보건학석사 학위논문

Effects of light condition and particle size on toxicity of nano-TiO_2 on *Daphnia magna*

나노 TiO_2 입자의 *Daphnia magna* 독성에 영향을 미치는 광조건과 입자크기의 영향

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Abstract

Background

Titanium dioxide (TiO_2) is known to have photocatalytic activity and ultraviolet absorbing property. Nano-sized TiO_2 has been widely used in photocatalysts, pigments, and cosmetic additives, and toothpaste. These a lot of utilities led to releasing nanometrerials into the environment or discharge to the environment.

As a result of ozone layer decrease, ultraviolet light could reach on the earth's surface. Because TiO_2 has photocatalytic activity, toxic effects could be changed. However little is known about the phototoxicity of TiO_2 in aquatic organisms such as *Daphnia magna* (*D. magna*).

Objectives

The aim of this study was to evaluate the effect of light condition and particle size of TiO_2 on *Daphnia magna*.

Methods

Aucte toxicity test and antioxidant enzyme toxicity test was performed. Acute toxicity test was performed on *D. magna* using pyrophosphated coated TiO_2 ($^{pyro\text{-}}TiO_2^{P25\text{-}70}$) and uncoated TiO_2 ($TiO_2^{P25\text{-}70}$) under different light condition such as fluorescent light, ultraviolet-B (UV-B) light and solar light. In addition, antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione s-transferase (GST), and lipid peroxidation (MDA) were analyzed to understand the mechanism of phototoxicity.

Results

TiO_2 toxicity changed with light condition. TiO_2 toxicity increased under UV-B and solar light condition. *D. magna* was more susceptible to $TiO_2^{P25\text{-}70}$ than $^{pyro\text{-}}TiO_2^{P25\text{-}70}$. Antioxidant enzymes, CAT and SOD were similarly expressed to mortality however

MDA expression shown reversely expressed pattern compared to CAT and SOD expression.

Conclusion

TiO_2 could be occurred phototoxicity thus when it released to real environmental condition, TiO_2 toxicity more increased. Micro size particles more toxic than nano size particle according to particle characteristics and test species. TiO_2 could mainly affects test species through oxidative stress mechanisms but other toxic mechanisms such as biological surface coating were also considered. Present study is worth than other toxicity test under laboratory condition because we conducted toxicity test under real environmental condition.

Keywords

Titanium dioxide; *Daphnia magna*; phototoxicity; ultraviolet light; antioxidant enzyme

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1. Introduction

Nanomaterials are defined as smaller than 100 nm in at one dimension which has a lot of special characteristics. Those characteristics which include size, chemical composition, surface structure, solubility, shape, and aggregation are important determined of the toxicity of nanomarterials (Nel et al., 2006; Skebo et al., 2007). Generally nanomaterials are more reactive compared with other materials results due to greater surface area to volume ratio (Navarro et al., 2008).

Titanium dioxide (TiO_2) is known to have photocatalytic activity and ultraviolet absorbing property. Nano-sized TiO_2 has been widely used in photocatalysts, pigments, and cosmetic additives, and toothpaste (Hall et al., 2009; Skebo et al., 2007). These a lot of utilities led to releasing nanomaterials into the environment or discharge to the environment (Wiesner et al., 2006). Titanium has been reported in surface water, and the concentration in surface run-off was as high as 3000 µg/L (Kaegi et al., 2008). In raw sewage, Ti concentrations reported ranged from 181 to 1233 µg/L (Median 321 µg/L, N=26) (Westerhoff et al., 2011). In WWTP effluents, Ti concentrations ranged from <5 µg/L to 15 µg/L in central Arizona, USA (Kiser et al., 2009). These amounts of Ti could affect environmental species.

Nanomaterials toxicity might be changed when it entered aquatic ecosystem. Natural organic matters (NOM) in the aquatic ecosystem may affect the surface charge of nano-sized materials and can cause precipitation (Navarro et al., 2008). When nanomaterials were aggregated or adsorbed to NOM, they would have less mobility and sink thus organisms like waterfleas which are filter feeders live in the aquatic phase can be affected (Farre et al., 2009). In the test condition, nano-TiO_2 can easily aggregates each other thus it is important to measure particle size in the test media after experiment to ensure particle size whether the hydrodynamic sizes were not changed or not during test period, but in most of previous

studies, did not shown measured particle size after experiments. However in the present study, we measured particle size of the TiO_2 in test media before and after experiment to confirm particle size effect.

In present study, we performed toxicity test under actual solar light condition. Global warming became important issue, and changing in aquatic environmental factors such as water temperature, dissolved oxygen concentration, intensity of rainfall, pH, salinity, and ultraviolet light intensity thus global warming can be one of the important points for aquatic toxicity and ecosystem. These factors might interact with toxicants and could affect the dose-response of the contaminants (Kim et al., 2010a). Among many factors, ultraviolet-B (UV-B) light is one of the most important factors because certain kinds of chemicals (i.e. polycyclic hydrocarbons) can absorb energy from ultraviolet light, thereby resulting in excited singlet and triplet state molecules (Ankley et al., 1995). UV-B light can be considered to make more severe interaction effects in real aquatic system by global warming. Because intensity of UV-B light has increased as a result of stratospheric ozone depletion (Huovinen et al., 2001). Because TiO_2 has photocatalytic activity, the toxicity might be changed under UV-B light. However, most toxicity tests were performing under normal laboratory condition like fluorescent light, their results could be underestimated actual toxic effects of TiO_2.

TiO_2 phototoxicity mechanisms are very little known. (Kim et al., 2009). Some ecological studies showed that TiO_2 exposure in aquatic species caused oxidative damage. TiO_2 particles have been studied with respect to reactive oxygen species (ROS) production and oxidative stress in toxicity studies (Kim et al., 2010b). The exposure of *D. magna* to TiO_2 caused oxidative stress by measuring of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione s transferase (GST) (Kim et al., 2010b), and caused lipid peroxidation (MDA) in the gill, intestine, brain, liver of rainbow trout (*Oncorhynchus mykiss*). However, previous studies have remained limited to relate to

actual materials size in the test media after experiment and phototoxic effect of actual solar light.

