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Bioaccumulation and Toxicity of Titanium Dioxide Nanoparticles in Epigeic Earthworm Species *Eudrilus eugeniae*

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Abstract: The production of titanium dioxide nanoparticles (TiO_2) for commercial applications has greatly increased over the last years and consequently the potential risk for living organisms. There is a growing awareness of the need to understand the behavior and influence these nanoparticles exert on the environment. Bioaccumulation serves as a good integrator to assess chemical exposure in soil. This research focused on the experimental bioaccumulation capability of titanium dioxide nanoparticles (TiO_2) by Earthworms *Eudrilus eugeniae*. It is found that TiO_2 exerted minimal toxicity within the traditional 48 h exposure time, but caused high toxicity when the exposure time was extended to 72 h. This demonstrated that exposure duration may be a contributing factor in NP-mediated toxicity. Moreover, upon chronic exposure to TiO_2 for 21 days, earthworm displayed severe growth retardation and mortality, as well as reproductive defects. A significant amount of TiO_2 was found accumulated in *Eudrilus eugeniae*. However, *Eudrilus eugeniae* displayed difficulty in eliminating TiO_2 from their body, presenting increased bioconcentration factor (BCF) values. This high level of bioaccumulation may interfere with food intake and ultimately affect growth and reproduction. In a nutshell, long-term exposure of soil organisms to TiO_2 may alter the growing status of these organisms at both individual and population levels, posing risks to ecosystems. The results of this study contribute to the current understanding of the potential ecotoxicological effects of nanoparticles and highlight the importance of characterization of NPs in understanding their behavior, uptake, and effects on organisms in soil.

Keywords: Bioaccumulation, Aggregation, Stability, Titanium dioxide nanoparticles, *Eudrilus eugeniae*

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Introduction

Nanoparticles (1–100 nm) are important components of the biogeochemical system, but human impact on the environment has altered their natural cycle by introducing engineered nanoparticles with several physicochemical characteristics and applications. Due to their peculiar characteristics (e.g. different optical properties, greater flexibility, resistance, reactivity, electrical conductivity, or absorption), nanoparticles (NPs) are finding more and more applications in many consumer products, such as conductors, cosmetics, plastics, and agents used in environmental recovery, as well as in various sectors such as the pharmaceutical, food,

biomedical, military, and automotive industries (Fisher and Egerton, 2001; Kaida *et al.*, 2004). The significant increase in the production and variety of nanoparticles has led to their release into the environment, especially into soil. Once they arrive in the soil environment, they can affect the living organisms. Hence, monitoring and assessing the effects of these nanoparticles is required (Lead *et al.*, 2018; Nowack *et al.*, 2015).

To date, despite the existing numerous applications of TiO_2-NPs, little is known about the bioaccumulation potential of TiO_2-NPs along the food chain (Esterkin *et al.*, 2005; Choi *et al.*, 2006) . Nevertheless, as this compound has a limited elimination rate and as it can be absorbed by the gastrointestinal tract in a size-dependent manner and pass through the mucus pores to enter other organs, some studies suggested a potential health risk such as disorders of the gut microbiota and gut-associated metabolism *in vivo* , apoptosis induction, hepatic toxicity , a potential risk for liver, ovaries, and testes , an impact on lipid metabolism, and an increased abundance of pro-inflammatory immune cells and cytokines in the colonic mucosa. Studies showed that TiO_2-NPs cause significant mitochondrial dysfunction by increasing mitochondrial ROS levels and decreasing ATP generation in macrophages. Moreover, TiO_2-NPs exposure activated inflammatory responses and attenuated macrophage phagocytic function. TiO_2 NPs interacted with sea urchin immune cells and increased the antioxidant metabolic pathway *in vitro*. In earthworms, only increased apoptosis was observed following TiO_2 nano-composites exposure. Thus, nanoparticle toxicity risk assessment is extremely important, as the adverse health effects remain poorly characterized for many nano-materials (Service, 2005; Nel *et al.*, 2006).

Since the soil ecosystem is highly sensitive to contamination by TiO_2-NPs, this work aimed to give an assessment of the bioaccumulation of TiO_2-NPs in earthworms. This allowed providing a chemical characterization and quantification of TiO_2-NPs in earthworms (Xu *et al.*, 2015; Shao *et al.*, 2019). The new emerging technique called Single-Particle Inductively Coupled Plasma Mass Spectrometry (spICP-MS) was applied, which allows the determination of nanoparticle number-based concentration, as well as the dissolved titanium. *Eudrilus eugeniae* was selected as a model organism for this study due to it being an important species in toxicity testing of soil. Various nanoparticles were described to impair earthworm defense mechanisms.

The objective of this study was to analyze the exposure of TiO_2 to soil and detect the biomass changes and bioaccumulation of TiO_2 in *Eudrilus eugeniae.* Analysis of physicochemical parameters such as pH, electrical conductivity total organic carbon, total nitrogen, total phosphorus, and total potassium were also done during the experimental period.

Materials and Methods

Collection of earthworm species:

Earthworms *Eudrilus eugeniae* (Fig. 1) were used for the study. These earthworms were purchased from Mani Organic Farm, Salem District, Tamil Nadu, India and cultured in cement tanks for further studies. The earthworms were reared in garden soil and garden waste in a vermibed of dimension 4 x 2 x 4.4 feet (length x breadth x height) sufficient for 2,000-3,000 worms with a controlled moisture content of 60-70% and temperature between 26 and 28 C. Nylon net was used to cover the bed to prevent the entry of predators. Adequate watering was done daily to maintain optimal moisture conditions in the vermibed.

Collection of Soil:

Top soil is collected from Periyar University garden not exceeding a depth of five inches. Then the collected soil was sun dried by spreading it on a flat, clean broad surface for 48 h. The dried soil was sieved using a 2 mm diameter sieve to remove debris (Khan *et al.*, 2012) and also was done soil spiking stainless steel spoon method (Doick *et al.* 2003). Physico-chemical characteristics of soil:

pH: 7.2 ± 0.01; EC: 1.8 ± 0.05 (dsm^{-1}); OC: 6.8 ± 0.05 (%); N: 0.3 ± 0.02 (%); K: 0.1 ± 0.01 (%); P: 0.04 ± 0.01 (%); C:N Ratio: 20.3.

Fig. 1: Earthworm species *Eudrilus eugeniae.*

Collection of cow dung:

Cow dung (CD) was collected from a Cowshed in Karuppur, Salem, Tamil Nadu, India. CD was freshly used for further experimentations. Physico-chemical characteristics of CD were-- pH: 8.2 ± 0.10; EC: 0.11 ± 0.02 (dsm^{-1}); OC: 4.9 ± 0.15 (%); N: 0.3±0.01 (%); K: 0.008 ± 0.005 (%); P: 0.03 ± 0.004 (%); C:N Ratio: 15.6.

