



• Brief Report

# Accelerated ecotoxicity of photoreactive nanoparticles on *Moina macrocopa*

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Zinc oxide nanoparticles (ZnO NPs) and titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) are well known as photoreactive nanoparticles (NPs). Various phototoxicities of ZnO NPs and TiO<sub>2</sub> NPs were reported on several organisms. It was still necessary to evaluate the toxicity of photoreactive ZnO NPs and TiO<sub>2</sub> NPs due to species-specific effects under various irradiation conditions. We compared the acute toxicity of *Moina macrocopa* under visible, ultraviolet (UV) A, and B irradiations, according to the Organization for Economic Cooperation and Development guidelines for the testing of chemicals (Test No. 202). The sensitivity of ZnO NPs for *M. macrocopa* was UVB > UVA > visible light irradiation. There were no significant lethal and immobile effects of TiO<sub>2</sub> NPs on juveniles under all irradiations and in the tested concentrations of TiO<sub>2</sub> NPs. Photoreactive NPs have a potential and accelerated toxicity on organisms in the ambient environments.

**Keywords:** *Moina macrocopa*, Phototoxicity, Titanium dioxide nanoparticles, Ultraviolet, Zinc oxide nanoparticles

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## INTRODUCTION

Zinc oxide nanoparticles (ZnO NPs) and titanium dioxide nanoparticles (TiO<sub>2</sub> NPs), well known as photoreactive nanoparticles (NPs), are increasingly found in personal care products and distributed in the environment due to their easy biodegradability [1,2]. In prior literature, under ultraviolet (UV) irradiation, the increased phototoxicity of ZnO NPs and TiO<sub>2</sub> NPs on fish (*Oryzias latipes* and *Danio rerio*), waterflea (*Daphnia magna* and *Daphnia similis*), nematodes (*Caenorhabditis elegans*), amphipod (*Hyalella azteca*), frogs (*Xenopus laevis*), and bacteria (*Escherichia coli* and *Bacillus subtilis*) was reported [2-9], while no significant phototoxicity of ZnO NPs and TiO<sub>2</sub> NPs on fish (*Danio rerio*), waterflea (*D. magna*), and algae (*Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*) [1,10,11] was reported. It was still necessary to evaluate the toxicity of photoreactive ZnO NPs and TiO<sub>2</sub> NPs due to species-specific effects under various irradiation conditions. However, the limited toxicity data of photoreactive ZnO NPs and TiO<sub>2</sub> NPs on waterflea (especially, *D. magna* or *D. similis*) exist. Therefore, it is requisite to

expand various waterflea in order to assess the toxicity of photoreactive ZnO NPs and TiO<sub>2</sub> NPs. *Moina macrocopa* is a representative Korean native species and used to assess ecotoxicity in various literatures.

## METHODS

### Test Chemicals

ZnO NPs (particle size < 100 nm) and TiO<sub>2</sub> NPs (particle size 21 nm, P25, white powder form) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Evonik Degussa (Bitterfeld-Wolfen, Germany), in a white powder form. A field emission-transmission electron microscope (FE-TEM; JEM2200FS, JEOL, Tokyo, Japan) was used to measure the morphology of the NPs. A particle size analyzer (UPA-150, Microtrac, Montgomeryville, PA, USA) was used to measure the specific surface area of the NPs. Particle size distributions of hydrodynamic diameters and zeta potential of 10 mg/L ZnO NPs and TiO<sub>2</sub> NPs suspended in deionized water (DW) and M4 medium [7] were measured using an electrophoretic light scattering spectropho-

tometer (ELS-8000, Otsuka Electronics Co., Osaka, Japan). A UV/Vis spectrophotometer (Libra S32 PC, Biochrom Ltd., Cambridge, UK) was used to observe the UV absorption spectra of 10 mg/L ZnO NPs and TiO<sub>2</sub> NPs suspended in M4 medium at 10 minutes intervals for 60 minutes.

### Test Organism

*M. macrocopa* was obtained from the Korea Institute of Toxicology (Daejeon, Korea). Cultures of test species were kept in M4 medium at 22 ± 1°C, respectively, with a photoperiod of 16:8 hours (light: dark). Green algae *P. subcapitata* was provided daily as a food source, with the concentration of 2 × 10<sup>4</sup> cells/mL. Neonates (less than 24 hours) were used for acute toxicity testing.