The aim of this study is identify the size effect and light condition effect of TiO_2 to *Daphnia magna*. In order to identify size effect, we used coated TiO_2 in order to prevent aggregating materials during test period and uncoated TiO_2 for comparison size effect. We exposed test organisms with actual solar light for the purpose of estimating phototoxicity. According to this test, findings could be more applied to estimate of TiO_2 toxicity to the aquatic species in real environment than other toxicity results which just performed in the laboratory condition.

2. Materials and Methods

2.1 Test materials

Uncoated P25 TiO_2 (TiO_2^{P25-70}) (Evonik GmbH, Germany) with average particle size of 70nm used in this study was kindly provided from Hanyang University (Seoul, Korea). In order to maintain particle size in the test media, Elendt M4 media (M4), we used pyrophosphate-coated P25 TiO_2 ($^{pyro-}TiO_2^{P25-70}$). In $^{pyro-}TiO_2^{P25-70}$, Pyrophosphate did not toxic to *D. magna* result by pyrophosphate pre-toxicity test at coating concentration.

2.2 Maintenance of *D. magna*

The animals used in this study were provided from Korea Institute of Toxicology (Daejeon, Korea) and maintain in our lab since 2003. *D. magna* were cultured in 3L glass jar with 25 ea/L density, by using M4 media prepared following US Environmental Protection Agency (US EPA) under 21±1 ℃ water temperature, 16/8 hour photo period. They were fed with algae (*Chlorella*) 5mL *chlorella* ($1x10^6$ units/mL)/1L and YCT (1:1:1 mixture of Yeast:Ceropyl® :Tetramin®) once a day.

2.3 Toxicity test design

D. magna acute toxicity test

In order to evaluating TiO_2 toxicity to *D. magna*, we performed acute toxicity test according to USA EPA guidelines (2002). To explore appropriate test concentrations, we performed

range finding test first. $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ were prepared from stock solution to achieve the concentration of 0, 0.31, 0.93, 2.78, 8.33, and 25 mg/L respectively. Acute toxicity test was performed in a 40ml test volume under static non-renewal condition. Five neonates (<24 h old) were exposed to test solution with four replicates per each test concentration. Before and after test, we measured water chemistry parameters such as pH, dissolved oxygen concentration, temperature, conductivity to ensure other factors did not affect *D. magna*. No movement for 15 s after gentle shaking of the test vehicle was regarded as immobilization, which was endpoint of this study. After experiment we calculated effective concentration at 50% inhibition (EC_{50}).

Light conditions

To estimate phototoxicity of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$, we chose three kinds of light condition such as fluorescent light, UV-B light, and solar light. Fluorescent light (12.1 μmol/m^2/s) is considered control condition to compare with both UV-B and solar light. We conducted solar light exposure test under the actual solar light in a temperature-controlled water bath (23±2 ℃) from 5th August to 7th August, 2011. Exposure to solar light was limited to 4 h per day, after which the cultures were placed in a temperature controlled incubator under the fluorescent light condition. During the solar light exposure, UV-B intensity (total 74.5-102 μW/cm^2 for 4 h/d) was measured two times in a hour by using VLX3W radiometer (UV-B sensor: 280-320nm; Cole-parmer Instrument So., IL, USA). The basis of the amount of measured UV-B light intensity during solar light exposure, we also conducted continuous UV-B light exposure (12-14 μW/cm^2, 24 h/d) in a temperature-controlled incubator. In antioxidant enzyme test, light condition was set up equally with acute toxicity test condition.

Antioxidant enzyme analysis

To describe $^{pyro-}TiO_2^{P25-70}$ and TiO_2^{P25-70} toxicity mechanism, because we consider TiO_2 toxic mechanism is oxidative stress, we measured antioxidant enzyme. For the antioxidant enzyme test, test solutions were prepared from stock solution to achieve the concentration 0, 0.034, 0.103, and 0.310 mg/L. Antioxidant enzyme test was performed in a 200 mL test volume under static non-renewal condition. Five adults (age from 30 to 35 days) were exposed to test solution with three replicates per each concentration except for control, 4 replicates. Before and after test, we also measured water chemistry parameters such as pH, dissolved oxygen concentration, temperature, conductivity to ensure other factors did not affect *D. magna*. After 48 h exposure under the three different kinds of light condition, *D. magna* were collected and then analysis for antioxidant enzymes such as catalase (CAT), glutathione s-transferase (GST), superoxide dismutase (SOD) and malondialdehyde (MDA), a product of lipid peroxidation level on *D. magna*. To determine the optimal sample size for the antioxidant enzyme biomarker analysis and to adjust enzyme activity per same protein unit, protein assay was conducted according to Bradford assay (Bradford, 1976). The CAT activity was measured by the decrease in absorbance at 240nm due to H_2O_2 consumption (ε^{mM}= 0.0436) following (Aebi, 1974). GST activity towards CDNB (1-chloro-2,4-dinitrobenzene) was measured as described by (Habig et al., 1974). SOD activity was determined by an indirect method involving the inhibition of cytochrome-c reduction (McCord and Fridovich, 1969). We performed MDA analysis using commercial thiobarbituric acid reactive substances (TBARS) kit (Cell Biolabs, Inc., San Diego, CA, USA) with provided manual.

3. Statistical analysis

Results of repeated experiments are expressed as the mean value ± standard deviation. We carried out student's t-test and a one-way analysis of variance with Dunnett's test and linear regression analysis using SPSS 15.0 for windows® (SPSS, Chicago, IL, USA) and ToxStat (ver 3.5. West, Cheyenne, WY, USA). *P*-values less than 0.05 were considered statistically significant.