Experimental design:

The earthworms were acclimatized to the laboratory conditions for a period of 60 days before the commencement of the experiment. Six circular buckets (33 cm length × 24 cm height) were used for the present study. The circular bucket was weighed with a Digital Sensitive Weighing balance (Model-CG203).

The test substrates were prepared according to the ISO guidelines for earthworm toxicity testing (ISO 11268-1, 1993). The soil sample collected was sieved (≤ 5 mm) to remove coarse stones and to homogenize. 1 kg of soil was weighed into a bucket. The soil was made up 21 to 60% water holding capacity using deionized water. The soil samples were contaminated with various concentrations of the TiO_2. The mixture was thoroughly mixed manually. Furthermore, the buckets were marked as T1, T2, T3 and T4. Soil and TiO_2 were taken in the following proportion:

S. No.	Treatments	Earthworms *Eudrilus eugeniae* (n)	Soil (kg)	Cow dung (g)	TiO_2 (mg/ kg)
1	T1	10	1	50	0
2	T2	10	1	50	5
3	T3	10	1	50	50
4	T4	10	1	50	500

The above treatments with TiO_2-contaminated soil were left for 10 days in the laboratory exposed to the elements. After 10 days, freshly collected cow dung of about 50 g for each treatment was thoroughly mixed into the bucket with TiO_2-contaminated soil.

Immediately after addition of additives, earthworms were sorted out from the holding containers, washed with clean water and ten earthworms of the species *Eudrilus eugeniae* were measured, weighed and inoculated into each container with contaminated soil. A netting material was placed on top of each of the containers and the cover lid frame was used to hold the containers firmly. This is done to avoid escape of the earthworms and to allow free flow of oxygen into the treatment during the course of the experiment. The setup was placed inside the laboratory and checked in morning and evening on a daily basis for 21 days.

Analysis of Soil Samples:

pH and Electrical conductivity (EC):

pH and EC of vermin compost samples were measured using pH (Elico, Model-Li 120) and EC meter (Amber Science Inc. Model 1056). 20 ± 0.1 g of vermicompost was added in 100 ml of distilled water and then stirred for 10 min and left for 30 min and pH was measured in supernatant liquid using pH meter. For electrical conductivity, the above mixture was left for 1 h and then measured for conductivity using an electrical conductivity meter.

Total organic carbon:

15 g of sucrose was dried at 105 C for 2 h, cooled and then kept in a desiccator. 11.886 g of dry sucrose was dissolved in water and made upto

100 ml in a volumetric flask and this solution contains 50 mg/ml of carbon. 0, 5, 10, 15, 20 and 25 ml of the carbon stock solution was pipetted into the labeled 100 ml volumetric flasks and made upto the mark with water and mixed well. These were the working standards, and contain 0, 2.5, 5.0, 7.5, 10.90, 12.5 mg/ml of carbon. 2 ml of each working standard was pipetted into labeled 100 ml conical flasks and dried at 105 C. 5% potassium dichromate solution was added to the respective conical flasks and about 20 ml of H_2SO_4 was added from a fast burette by gently swirling the mixture. After cooling the mixture, about 50 ml of 0.4% barium chloride was added and mixed thoroughly and allowed to stand overnight or centrifuged. Finally, the aliquot of the clear supernatant solution was transferred into a colorimeter cuvette and measured for each standard and sample absorbance at 600 nm. About 0.5 g of ground vermicompost was used to determine organic carbon content using the above procedure

Calculation: A graph was plotted for absorbance against standard concentration. Solution concentration was determined for each unknown and the blanks. The mean blank value was subtracted from the unknowns which gives a value for corrected concentration K.

$$\% \text{ total organic carbon} = \frac{K \times 0.1}{W \times 0.74}$$

Where, K is corrected concentration; W is the weight of the vermicompost.

Total nitrogen:

Firstly, mixed indicator (bromocresol green + methyl red) was prepared by taking 99 mg of bromocresol green and 66 mg of well-powdered methyl red and dissolved together in 100 ml of ethyl alcohol. Secondly, working boric acid solution was prepared by adding 20 ml of the mixed indicator to 1 liter of 2% boric acid solution and pH was adjusted to 5.0 after shaking until the solution assumes to reddish purple tangerine red colour. Finally, standard sulphuric acid (0.02N) was prepared using NaOH. About 20 g of sieved vermicompost sample was taken into 1 liter round bottom flask and about 10 ml of distilled water was added. 2 to 3 glass beads were added to prevent bumping and 1 ml of liquid paraffin was added to prevent frothing. About 100 ml of 0.32% potassium permanganate and an equal volume of 92.5% sodium hydroxide solution was added to the flask and distilled. The distillate was collected in a beaker containing 20 ml of 2% boric acid working solution and about 150 ml of distillate was collected and titrated with standard 0.02N sulphuric acid till the colour changes from green to red and burette readings were recorded. Blank is carried out without vermicompost.

Calculations were performed as follow:

$$N\% = (A - B) \times \text{Normality of } H_2SO_4 \times \text{Equi. Wt. of N.} \times \frac{100\text{ g}}{\text{vermicompost sample}} \times \frac{1}{100 \text{ to convert N mg into g}}$$

$$N\% = (A - B) \times \text{Normality of } H_2SO_4\ 0.02N \times \text{Equi. Wt. of N} \times \frac{100\text{ g}}{\text{vermicompost sample (g)}} \times \frac{1}{100 \text{ to convert N mg into g}} \quad (14)$$

$$N\% = (A - B) \times \text{normality of } H_2SO_4 \times 0.014 \times \frac{100\text{ g}}{\text{vermicompost sample (g)}}$$

Where, A is the volume of standard H_2SO_4 required for vermicompost sample, B is the volume of standard H_2SO_4 required for the blank sample.

Total phosphorus:

At first, molybdate reagent was prepared by separately dissolving 538 mg of ammonium molybdate in 50 ml of distilled water and 50 mg of antimony sodium tartrate in 50 ml of distilled water and then the two solutions were mixed well. To this, about 6.8 ml of conc. H_2SO_4 was added and made upto 125 ml by adding distilled water. This solution remains stable for 4 weeks at 2 C. Secondly, digestion mixture was prepared by adding 0.054 g selenium powder and 1.898 g lithium sulfate to 45.45 ml of 30% hydrogen peroxide and mixed well. Slowly, 54.54 ml of conc. H_2SO_4 was added while cooling in an ice bath. This mixture remains stable for 4 weeks when stored at 2 C. 0.05 g of vermicompost was taken in a flask, and 4.4 ml of digestion mixture was added and then digested at 360 C for 2 h. The resulting

solution should be colorless. To this, 25 ml of distilled water was added and mixed well until no more sediments dissolve. The contents were allowed to cool and made upto 50 ml with distilled water and mixed well. About 1 ml of the sample or distilled water standard was taken in a test tube and 4 ml of 1% freshly prepared ascorbic acid solution and 3 ml of molybdate reagent was added and mixed well. Test tubes were left for 1 h for the color to develop fully. Finally, the samples and standard absorbances were read in a colorimeter at 880 nm.