### Phototoxicity Test

Stock solutions of 100 mg/L test NPs dispersed in M4 medium were vigorously shaken by hand and sonicated for 10 minutes at 40 kHz in a water bath sonicator (Powersonic 420, Hwashin Technology, Seoul, Korea). Serial exposure solutions (0, 1, 2, 3, 4, and 5 mg/L for ZnO NPs and 0, 1, 3, 5, and 10 mg/L for TiO<sub>2</sub> NPs) were diluted with M4 medium. At this time, ethylenediaminetetraacetic acid was excluded from the M4 medium to prevent the chelating effect [13]. Exposure solutions were placed in a photoreactor (1 LZC-4, Luzchem Research Inc., Ottawa, Canada) under a UV lamp for UV irradiation and in the incubator under standard fluorescent lamps for visible light irradiation, then irradiated for 20 minutes. The photoreactor has a UV lamp with a spectral distribution of 316 to 400 nm for UVA and 281 to 315 nm for UVB. The light intensity measured using a spectroradiometer (SPR 4001, Luzchem Research Inc.) was 8.2 mW/m<sup>2</sup> for UVA and 5.68 mW/m<sup>2</sup> for UVB.

The acute toxicity test was conducted according to the Organization for Economic Cooperation and Development (OECD) guidelines (No. 202) for chemical testing [12]. Tests were performed with 2 mL of exposure solution in a 24-well microplate (inner diameter 17 mm × height 17 mm, volume 3.8 mL/well). Each test unit contained 5 neonates, with 4 replicates. The test duration was set as 48 hours. Survival and mobilization were measured as toxicity endpoints. The microplate was incubated under the same conditions as that of pre-incubation.

### Nanoparticles Dissolution and Ion Toxicity

To estimate the dissolution of ZnO NPs in M4 medium, minimum and maximum exposure concentrations of ZnO NPs were exposed to the same conditions as those of the phototoxicity test. At 0 and 48 hour, each exposure solution was filtered sequentially through 200-nm nylon membrane filters (Whatman,

Maidstone, UK) and 50-nm membrane filters (Millipore, Darmstadt, Germany). Zinc (Zn) ion concentrations were then measured using inductively coupled plasma-atomic emission spectroscopy (Jobin Yvon, Longjumeau, France). To estimate the effect of Zn ions released from the ZnO NPs, ion toxicity test was carried out in a same way as that of the ZnO NPs toxicity test, with Zn chloride (Sigma-Aldrich) and exposure concentrations of 0, 0.5, 1, and 2 mg/L.

### Statistics

Multiple comparisons were conducted using Dunnett's test and differences were considered statistically significant at  $p < 0.05$ . Trimmed-Spearman-Kärber program was used to calculate the lethal concentration at 50% (LC<sub>50</sub>) and effective concentration at 50% (EC<sub>50</sub>) values, and their corresponding 95% confidence limits.