4. Results

D. magna acute toxicity test

In the acute toxicity test, water chemistry parameters were not changed during test period (data not shown) thus we considered other factors were not affect *D. magna*. Particle size of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ after experiment summarized at Table 1 depending on different light condition. After 48 h exposure, particle size of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ was measured from 103 nm to 202 nm under fluorescent light, 84 nm to 319 nm under UV-B light and 104 nm to 381 nm under solar light. However $TiO_2^{P25\text{-}70}$ were aggregated in the test media and particle size has grown (>1000 nm) under all light condition (Table 1).

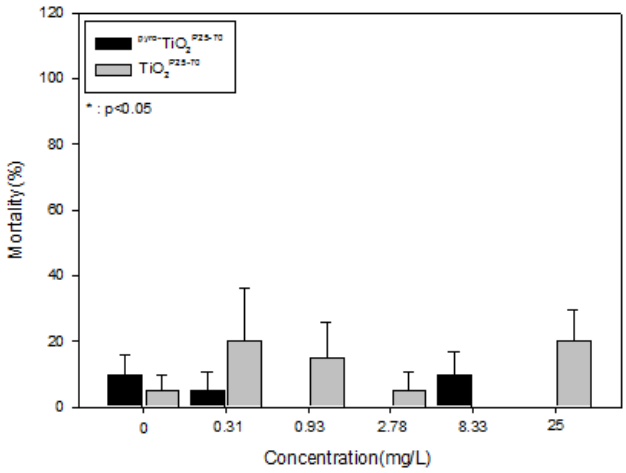
EC_{50} value of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ under fluorescent light was very low (EC_{50}>25 mg/L). However significant mortality occurred at the highest concentration of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ under UV-B and solar light (Figure 1 (b), and (c)). EC_{50} was calculated to 24.96, 13.64 mg/L under UV-B and solar light, respectively. $TiO_2^{P25\text{-}70}$ toxic effect did not occurred under fluorescent light (EC_{50}>25 mg/L) even at the highest concentration. However mortality was increased even lowest concentration (0.31 mg/L) when exposed UV-B (EC_{50} 0.20 mg/L) and solar light (EC_{50} 0.72 mg/L). These results suggested that toxicity of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ was changed under light condition and $TiO_2^{P25\text{-}70}$ was more toxic than $^{pyro\text{-}}TiO_2^{P25\text{-}70}$.

Table 1. Acute toxicity test results and particle size after experiment by exposure of $^{pyro-}TiO_2^{P25-70}$ and TiO_2^{P25-70} with different light condition.

Materials	Light condition	48 h EC_{50} (mg/L)	Size[a] (nm)		
			2 mg/L	8 mg/L	25 mg/L
$^{Pyro-}TiO_2^{P25-70}$	**Fluorescent**	>25	202	572	103
	UV-B	24.96 (11.42-38.47)	319	142	84
	Solar	13.64 (10.20-17.08)	381	270	104
TiO_2^{P25-70}	**Fluorescent**	>25	>1000	>1000	>1000
	UV-B	0.20 (-0.02-0.42)	>1000	>1000	>1000
	Solar	0.72 (0.34-1.01)	>1000	>1000	>1000

[a]Size indicates hydrodynamic size of particles in test media after experiment

(a)



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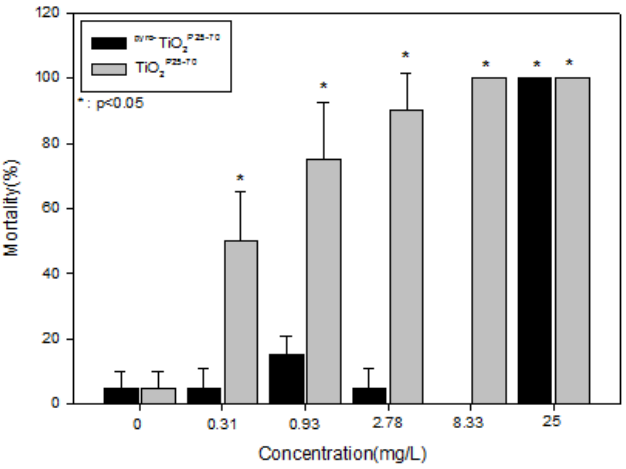

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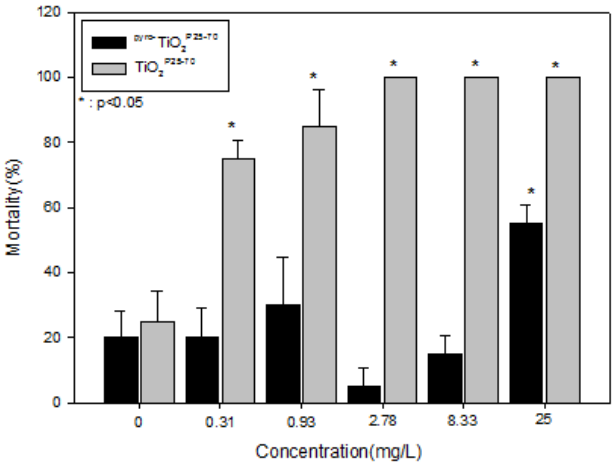


Figure 1. Toxicity results by exposure of $^{pyro-}TiO_2^{P25-70}$ and TiO_2^{P25-70} with different light condition. (a) fluorescent light, (b) ultraviolet B light and (c) solar light.

Antioxidant enzyme activity

Mortality of exposure to sublethal concentration for antioxidant enzyme test after 48 h was presented figure 2. Mortality of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ did not occurred at low concentration (0.034 and 0.103 mg/L) under fluorescent, UV-B and solar light, however significant mortality observed at the highest concentration (0.310 mg/L) under UV-B light condition ($p<0.05$) (Figure 2 (a)).