Calculation: Total phosphorus (%)

$$= \frac{\text{O.D. of sample} \times 0.1}{\text{Wt. of sample}}$$

Total potassium:

About 0.05 g of vermicompost was taken and 10 ml of N-neutral ammonium acetate solution (7.78 g in 100 ml of distilled water) was added and shaken for 5 min and then filtered through Whatman No.1 filter paper. The filtrate was atomized in a flame photometer and read. Zero setting of flame photometer was done using ammonium acetate solution (77.80 g of N-neutral ammonium acetate in 1000 ml of distilled water).

Calculation: Total potassium (%) =

$$\frac{0.1 \times \text{flame value}}{10 \text{ X wt. of vermicompost sample}}$$

Statistical analysis:

All experiments were carried out in duplicate. Data were expressed as Mean ± Standard Error. The statistical significance for various treatments was evaluated by one-way analysis of variance (ANOVA) using SPSS version 18 (SPSS Inc., Chicago, USA). When there was a significant difference, Tukey's honestly significantly different (HSD) multiple comparison tests were performed by fixing the significance at level $P<0.05$.

Bioaccumulation Assessment:

E. eugeniae from taxonomically verified in-house cultures were exposed to test soil for a 21 day uptake period. Soils (spiked for the uptake phase and clean for the elimination phase and controls) were distributed into 125 ml glass jars (100 g wet wt ea.). One adult *E. eugenia* (mean wet mass = 444 ± 119 mg) was added to each test vessel, and fed Magic Worm Food® (1 ml) on day 0 and 14 in each the uptake and elimination phases. Test vessels were sampled on 1, 2, 3,4 in triplicate for soil and tissue analysis throughout the test.

Bioaccumulation tissue analysis:

Earthworms sampled during bioaccumulation testing were depurated for 24 h in the dark on a moistened filter paper, then individually frozen at -20 C before being lyophilized (Labconco Free Zone) for total TiO_2 analysis in tissues. The lyophilized worm's mass was recorded (to 0.001 g) and 2 ml of HNO_3 (CAS 7697–37–2) was added to each tube containing a worm then heated to 110 C for 5 h. The digested tissue was then cooled and then diluted to 15 ml with nanopure water. Analysis for total TiO_2 in earthworm tissue was performed using a Perkin Elmer NexION ICP-MS. Additional replicates were added at the end of the uptake phase (day 21) for earthworm samples to be processed for imaging via enhanced dark-field hyper-spectrophotometry. Earthworms were depurated for 24 h in the dark on moistened filter paper, and then fixed with a 2% formaldehyde in 200 mM phosphate buffer (pH 7.2) solution. Three millimeter sections from the posterior of each preserved earthworm were sampled for processing using a tissue processor (Leica EM TP automatic tissue processor). The fixed tissue sections were processed as follows: 70% ethanol (1 h); 95% ethanol (1 h); 4x ethanol (increasing holding time from 1 to 2 h); 2x xylene (1 h each); 2x paraffin wax (1 h each). Processed tissues were embedded in paraffin blocks and sectioned at 5 µm using a Leica UC7 ultra-microtome. Sections were stained using hematoxylin and eosin, and mounted on polystyrene slides with a glass cover slip. Prepared sections were analyzed with Cytoviva enhanced dark-field hyperspectral microscopy (Zamora-Perez *et al.*, 2018). Briefly, enhanced dark-field hyperspectral microscopy involves the use of an optical microscope fitted with dark-field illuminator and hyperspectral light

(halogen) to capture spectrum from the sample in the visible to near infrared range (400 – 1000 nm) (Zamora-Perez *et al.*, 2018). The data collected by this system is analyzed by a geospatial software, ENVI. Spectral libraries were created by collecting spectral data from samples of TiO_2 nano-powder. The earthworm library was based on the negative control that was filtered out of test sample data using the ENVI software. This method was used to qualitatively determine if TiO_2 could be observed in earthworm tissues.

Assessment of Earthworm Biomass:

The increase or decrease in number of earthworms and total weight of earthworms were recorded separately in various treatments during vermin remediation period.

Assessment of Earthworm Mortality:

Eudrilus eugeniae earthworms (n =10) were introduced into the circular bucket. At certain time intervals, the number of living earthworms in each bucket was recorded. The moisture content of 60% water holding capacity was maintained throughout the test period by weighing, to determine moisture loss, and adding deionized water whenever necessary. The samples were monitored for a total of 21 days.

Results and Discussion

Bioaccumulation is the gradual accumulation of substances, such as pesticides or other chemicals, in an organism. Bioaccumulation serves as a good integrator to assess chemical exposure in soil and is dependent on factors, such as the exposure routes, diet and the medium. The study focused on the experimental bioaccumulation capability of TiO_2 NPs by earthworm through bioconcentration factors (BCFs) after 21 days of exposure.

Physicochemical parameters such as pH, electrical conductivity, total organic carbon, total nitrogen, total phosphorus, and total potassium during the experimental period was analyzed and was found altered due to the exposure of Titanium dioxide nanoparticles in the substrate. The change in pH is highly deviated in T4 where the concentration of $nTiO_2$ was 500 mg kg^{-1} (Table 1). Figure 2 shows the change in pH in the substrate. There is a change in Electrical Conductivity in the substrate as the TiO_2 substrate increases from 5 mg kg^{-1} to 500 mg kg^{-1} (Fig. 3). Total organic carbon was found to be reduced in the substrate where high level of TiO_2 was administered (Fig. 4). The presence of Total Nitrogen, Total Phosphorous and Total Potassium were also altered as the concentration of TiO_2 increased from 5 mg kg^{-1} to 500 mg kg^{-1} (Figs. 5-7).

In this study, *Eudrilus eugeniae* was observed to ingest TiO_2 from the substrate, and also cleared out some $nTiO_2$ aggregates. At the end of the toxicity studies, some of $nTiO_2$ was found to remain in the body. These findings suggest that *E. eugeniae* might fail to excrete all ingested $nTiO_2$, and the observed toxicity may be related to the accumulation of $nTiO_2$ in the body.

Therefore, to elucidate the possible mode of action for $nTiO_2$-mediated toxicity, further characterized the uptake and depuration of $nTiO_2$ by *E. eugeniae*. In these experiments, *E. eugeniae* were exposed to either a low or a high concentration of $nTiO_2$ for 24 h followed by 72 h of depuration. As shown in Table 2, the amount of $nTiO_2$ accumulated in *E. eugeniae* increased with the extended exposure time, reaching a plateau. During the depuration phase, whole body $nTiO_2$ decreased with time extended. After 72 h of depuration, however, a considerable amount of $nTiO_2$ was still present in the bodies of *E. eugeniae*, suggesting that elimination of $nTiO_2$ is relatively difficult.