## RESULTS

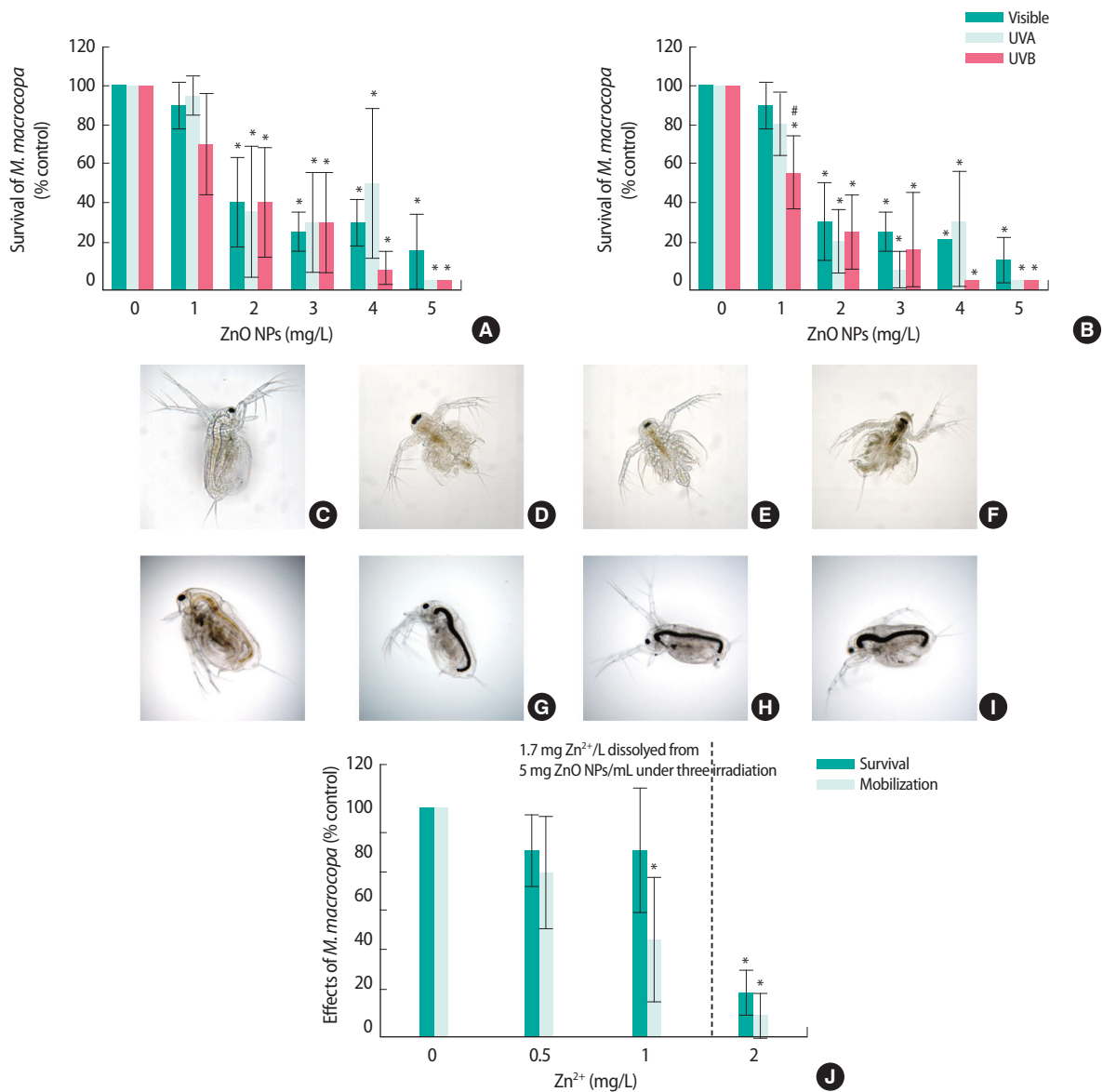
Figure S1 (A and B) show the morphological TEM images of test NPs. Table S1 shows the specific surface area, mean hydrodynamic diameter, and zeta potential of ZnO NPs and TiO<sub>2</sub> NPs dispersed in M4 medium and DW. The zeta potential indicates that ZnO NPs are incipiently unstable and TiO<sub>2</sub> NPs rapidly coagulate or flocculate in solution. The UV absorption spectra of 10 mg/L ZnO NPs and 10 mg/L TiO<sub>2</sub> NPs suspended in M4 were determined at 10-minute intervals for 60 minutes (Figure S1C-S1F). Both ZnO NPs and TiO<sub>2</sub> NPs absorbed light (320 to 400 nm) and peaked at 370 to 380 nm for ZnO NPs and 330 to 340 nm for TiO<sub>2</sub> NPs. The absorbance of TiO<sub>2</sub> NPs was higher than that of ZnO NPs, indicating that TiO<sub>2</sub> NPs more easily absorb light and react with it, relative to ZnO NPs. The absorbance of ZnO NPs and TiO<sub>2</sub> NPs decreased as a function of increased irradiation time. Figure S1 (G and H) shows that the ions released from the ZnO NPs increased as a function of increased exposure concentrations. At 0 hour, the number of ions released from the ZnO NPs under UV irradiation was greater than that under visible light irradiation ( $p < 0.5$ ).