$TiO_2^{P25\text{-}70}$ exposure led to significant mortality at the lowest concentration (0.034 mg/L) and middle concentration (0.103 mg/L) under fluorescent light (Figure 2 (b)). However mortality decreased with increasing concentration. UV-B light exposure led to significant mortality at the lowest concentration (0.034 mg/L) however mortality decreased with increasing concentration. Under solar light, mortality just little observed at the middle concentration (0.103 mg/L).

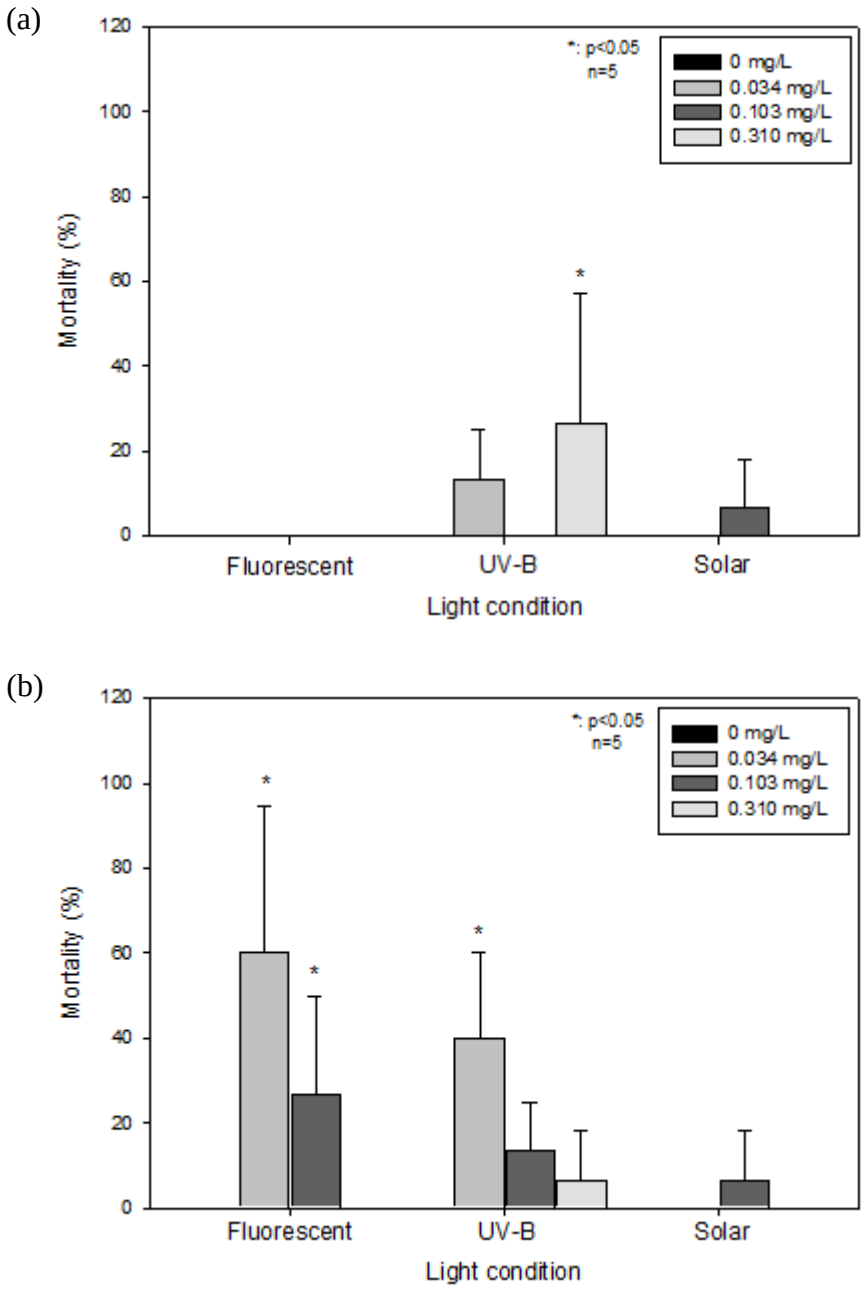
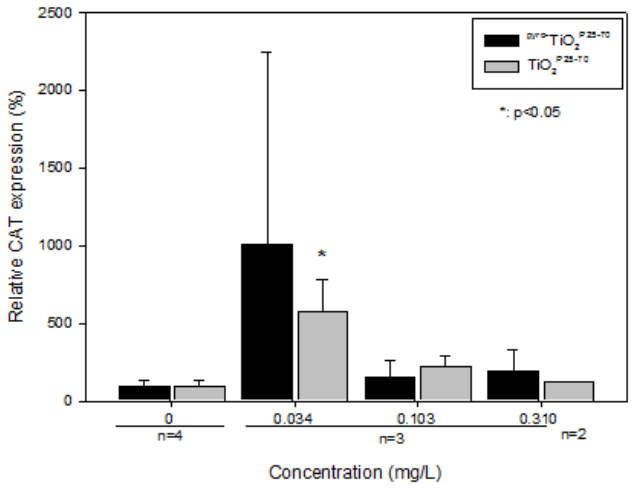


Figure 2. Mortality rate result by exposure of $^{pyro-}TiO_2^{P25-70}$ and TiO_2^{P25-70} with different light condition. (a) $^{pyro-}TiO_2^{P25-70}$, (b) TiO_2^{P25-70}

Figure 3 indicates relative CAT expression (%) result by exposure to $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ with different light condition. CAT expression under fluorescent light was shown similar pattern between $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ that increased at the lowest concentration however decreased with increasing concentration (Figure 3 (a)). CAT expression under UV-B light was significantly increased at the lowest concentration of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ exposure also increased at the lowest concentration of $TiO_2^{P25\text{-}70}$ exposure (Figure 3 (b)). CAT expression under solar light was just increased at the lowest concentration of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ exposure (Figure 3 (c)).