The acute test, included both the traditional 48 h and 72 h to determine whether the exposure duration could influence the toxicity of $nTiO_2$ to soil organisms. After incubation for 48 h, high $nTiO_2$ concentrations caused only 10% and 20% immobilization, respectively, and no significant mortality was found. However, high toxicity was found when the exposure time was extended to 72 h. Significant immobilization and 2% mortality were observed at the high concentration of $nTiO_2$ (Table 3).

Table 1: Change of pH, electrical conductivity, total organic carbon, total nitrogen, total phosphorus, and total potassium after treatment

Treatments	pH		EC (dsm^{-1})		TOC (%)		TN (%)		TP (%)		TK (%)	
	0 day	21st day	0 day	21st day	0st day	21st day	0 day	21st day	0 day	2st day	0 day	21st day
T1	6.55 ± 0.05	6.75 ± 0.05	1.57 ± 0.05	1.71 ± 0.05	18.25 ± 0.05	14.85 ± 0.45	0.39 ± 0.00	0.43 ± 0.01	0.20 ± 0.00	0.24 ± 0.00	0.09 ± 0.00	0.12 ± 0.00
T2	6.56 ± 0.05	7.81 ± 0.05	1.55 ± 0.05	1.79 ± 0.05	18.05 ± 0.05	14.25 ± 1.05	0.39 ± 0.00[a-]	0.43 ± 0.00	0.20 ± 0.00	0.25 ± 0.00	0.08 ± 0.00	0.13 ± 0.00
T3	6.58 ± 0.05	7.85 ± 0.05	1.56 ± 0.05	1.85 ± 0.05	18.05 ± 0.05	14.92 ± 0.45	0.38 ± 0.01[a-]	0.44 ± 0.01	0.22 ± 0.02	0.25 ± 0.00	0.08 ± 0.00	0.15 ± 0.00
T4	6.55 ± 0.05	6.70 ± 0.10	1.55 ± 0.05	1.72 ± 0.05	18.20 ± 0.40	16.75 ± 0.45	0.37 ± 0.00	0.40 ± 0.01	0.21 ± 0.01	0.23 ± 0.01	0.08 ± 0.00	0.11 ± 0.00

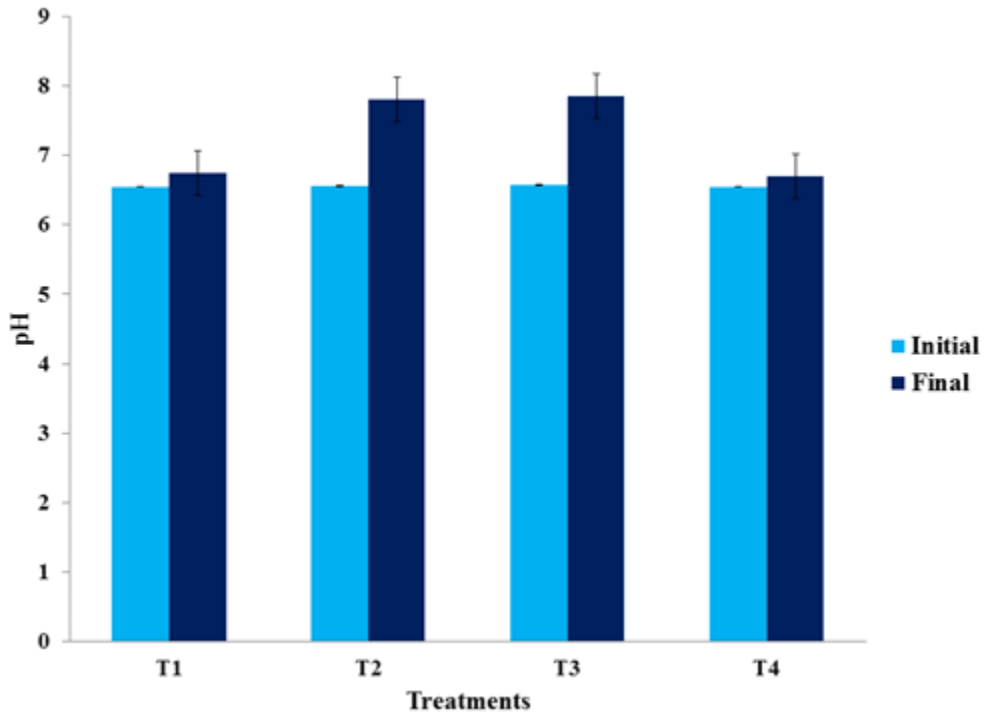

Fig. 2: Changes of pH during the experimental period.

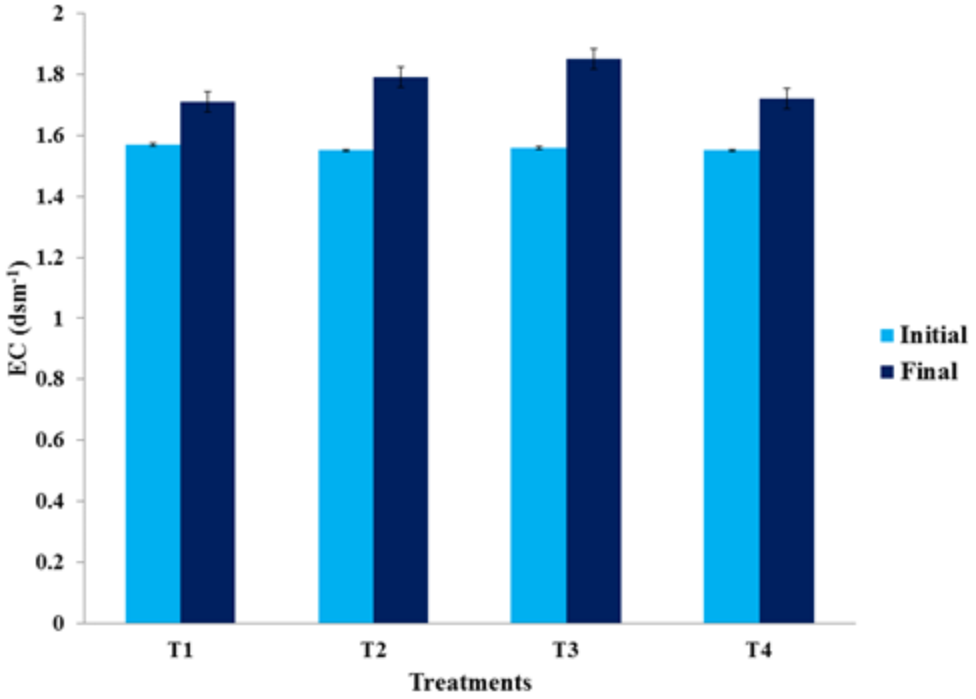

Fig. 3: Changes of EC during the experimental period.