Figure 1 (A and B) shows the survival and mobilization of juveniles at 48 hours of exposure to ZnO NPs and TiO<sub>2</sub> NPs, under either visible light or UV irradiation. Figure 1C shows the control juveniles. Figure 1 (D-I) shows the lethal juveniles exposed to ZnO NPs, while Figure 1 (G-I) shows the normal juveniles exposed to TiO<sub>2</sub> NPs. At 5 mg/L ZnO NPs, gut and appendages of juveniles were adsorbed by aggregates assumed as ZnO NPs (black particles in Figure 1F). Gut of all TiO<sub>2</sub> NPs-treated juveniles was occupied by aggregates assumed as TiO<sub>2</sub> NPs (black particles in Figure 1G-I). With exposure to ZnO

**Table 1.** Toxicity values of *M. macrocopa* after exposure to ZnO NPs and TiO<sub>2</sub> NPs using acute toxicity test

Endpoints	Exposure duration (hr)	ZnO NPs			TiO <sub>2</sub> NPs		
		Visible	UVA	UVB	Visible	UVA	UVB
LC <sub>50</sub>	24	> 5	> 5	> 5	> 10	> 10	> 10
	48	2.71 (2.31, 3.17)	2.15 (1.99, 2.33)	1.62 (1.38, 1.91)	> 10	> 10	> 10
EC <sub>50</sub> (immobilization)	24	1.96 (1.78, 2.16)	> 5	3.67 (3.37, 4.00)	> 10	> 10	> 10
	48	1.79 (1.65, 1.94)	1.41 (1.32, 1.51)	1.12 (0.92, 1.36)	> 10	> 10	> 10

ZnO NPs, zinc oxide nanoparticles; TiO<sub>2</sub> NPs, titanium nanoparticles; UV, ultraviolet; LC<sub>50</sub>, lethal concentration at 50%; EC<sub>50</sub>, effective concentration at 50%.



**Figure 1.** Effects of ZnO NPs, TiO<sub>2</sub> NPs, and Zn ions under visible light, UVA, or UV irradiation on *M. macrocopa*. (A) Survival of ZnO NPs-treated *M. macrocopa*, (B) mobilization of ZnO NPs-treated *M. macrocopa*, (C) control of *M. macrocopa*, (D-F) 1, 3, and 5 mg/L of ZnO NPs-treated lethal *M. macrocopa*, respectively, (G-I) 1, 5, and 10 mg/L of TiO<sub>2</sub> NPs-treated *M. macrocopa*, respectively, and (J) effects of Zn ions-treated *M. macrocopa*. ZnO NPs, zinc oxide nanoparticles; TiO<sub>2</sub> NPs, titanium dioxide nanoparticles; Zn, zinc; UV, ultraviolet. #*p*<0.05: significant differences from visible light at the same exposure concentrations. \**p*<0.05: significant differences from the control under visible light.

NPs, the survival and mobilization of juveniles were noticeably decreased from 2 to 5 mg/L NPs under all irradiation (Figure 1A and 1B). At concentrations of 1 mg/L ZnO NPs, ZnO NP-

treated juveniles under UVB irradiation exhibited significantly decreased mobilization compared with those under visible light irradiation (*p* < 0.05). According to 48 hours-LC<sub>50</sub> and 48

hours-EC<sub>50</sub> presented in Table 1, the sensitivity of ZnO NPs for *M. macrocopa* was UVB > UVA > visible light irradiation. Meanwhile, there were no significant lethal and immobile effects of TiO<sub>2</sub> NPs on juveniles under all the irradiations, at any of the tested TiO<sub>2</sub> NP concentrations.

To assess the adverse effects of ions released from ZnO NPs, we conducted an ion toxicity assay (0.5, 1, and 2 mg Zn<sup>2+</sup>/L) using 1.7 mg Zn<sup>2+</sup>/L released from the maximum concentrations of ZnO NPs under all the irradiations (Figure 1J). Zn ions induced mortality and immobilization of juveniles. Toxicity values for Zn ions were calculated as 1.38 (1.30 to 1.46) mg Zn<sup>2+</sup>/L for 48 hours-LC<sub>50</sub> and 0.79 (0.68 to 0.92) mg Zn<sup>2+</sup>/L for 48 hours-EC<sub>50</sub>, respectively.