(a)



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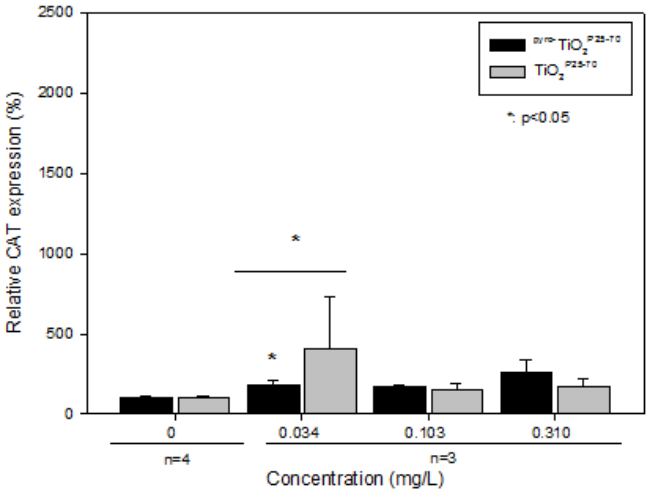

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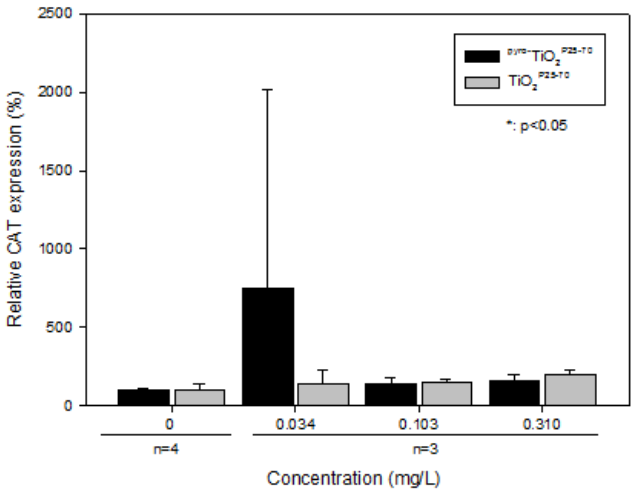
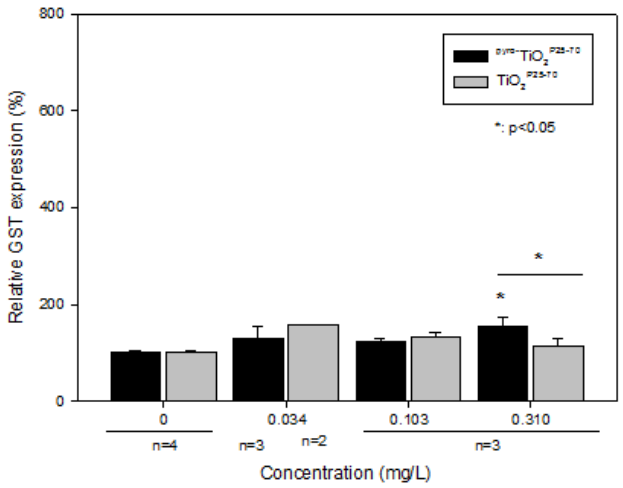


Figure 3. Catalase enzyme activity by exposure $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ with different light condition. (a) fluorescent light, (b) ultraviolet B light and (c) solar light.

Figure 4 indicates relative GST expression (%) result by exposure of $^{pyro-}TiO_2^{P25-70}$ and TiO_2^{P25-70} with different light condition (Figure 4 (a), (b) and (c)). GST expression under fluorescent light was significantly increased at the highest concentration of $^{pyro-}TiO_2^{P25-70}$ exposure (Figure 4 (a)). GST expression under UV-B light was increased with increasing concentration of $^{pyro-}TiO_2^{P25-70}$ but TiO_2^{P25-70} exposure little affect GST expression (Figure 4 (b)). Under solar light, GST expression was increased at the lowest concentration of $^{pyro-}TiO_2^{P25-70}$ exposure but decreased with increasing concentration and TiO_2^{P25-70} exposure little affect GST expression (Figure 4 (c)).

(a)



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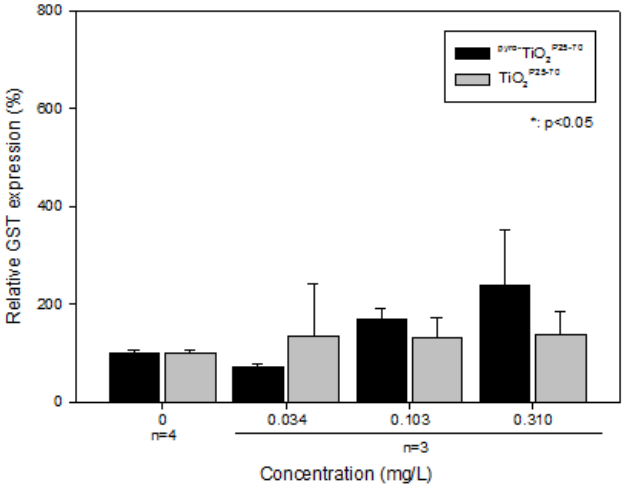