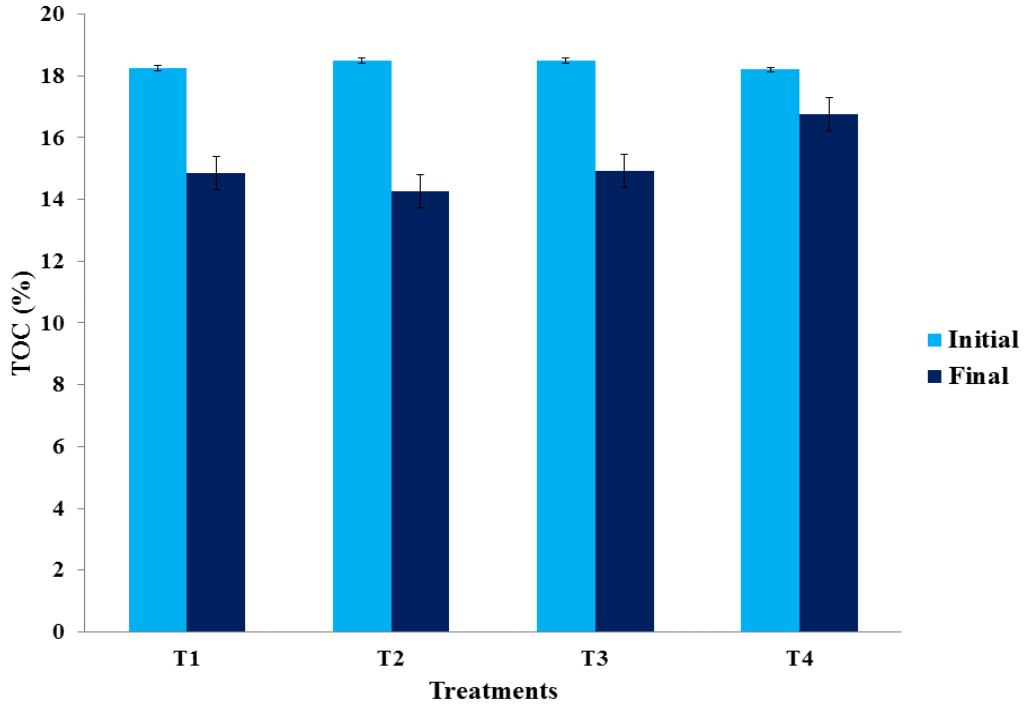

Fig. 4: Changes of TOC during the experimental period.

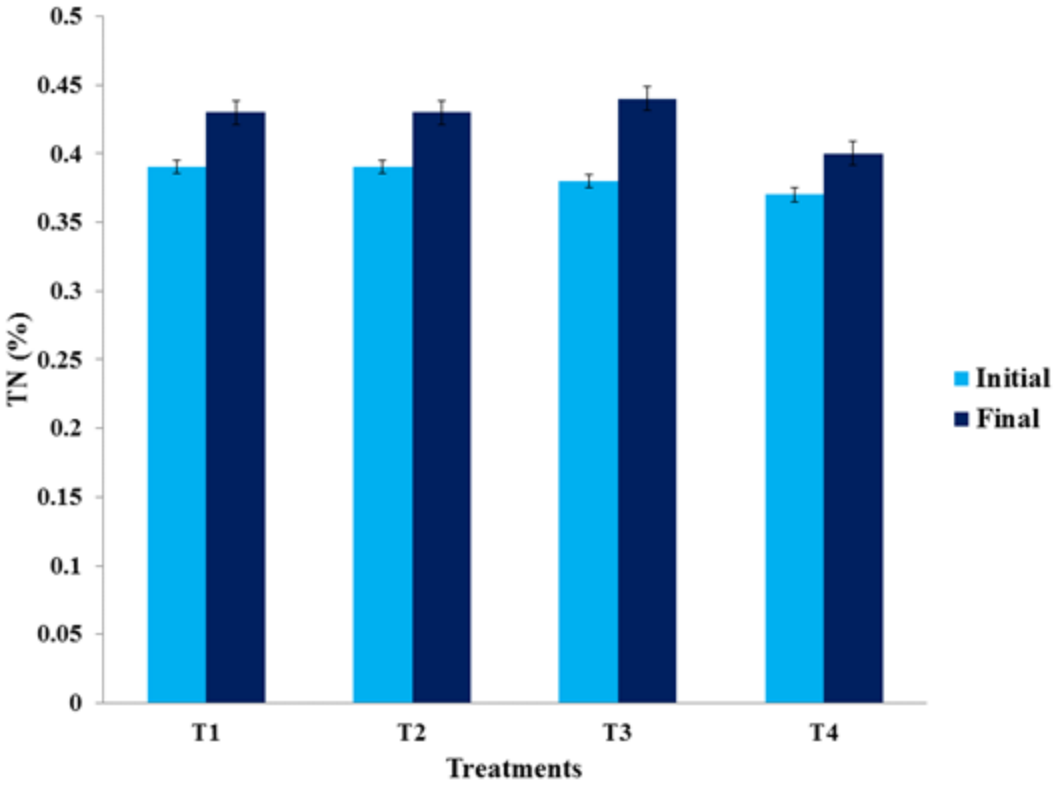

Fig. 5: Changes of TN during the experimental period.

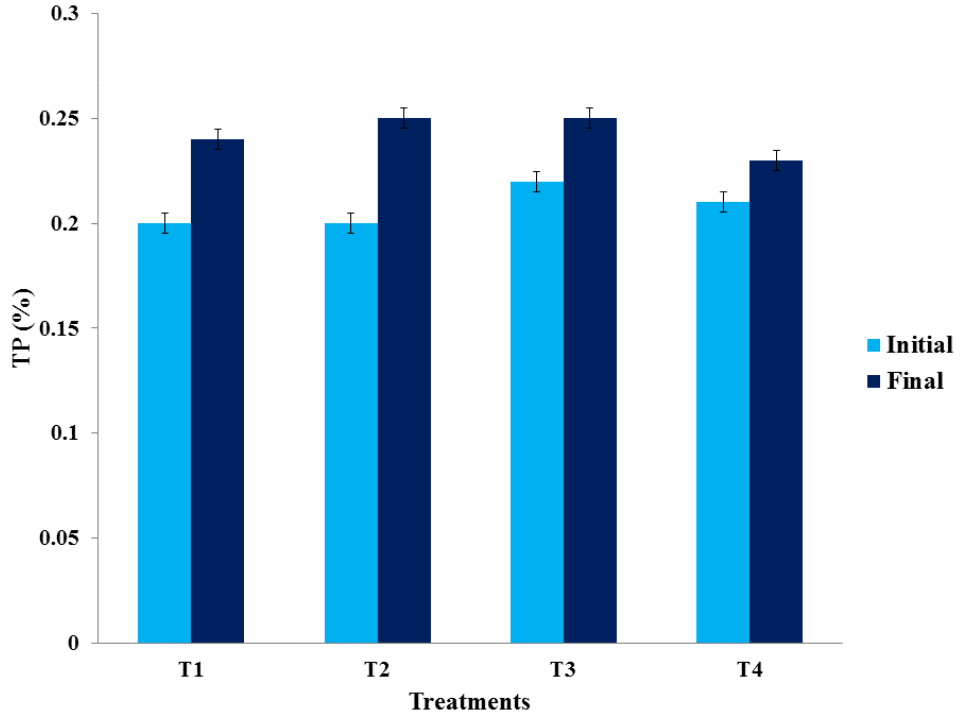

Fig. 6: Changes of TP during the experimental period.

