a), photocatalysts (He et al., 2016a,b), coating/surfaces, and additives in paints, rubbers and ceramics, (Ma et al., 2009) etc. The fates of nanoparticles are affected by environmental factors such as light, oxidants, and microorganisms, which could exert their influence via chemical or biological modifications (He et al., 2014; He and Hwang, 2014).

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Dissolved organic matter (DOM) is composed of a heterogeneous mixture of organic compounds (Mostofa et al., 2013). Humic substances such as fulvic acid and humic acid (HA) are the dominant DOM fractions in freshwater and coastal seawater. Tannic acid (TA) is more dominant in swamps, determined by the brownish-tan coloration (Berner and Berner, 2012). As aquatic systems serve as the primary sink for the released nanomaterials (Gao et al., 2009), it is therefore necessary to understand the fate and ecological effects of these nanomaterials in natural aquatic environments (Dasari and Hwang, 2013; He et al., 2014). It has been reported that both HA (Wiszniewski et al., 2002) and TA (Kruthika et al., 2014) could be degraded by TiO<sub>2</sub> NPs. The implication here then, is that the TiO<sub>2</sub> NPs are themselves affected in their inherent properties by the encounter with these environmental constituents. The interaction between TiO<sub>2</sub> NPs and DOM is critical in determining the toxicity of TiO<sub>2</sub> NPs. However, the toxicological profile of TiO<sub>2</sub> NPs is not completely understood and several concerns have emerged on the potentially undesirable effects of TiO<sub>2</sub> NP properties, in regard to the harmful interactions with biological systems and the environment at the nano-bio-eco interface (He et al., 2014; He et al., 2015b). Hence, this study seeks to elucidate this profile for application in follow-on assessments of environmental impact.

Herein, the effects of selected DOMs on the photoinduced toxicity of TiO<sub>2</sub> NPs to *Escherichia coli* (*E. coli*) *in vitro* are examined. To the best of our knowledge, it is the first report on the role of TA on the phototoxicity of TiO<sub>2</sub> NPs.

## 1. Materials and methods

### 1.1. Chemicals

The nanoparticles used in this study were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and Sky Spring Nanomaterials Inc. (Katy, TX, USA) with chemical purity of 98% or higher. HA and TA were purchased from Sigma-Aldrich (Sigma-Aldrich Co., USA). HA or TA solutions were prepared in different concentrations with Millipore water. Stock solutions (2000 ppm) were sonicated (FS30, Fisher Scientific, USA) for 30 min prior to adding them to make the working solution. TiO<sub>2</sub> NPs were prepared to reach the final concentrations of 0, 1, 2.5, 5 ppm and 250, 500, 750 ppm under light and dark conditions, respectively.

### 1.2. Characterization

To assess the effect of TA and HA on physicochemical properties of TiO<sub>2</sub> NPs, the hydrodynamic size and zeta potential values were obtained via dynamic light scattering (DLS) and phase analysis light scattering (PALS), respectively, using a ZetaSizer Nano ZS (Malvern Instrument, USA) at 25°C and an applied voltage of 120 V. A viscosity of 0.8872 centipoise (cP), a dielectric constant of 78.5, and Henry function of 1.5 were the set parameters. Primary nanoparticle size was determined using a Jeol, JEM 1011 electron microscope working at 100 kV (Joel USA, Inc., MA) equipped with a Gatan camera model 785. Absorbance spectra of the photocatalysts were measured using a UV-vis spectrophotometer (UV-2600, Shimadzu, Japan).

### 1.3. Preparation of *E. coli*

*E. coli* was cultured in Luria-Bertani (LB) Broth and placed in a shaker for 18 hr at 37°C. Following incubation, the culture was washed three times with sterilized physiological saline (0.8%, w/v) in a centrifuge (Eppendorf Centrifuge 5810 R, Eppendorf AG, Germany) at 4°C and 1735 × *g* for 10 min. The bacterial suspensions were diluted (10<sup>6</sup> × dilution factor) and exposed to the TiO<sub>2</sub> in quartz test tubes (ACE Glass Inc., USA).

### 1.4. Qualitative assay: transmission electron microscopy (TEM) analysis

In this section, 10 ppm HA or TA (final concentration) was added into the quartz test tube at a final volume of 2 mL. One hundred microliter diluted bacteria suspension and 75 ppm (final concentration) TiO<sub>2</sub> NPs were added subsequently into the quartz test tubes. Afterwards, the quartz tubes were incubated outdoors in a tub under natural sunlight at noontime during sunny days in Jackson, MS, USA (32°19'N; 90°5'W). The incubation temperature for all the tubes was maintained by immersion in a tub of water (~25°C). All samples were incubated for a period of 30 min. Darkness incubation was achieved by wrapping the tubes with aluminum foil at the same temperature maintained by water bath. For each sample, a 20 μL aliquot was placed on the TEM grid (Ted Pella, USA) and dried at room temperature. TEM measurements were performed on a Jeol, Jem 1011 electron microscope (JEOL Limited, Japan) operating at 100 kV and equipped with a Gatan camera (Gatan Incorporated, USA).

### 1.5. Quantitative assay: LC<sub>50</sub> analysis

HA and TA solutions were prepared to reach the following final concentrations simulating the ambient levels: 10 and 40 ppm. Quartz tubes (13 mL capacity, ACE Glass Inc., USA) were then filled with various amounts of distilled water, bacteria (*E. coli*), DOM (terrestrial HA or TA), and TiO<sub>2</sub> NPs and mixed well. Same as the protocol in qualitative analysis, quartz test tubes with *E. coli*, DOM, and TiO<sub>2</sub> were then exposed to sunlight under the same condition. After exposure of 30 min, aliquots of 100 μL of all three replicates were used to determine the LC<sub>50</sub>, at 37°C for 24 hr. LC<sub>50</sub> values were calculated using the Probit Method using GraphPad Prism of GraphPad Software, Inc.

### 1.6. Determination of reactive oxygen species

The production of hydroxyl radicals (.OH), the reported dominant reactive oxygen species (ROS) induced by TiO<sub>2</sub> NPs under full spectrum sunlight, was determined by the fluorescence spectroscopy using terephthalic acid, described previously with slight modification (Ishibashi et al., 2000; He et al., 2015c; He et al., 2016b). Terphthalic acid can be specifically hydroxylated by .OH and lead to the formation of fluorescent 2-hydroxyterephthalic acid. Briefly, a stock solution of 5 × 10<sup>-3</sup> mol/L terephthalic acid with a concentration of 2 × 10<sup>-2</sup> mol/L NaOH was first prepared and stored at 4°C. TiO<sub>2</sub> samples were placed in 5 mL of 5 × 10<sup>-4</sup> mol/L terephthalic acid with a concentration of 2 × 10<sup>3</sup> mol/L NaOH and stirred for 1 hr in the dark. The solution was then exposed to sunlight for 30 min. The reaction solution was filtered

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