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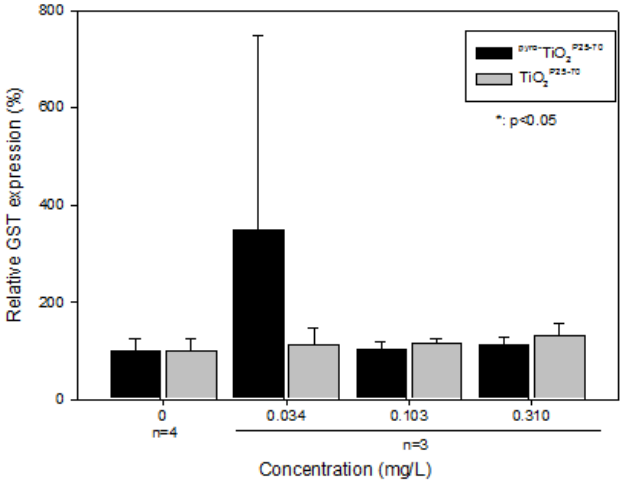
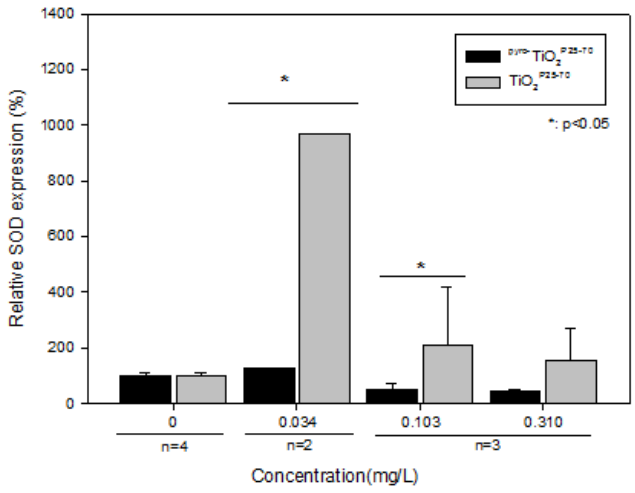


Figure 4. Glutathion S-transferses enzyme activity by exposure $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ with different light condition. (a) fluorescent light, (b) ultraviolet B light and (c) solar light.

Figure 5 indicates relative SOD expression (%) result by exposure to $^{pyro-}TiO_2^{P25-70}$ and TiO_2^{P25-70} with different light condition. SOD expression under fluorescent light was little affected by $^{pyro-}TiO_2^{P25-70}$ exposure however significantly increased at the lowest concentration (0.034 mg/L) of TiO_2^{P25-70} exposure (Figure 5 (a)). SOD expression under UV-B light was little increased with increasing concentration of $^{pyro-}TiO_2^{P25-70}$ exposure but increased at the lowest concentration of TiO_2^{P25-70} exposure (Figure 5 (b)). Under solar light exposure, SOD expression was increased at the lowest concentration of $^{pyro-}TiO_2^{P25-70}$ and TiO_2^{P25-70}, decreased with increasing concentration (Figure 5 (c)).

(a)



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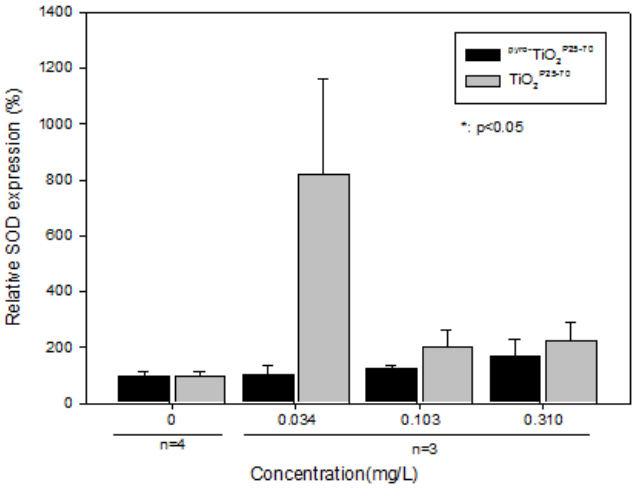

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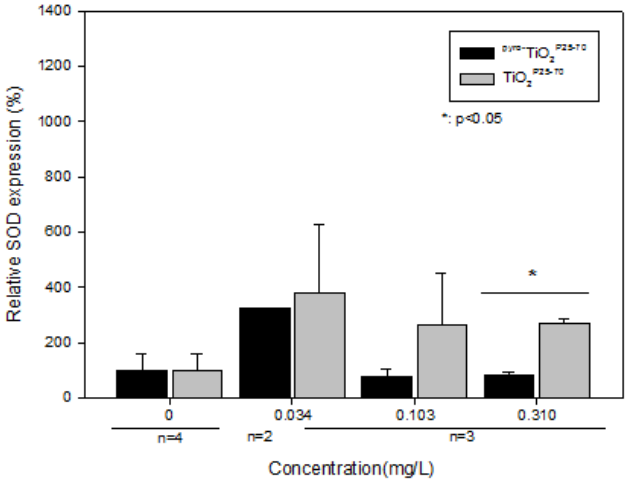
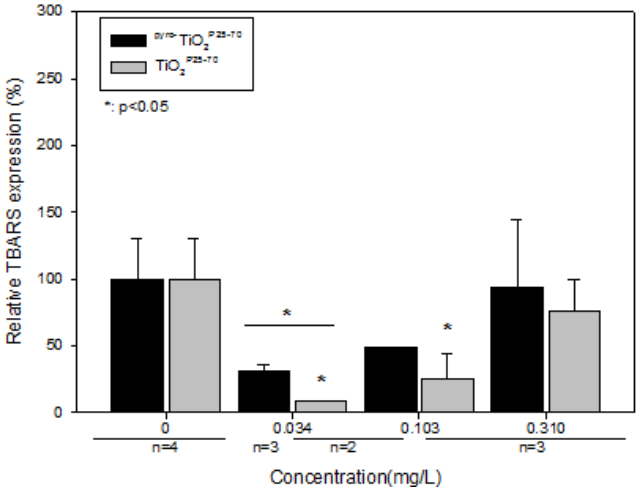


Figure 5. Superoxide dismutase enzyme activity by exposure $^{pyro-}TiO_2^{P25-70}$ and TiO_2^{P25-70} with different light condition. (a) fluorescent light, (b) ultraviolet B light and (c) solar light.

Figure 6 indicates relative TBARS expression (%) result by exposure to $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ with different light condition. TBARS expression under fluorescent light was decreased even at the lowest concentration of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ exposure but increased with increasing concentration (Figure 6 (a)). Under UV-B light, TBARS expression was shown decreasing pattern with increasing $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ concentration (Figure 6 (b)). Under solar light, TBARS was increased at the lowest concentration of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ exposure however did not shown significant difference because of large standard deviation. (Figure 6 (c)).