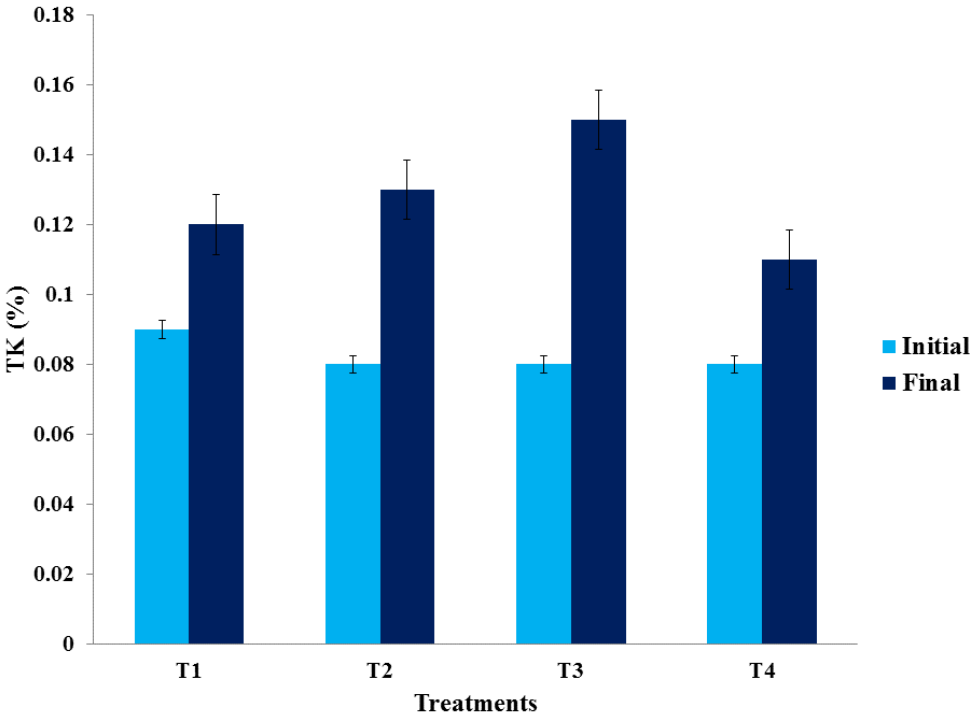

Fig. 7: Changes of TK during the experimental period.

Table 2: Bioaccumualtion of TiO_2

S. No.	Treatments	TiO_2 concentration (mg/kg)
1	T1	-
2	T2	0.31±0.00
3	T3	6.43±0.02
4	T4	18.76±0.05

Table 3: Earthworm Biomass and Mortality

S. No.	Treatments	Earthworms biomass (g)			Mortality Rate (%) (after 21 days)
		Initial	Final	Change (%)	
1	T1 (0 mg)	7.30± 0.10	13.33± 0.01a	+82	0%
2	T2 (5 mg)	7.35± 0.05	12.37± 0.00c	+68	0%
3	T3 (50 mg)	7.35± 0.15	12.98± 0.01a	+76	0%
4	T4 (500 mg)	7.50± 0.10	11.24± 0.01d	+54	2%

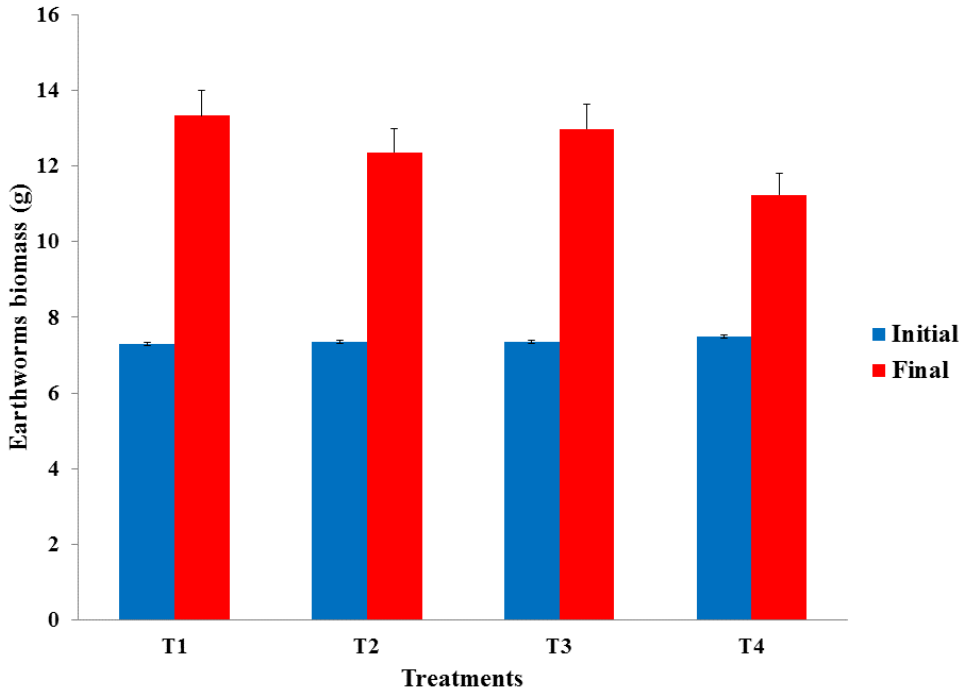

Fig. 8: Changes of Earthworm Biomass during the experimental period.

The present study indicated that in the low and medium concentrations, the mortality is minimum and Earthworm survival was only adversely affected at the highest TiO_2 concentration.

The experimental studies also showed that the biomass of earthworm increased in all experimental substrates after a few days but it is reduced in treatment groups as compared to T1. The increase in biomass was comparatively less in T4 because of the high concentration of TiO2 in T4 (Fig. 8). Mortality Rate of earthworms reached the pinnacle with increased use of TiO_2 in the soil (500 mg kg^{-1}). Effects caused by Titanium dioxide exposure was found to be proportionate to the concentration (Fig. 9).