## DISCUSSION

The absorbance of ZnO NPs and TiO<sub>2</sub> NPs determined by UV/Vis spectrophotometer showed a decrease dependent on increased irradiation time. This is possibly due to the aggregation and dissolution of NPs. When TiO<sub>2</sub> NPs were re-sonicated for 10 minutes, the absorbance of TiO<sub>2</sub> NPs increased (data not shown). However, the absorbance of ZnO NPs was unaltered after re-sonication, probably indicating dissolution of the ZnO NPs. The ions released from the ZnO NPs showed increase dependent on increased exposure concentrations. In addition, the ions released from the ZnO NPs under UV irradiation were greater than under visible light irradiation ( $p < 0.5$ ). This phenomenon may be due to the accelerated dissolution of ZnO NPs caused by UV irradiation [2,11], in the initial stages. In a previous study, Lee and An [11] and Shin et al. [2] reported the absorbance of ZnO NPs and TiO<sub>2</sub> NPs as a function of time until 1 hour. They reported the absorbance of ZnO NPs and TiO<sub>2</sub> NPs dispersed in OECD algal medium and M4 medium was decreased as a function of increased irradiation time, as observed in this study.

UV irradiations induced more serious effects of ZnO NPs on *M. macrocopa* compared to visible light irradiation, according to toxicity values presented in Table 1. Since 1.7 mg Zn<sup>2+</sup>/L released from maximum concentrations of ZnO NPs included in 1 to 2 mg Zn<sup>2+</sup>/L, the ions released from ZnO NPs is may be induced mortality and immobilization of juveniles, described in Figure 1J. Similar to what was found in this study, the increased dissolution of ZnO NPs was shown in various exposure solutions [2,3,14,15]. Therefore, the observed mortality and immobilization of juveniles were probably due to both the ZnO NPs and Zn ions released from the NPs. The different sensitivity of ZnO NPs on *M. macrocopa* under three irradiations may be caused by a dissolution rate of ZnO NPs in exposure solutions.

According to Figure 1G and 1H, more ions were released from the ZnO NPs under UV irradiation than under visible light irradiation, just after pre-irradiation of UVA or UVB (at 0 hour). At 48 hour, there were no significant differences in dissolution levels between visible and UV irradiation. The promoted toxicity of ZnO NPs under UV irradiation is shown in the bacterial growth inhibition and mortality of nematode [3,4]. According to a prior study, Zn ions induced the inhibition of survival and reproduction of *D. magna* [16]. Zn ions inhibited calcium uptake and the production of hypocalcaemia in *D. magna*, thereby inhibiting movement, filtration rate, food uptake, available energy, growth, and reproduction. Therefore, the different mortality and immobilization of juveniles was probably due to the accelerated dissolution of ZnO NPs just after pre-irradiation by UV light, compared to visible light irradiation.

Under UV irradiation, ZnO NPs show bioavailable dissolution to decrease the survival and mobilization of *M. macrocopa*. Our results suggest that the photoreactive NPs, under solar light during daytime, have a potential and accelerated toxicity on organisms in dark environments. Therefore, it is necessary to evaluate the altered toxicity of photoreactive NPs in various environments and investigate mechanisms for photoreactive NPs.

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## CONFLICT OF INTEREST

The authors have no conflicts of interest associated with the material presented in this paper.

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**Table S1.** Physicochemical properties of ZnO NPs and TiO<sub>2</sub> NPs