(a)



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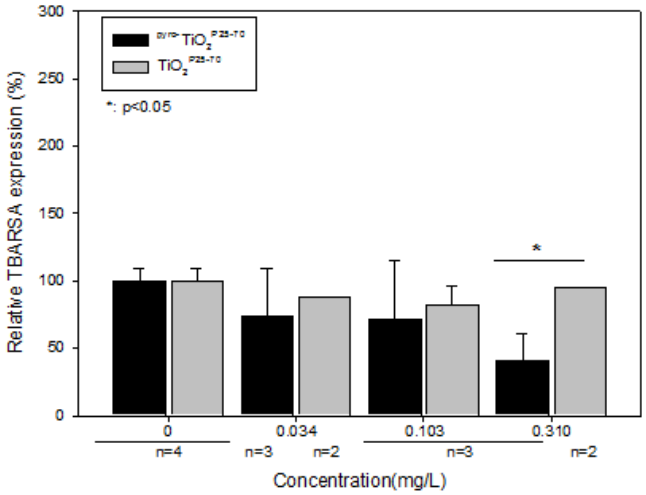

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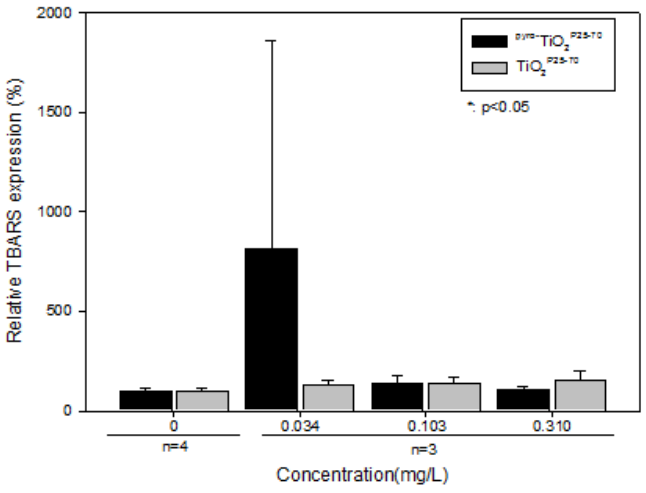

Figure 6. Thiobarbituric acid reactive substances expression by exposure $^{pyro-}TiO_2^{P25-70}$ and TiO_2^{P25-70} with different light condition. (a) fluorescent light, (b) ultraviolet B light and (c) solar light.

5. Discussion

Micrometer size particles were more toxic than nanometer size particles. In the acute toxicity test, measured particle size of $TiO_2^{P25\text{-}70}$ (>1000 nm) was bigger than $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ (84-572 nm) after 48hr test duration (Table 1), and EC_{50} value of $TiO_2^{P25\text{-}70}$ was lower than that of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ under UV-B or solar light condition (Figure 1 (a), (b)). It suggested that micrometer size particles were more toxic than nanometer size particle. However previous studies reported that nano size materials were more toxic than micro size materials. Algal (*Pseudokirchneriella subcapitata*) growth inhibition tests by using nano TiO_2 (25-70 nm) and bulk TiO_2 (Aruoja et al., 2009). The results indicated nano size TiO_2 was more toxic (EC_{50} 5.83 mg/L) than micro size TiO_2 (EC_{50} 35.90 mg/L). Acute test with uncoated TiO_2 which particle size was ~100 nm and ~200 nm size each (Dabrunz et al., 2011). The results revealed ~100 nm particles are more toxic (100 %) than ~200 nm particles (57 %). Acute toxicity tests using nano TiO_2 ($\leq$20 nm) and bulk TiO_2 (10,000nm), and EC_{50} of nano TiO_2 and bulk TiO_2 were 35.31 mg/L, 275.28 mg/L, respectively (Zhu et al., 2009). It was indicated that nano size particle was more toxic than micro size particle. However some toxicity test reported similar result to ours. Cytotoxicity test was performed by using human alveolar type II-like epithelial cell line, A549. The result showed micrometer particles of TiO_2 caused more DNA damage (24 %) compared to nanoparticles (19 %) (Karlsson et al., 2009). These conflicted results for toxicity of nano and bulk TiO_2 can be partly caused by changed hydrodynamic sizes after experiments. However previous studies didn't measured particle size in the test media. Nanoparticles could be changed particle size in the test media during experiments, thus it is important to measure particle size before and after experiment. In the present study, we measured particle size before and after experiment thus our results are more convincing than other toxicity test to explain size effects.

Particle size of TiO_2 was more important than TiO_2 concentration in the water phase to exhibit toxicity on *D. manga*. In the antioxidant enzyme test (low dose condition), mortality of *D. magna* did not shown dose response pattern exposing of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ but decreasing trend with increasing concentration of $TiO_2^{P25\text{-}70}$ (Figure 2). In other words mortality did shown dose response pattern thus it is suggested that particle concentration at the low dose condition was less important to exhibit toxicity. Previous studies have performed test which was relations between particle concentration and particle distribution in the water phase. Particle concentrations in water phase were decreased with increasing initial particle concentration (Keller et al., 2010). Doublet formation times (collision of two particles forming an agglomerate of the two primary particles) of ceria nonoparticle decreased with increasing concentration at the same size of particle (Limbach et al., 2005). It indicated that aggregation more occurred at the higher concentration than lower concentration thus lots of particles which had dispersed in the water phase settled down on the bottom of test beaker. Although we did not measure particle concentration of test media, according to this phenomenon it suggested that particles were well distributed at the low concentration than high concentration media because of slow doublet formation times.