This study focused on the analysis of the exposure of TiO_2 to soil and detected the biomass changes and bioaccumulation of TiO_2 in *Eudrilus eugeniae* . Analysis of physicochemical parameters such as pH, electrical conductivity, total organic carbon, total nitrogen, total phosphorus, and total potassium were also done during the experimental period. The analysis pointed out interesting and critical aspects of TiO_2 form, NP size, and surface reactivity (Chen and Fayerweather, 1988; Driscoll *et al.*, 1991; Warheit *et al.*, 1997). The relevance of the toxicological research in animals lies on the possibility to evaluate the type and entity of the adverse response induced by different doses of TiO_2 NPs and to extrapolate threshold levels aimed to protect exposed organisms. The test substrates were prepared according to the ISO guidelines for earthworm toxicity testing (ISO 11268-1, 1993). The soil sample collected was sieved (≤ 5 mm) to remove coarse stones and to homogenize. 1 kg of soil was weighed into a bucket. The soil was made up 21 to 60% water holding capacity using deionized water. The soil samples were contaminated with various concentrations of the TiO_2. The mixture was thoroughly mixed manually. Furthermore, the buckets were marked as T1, T2, T3 and T4. Soil and TiO_2 were taken in the proportion. Immediately after addition of additives, earthworms were sorted out from the holding containers, washed with clean water and

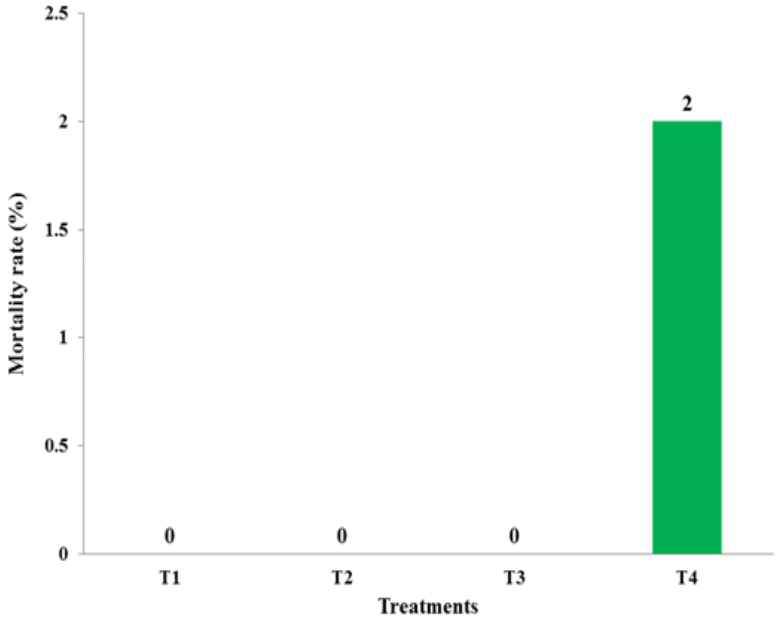

Fig. 9: Earthworm Mortality Rate during the experimental period.

ten earthworms of the species *Eudrilus eugeniae* measured, weighed and inoculated into each container with contaminated soil. Analysis of Soil Chemical parameters were done as described. The results of the study showed that the earthworm biomass changed as per the addition of TiO_2 nanoparticles. Earthworm mortality was higher in the sample where TiO_2 was present in higher concentration (Donaldson *et al.*, 2000; Oberdörster, 2000; Gumbleton, 2001; Tinkle *et al.*, 2003). In all the studies reviewed, the commonly proposed pathogenic mechanisms initiated by TiO_2 NPs are dominated by morphological abnormalities in earthworm. Coiling, curling, and mucous secretion leads to rupture of the cuticle and results in the degeneration of earthworm body and fragmentation of posterior segment once they are exposed to it.

Although qualitative, the use of the enhanced dark-field hyperspectral imaging technique showed promise as a new technique to isolate nano-particulate accumulation within tissue. Since the stability of TiO_2 is an important factor to consider with respect to toxicity and bioaccumulation, understanding how different engineered particulate properties (i.e., surface coating, shape, size) affect dissolution in various soil environments, and the biota's ability to regulate or eliminate any particles taken up, will become paramount to refining the assessment of risk posed by the proliferation of metallic nanoparticles in the environment (Oberdörster, 2004; Sayes *et al.*, 2004; Zhu *et al.*, 2007).

A short period of exposure of the TiO_2 NPs were not lethal to the earthworms. But abnormal physiological and behavioral changes occurred under the higher concentrations during the experimental period (Shao *et al.*, 2019; Xu *et al.*, 2015). Since TiO_2 NPs could cause oxidative stress and the decreases in the growth rate on earthworm, the release of TiO_2 NPs into the environment may pose potential risks to aquatic organisms (Zhu *et al.*, 2010). This needs to be considered against the context of a general lack of knowledge of the fate, behavior, and bioavailability of these types of particles in natural systems and suggests a need for longer-term and more environmentally realistic NP exposure regimes to

fully determine the transport capabilities of NPs in the aquatic environment (Tan *et al.* 2012). Data on the behavior and effects of NPs in the environmental food chain would be of primary importance for understanding their overall potential hazard for ecosystems.

Conclusion

Nanotechnology has become in the limelight of various interdisciplinary researches during the past few decades owing to their intriguingly appealing characteristics. It has considerably revolutionized many technological inventions, especially in electronics, optoelectronics, environmental remediation, agriculture, biomedical technology. Development, usage, and potential release of NPs have run ahead of possible risks they impose on the environment.

Most of the NPs discharged into the environment were provided with a path to enter into soil biota. Some metallic NPs can easily mimic the active site prosthetic groups of certain organisms and can deprive the enzymatic activity. The general adverse effects include oxidative stress and related biomarker expression, inflammation, etc.

Subsequently, its transport and bioaccumulation via the food chain is an indispensable concept. This bioaccumulation and tropic transfer of NPs has been documented about the aquatic ecosystem in several studies but very much restricted to terrestrial biota.

The experimental studies also showed that biomass of earthworms gradually reduces (as compared to T1) with the increased exposure to Titanium Dioxide nanoparticles. Mortality Rate of the earthworms reach the pinnacle with increased use of TiO_2 in the soil. The harmful effects caused by Titanium dioxide exposure was found to be proportionate to the concentration.

Though TiO_2 NPs have several applications, these cause pollution and toxic effects with improper handling, leading to hazardous effects to living organisms and the environment. The government of most countries has established a set of principles for the use, disposal, and treatment of nanoparticle wastes, and these principles belong more or less to the same dimension with alterations depending on the type of nanoparticle being synthesized or the level of contamination risk. Improper disposal of nanoparticles has witnessed a steep rise from 2005 to 2010 (Lee *et al.*, 2010).