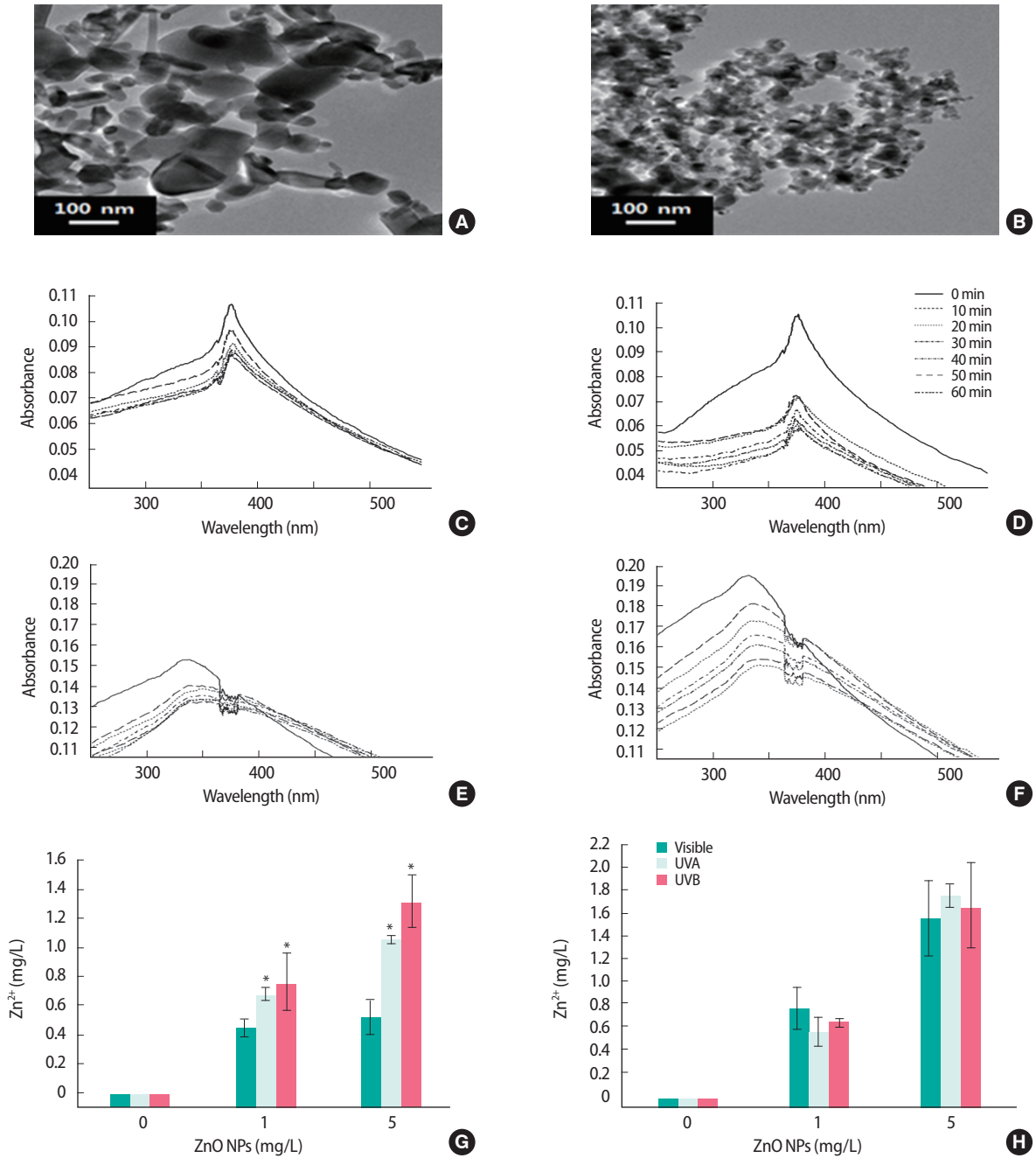
Properties	ZnO NPs	TiO <sub>2</sub> NPs
Manufacturer	Sigma-Aldrich	Evonik Degussa
Crystal structure <sup>a</sup>	-	Anatase 72.6%, Rutile 18.4%, Amorphous 9%
Surface coating <sup>a</sup>	No	No
Mean hydrodynamic diameter (nm) <sup>b</sup>		
Deionized water	211±11	354±15
M4 medium	622±30	1389±53
Zeta potential (mV) <sup>b</sup>		
Deionized water	-13.3±1.1	-2.0±1.4
M4 medium	-10.1±2.4	-0.4±0.3
Specific surface area (m <sup>2</sup> /g) <sup>c</sup>	12.0±0.0	55.0±0.4

ZnO NPs, zinc oxide nanoparticles; TiO<sub>2</sub> NPs, titanium dioxide nanoparticles.

<sup>a</sup>Supplied from the manufacturer.

<sup>b</sup>Measured by electrophoretic light scattering spectrophotometer .

<sup>c</sup>Measured by particle size analyzer.



**Figure S1.** Physicochemical characterization of ZnO NPs and TiO<sub>2</sub> NPs and Zn ions released from the exposure concentration of ZnO NPs dispersed in M4 medium (A and B). Transmission electron microscope images of ZnO NPs and TiO<sub>2</sub> NPs (C and D). Absorption spectra of ZnO NPs under UVA and UVB (E and F). Absorption spectra of TiO<sub>2</sub> NPs under UVA and UVB, as a function of UV irradiation in M4 medium at 10 mg/L NPs (G and H). Zn ions released from the exposure concentrations of ZnO NPs at 0 and 48 hours. ZnO NPs, zinc oxide nanoparticles; TiO<sub>2</sub> NPs, titanium dioxide nanoparticles; Zn, zinc; UV, ultraviolet. \* $p < 0.05$  significant differences from visible light at the same exposure concentrations.