The lack of strict regulations and policies regarding the usage and disposal of NPs, has become a great threat to the environment. The impact of NPs under the current and future exposure extremities on various levels of ecosystems needs to be elucidated further. On the other side, their functions and interactions across multiple concurrent ecosystems need special attention. Particularly, NPs having longer half-life in the ecosystem, are susceptible to long-term deposition in various environmental sediments. They need special focus as they are likely to cause more potential harm to both aquatic and terrestrial biota. Nonetheless, various analytical techniques are still under development to much effectively detect and characterize NPs quantification in complex environmental strata, but its efficacy in proper management is still a controversial topic. Nanotechnology is growing in an unforeseen exponential manner, and it is also an authentic fact that issues related to notoriously difficult nano waste disposal will grow at an even faster rate if unchecked.

References

Adams LK, Lyon DY and Alvarez PJJ. (2006) Comparative ecotoxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions. Water Res. 40(19): 3527-3532.

Alijagic A, Gaglio D, Napodano E, Russo R, Costa C, Benada O, Kofronova O and Pinsino A. (2020) Titanium dioxide nanoparticles temporarily influence the sea urchin immunological state suppressing inflammatory-relate gene transcription and boosting antioxidant metabolic activity. J Hazard Mater. 384: 121389.

Ardestani MM, Van S traalen NM and Van Gestel CAM. (2014) Uptake and elimination kinetics of metals in

soil invertebrates: a review. Environ Pollut. 193: 277-295.

Brunelli A, Pojana G, Callegaro S and Marcomini A. (2013) Agglomeration and sedimentation of titanium dioxide nanoparticles (n-TiO_2) in synthetic and real waters. J Nanoparticle Res. 15: 1684.

Chen Q, Wang N, Zhu M, Lu J, Zhong H, Xue X, Guo S, Li M, Wei X, Tao Y and Yin H. (2018) TiO_2 nanoparticles cause mitochondrial dysfunction, activate inflammatory responses, and attenuate phagocytosis in macrophages: A proteomic and metabolomic insight. Redox Biol. 15: 266-276.

Crane M and Handy RD. (2007) An assessment of regulatory testing strategies and methods for characterizing the ecotoxicological hazards of nanomaterials. Report for Defra, Department for Environment, Food and Rural Affairs, London, UK.

Donaldson K, Stone V, Gilmour PS, Brown DM and MacNee W. (2000) Ultrafine particles: mechanisms of lung injury. Philos Trans Royal Soc London 358: 2741-2749.

Esterkin CR, Negro AC, Alfano OM and Cassano AE. (2005) Air pollution remediation in a fixed bed photocatalytic reactor coated with TiO2. AIChE J. 51: 2298-2310.

Federici G, Shaw BJ and Handy RD. (2007) Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects. Aquat Toxicol. 84: 415-430.

Fisher J and Egerton T. (2001) Kirk–Othmer Encyclopedia of Chemical Technology. John Wiley, New York.

Garner KL, Suh S and Keller AA. (2017) Assessing the risk of engineered nanomaterials in the environment: development and application of the nanofate model. Environ Sci Technol. 51(10): 5541-5551.

Griffitt RJ, Luo J, Gao J, Bonzongo JC and Barber DS. (2008) Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem. 27: 1972-1978.

Gurr J, Wang ASS, Chen C and Jan K. (2005) Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 213: 66-73.

Hall S, Bradley T, Moore JT, Kuykindall T and Minella L. (2009) Acute and chronic toxicity of nano-scale TiO2 particles to freshwater fish, cladocerans, and green algae, and effects of organic and inorganic substrate on TiO_2 toxicity. Nanotoxicol. 3(2):. 91-97.

Handy RD and Shaw BJ. (2007) Toxic effects of nanoparticles and nanomaterials: implications for public health, risk assessment and the public perception of nanotechnology. Health Risk Soc.. 9(2): 125-144.

Heckmann LH, Hovgaard MB, Sutherland DS, Autrup,H, Besenbacher F and Scott-Fordsmand JJ. (2011) Limit-test toxicity screening of selected inorganic nanoparticles to the earthworm *Eisenia fetida*. Ecotoxicol. 20(1): 226-233.

Hou WC, Westerhoff P and Posner JD. (2013) Biological accumulation of engineered nanomaterials: a review of current knowledge. Environ Sci Process Impacts 15 (1): 103-122.

Hu CW, Li M, Cui YB, Li DS, Chen J and Yang LY. (2010) Toxicological effects of TiO_2 and ZnO nanoparticles in soil on earthworm *Eisenia fetida*. Soil Biol. Biochem. 42: 586-591.

Kwak JI and Youn-Joo A. (2015) Ecotoxicological effects of nanomaterials on earthworms: a review. Hum Ecol Risk Assess Int J. 21: 1566-1575.

Lead JR, Batley GE, Alvarez PJJ, Croteau MN, Handy RD, McLaughlin MJ, Judy JD and Schirmer K. (2018) Nanomaterials in the environment: behavior, fate, bioavailability, and effects- an updated review. Environ Toxicol Chem. 37: 2029-2063.

Moore MN. (2006) Do nanoparticles present ecotoxicological risks for the health of the aquatic environment?" Environ Int. 32(8): 967-976.

Oberdörster E. (2004) Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. Environ. Health Perspect. 112: 1058-1062.

Reeves JF, Davies SJ, Dodd NJF and Jha AN. (2008) Hydroxyl radicals (_OH) are associated with titanium dioxide (TiO_2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells. Mutat Res. 640: 113-122.

Santorufo L, Van Gestel CAM and Maisto G. (2012) Ecotoxicological assessment of metal-polluted urban soils using bioassays with three soil invertebrates. Chemosphere 88 (4): 418-425.

Service RF. (2005) Nanotechnology. Calls rise for more research on toxicology of nanomaterials. Science 310: 1609.

Shao X, He J, Liang R, Lu Y, Shi Y, Wang Y, Zheng X, Zhang S and Wang T. (2019) Mortality, growth and metabolic responses by 1H NMR-based

metabolomics of earthworms to sodium selenite exposure in soils. Ecotoxicol Environ Saf. 181: 69-77.

Tan C, Fan WH and Wang WX. (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.

Tourinho PS, van Gestel CAM, Lofts S, Svendsen C, Soares AMVM and Loureiro S. (2012) Metal-based nanoparticles in soil: fate, behavior, and effects on soil invertebrates. Environ Toxicol Chem. 31(8): 1679-1692.

Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL and Sayes CM. (2007) Development of a base set of toxicity tests using ultrafine TiO_2 particles as a component of nanoparticle risk management. Toxicol Lett.. 171(3): 99-110.

Xiong D, Fang T, Yu L, Sima X and Zhu W. (2011) Effects of nanoscale TiO_2, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. Sci Total Environ. 409(8): 1444-1452.

Zhu X, Zhu L, Duan Z, Qi R, Li Y and Lang Y. (2008) Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to Zebrafish (*Danio rerio*) early developmental stage. J Environ Sci Hlth. A 43(3): 278-284.

Zamora-Perez P, Tsoutsi D, Xu R and Rivera-Gil P. (2018) Hyperspectral-enhanced dark field microscopy for single and collective nanoparticle characterization in biological environments. Materials 11 (2): 243.