Phototoxicity in present study might be due to oxidative stress result of reactive oxygen species. In the present study TiO_2 toxicity was increased under UV-B and solar light condition (Figure 1 (b) and (c)). In our previous study, no significant mortality to *Moina macrocopa* was noted for any TiO_2 test solution under no UV-A light, however presence of UV-A exposure mortality was significantly increased compared to control group. TiO_2 absorbs UV-A light catalyzing the generation of reactive oxygen species, such as superoxide anion radicals, hydrogen peroxide, free hydroxyl radicals, and singlet oxygen in aqueous media (Hirakawa et al., 2004). The adverse sublethal and lethal effects on gammarids can be caused by combined application of nano TiO_2 and ambient UV-irradiation, and this result

could be supported by the formation of reactive oxygen species in gammarids (Bundschuh et al., 2011).

Antioxidant enzymes have been proposed as an early warning indicator of the population-level effect from sublethal concentration exposure. In the present study some antioxidant enzymes were expressed at the lowest concentration of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$ exposure under all light condition especially under fluorescent condition with $TiO_2^{P25\text{-}70}$ exposure (Figure 3 and 6). The reason why SOD and CAT sensitive to TiO_2 exposure was that SOD-CAT system provides the first defense against oxidative toxicity at a cellular level (Hao et al., 2009). Because SOD plays a critical antioxidant role in the dismutation of $O_2^{-}\cdot$ and CAT dismutates to form H_2O_2 to water and oxygen (Kim et al., 2010b), thus SOD and CAT were expressed at the lowest concentration which significantly affects mortality of *D. magna* for the purpose of defense from oxidative stress. Expression of CAT and SOD more expressed than that of $^{pyro\text{-}}TiO_2^{P25\text{-}70}$ because $TiO_2^{P25\text{-}70}$ was more toxic than pyro-$TiO_2^{P25\text{-}70}$. GST expression little affected mortality of *D. magna* (Figure 4).

Oxidative stress was determined by the TBARS assay with malondialdehyde (MDA) as the principal product from lipid peroxidation. MDA expression showed different expression pattern compare CAT and SOD expression (Figure 6). MDA expression was significantly decreased at the lowest concentration under fluorescent light condition which CAT and SOD expression was highly expressed (Figure 6 (a)). In other words MDA expression pattern shown reverse pattern of CAT and SOD. Among fixed amount of oxidative stress, large amount of oxidative stress was detoxified by CAT and SOD. As a result of this detoxification response of CAT and SOD, MDA expression was effected. There are similar results about expression of antioxidant enzyme. CAT and SOD expressions were reduced exposed to TiO_2 nanoparticles but MDA expression increased (Hao et al., 2009). CAT and MDA showed different expression pattern at the same concentration of microcystin-LR (Ortiz-Rodriguez

and Wiegand, 2010).

Biological surface coating mediated toxicity was another possible toxic mechanism of nanoparticles. In the present study biological surface coating could be observed at the outer surface of *D. manga*. (Data not shown). It suggested biological surface coating might affect toxicity of $^{pyro}TiO_2^{P25\text{-}70}$ and $TiO_2^{P25\text{-}70}$on *D. magna*. Several studies confirmed that biological surface coating was affected on test organisms (Dabrunz et al., 2011). Physical effects are major importance of toxicity on *D. magna*. Adsorption of the material to the exterior surface of the organisms can be considered as the first step in the biological uptake of any NP (Dabrunz et al., 2011). Apart from zooplankton, NP adhesion to biological surface coating has also been shown in other organisms. Examined respiratory toxicity test, organ pathologies, and other physiological effects by using of single walled carbon nanotube (SWCNT) (Smith et al., 2007). The results showed SWCNT existed in the gill and observed an entrapping of algae cells (*P. cubcapitata*) by TiO_2 aggregates formed during the 72 h toxicity test phytoplankton (Aruoja et al., 2009). Biological surface coating results in the increase of both the specific weight and the physical resistance during swimming movements, thus it is likely that the energy demand strongly increases (Dabrunz et al., 2011) eventually offered critical effects.

It is possible that surface properties of coating materials might affect toxicity of TiO_2 to *D. magna*. Some reports revealed that the toxicity of QDs in biological systems is not dependent on the nanoparticle itself but on the surface molecules (Hoshino et al., 2004). No cytotoxicity was detected from their ingredients or the QD core itself, suggesting that surface processing will overcome the toxicity of nanomaterials. Stabilized silver nanoparticles were more toxic than non stabilized silver nanoparticles (Panacek et al., 2009). However these results came from toxicity of surfactant itself. In the present study, we used pyrophosphate as coating material which not affects *D. manga* thus it is not possible surfactant affect test organism.

Other reports revealed that coated nano silver release silver ions at the greater rate than uncoated nanosilver. However uncoated silver particles had greater toxicity than the same size coated particles, indicating silver ion release at most makes a small contribution to toxicity (Posgai et al., 2011). In the present study it is possible that titanium ions released from materials however the low solubility and the low intrinsic toxic potential of titanium itself, it was likely that *D. magna* which exposed to releasing Ti was not little affected to *D. magna* by Ti ion.

6. Conclusion

In the present study, we could confirmed that nano size materials were not always toxic than micro size materials therefore consideration between test materials and test species is required to accurately estimate of nano material toxicity. TiO_2 could mainly affects test species through oxidative stress mechanisms but other toxic mechanisms such as biological surface coating were considered. In the real environmental condition, TiO_2 material exposed solar light. Because we conducted toxicity test under real environmental condition, thus it is worthwhile than other toxicity test under laboratory condition. We did not measured particle concentration and Ti ion concentration of test media thus it is hard to explain accurate TiO_2 toxic effects and toxic mechanisms thus further studies required to identify TiO_2 concentration effects and Ti ion effects.

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국문초록

나노 TiO_2 입자의 *Daphnia magna* 독성에 영향을 미치는 광조건과 입자크기의 영향

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연구배경

TiO_2는 광촉매 특성과 자외선을 흡수하는 특성을 가진 것으로 알려져 있다. 나노 크기의 TiO_2는 여러 상업 분야에 많이 사용되는데, 광촉매제, 안료, 화장품의 첨가제 그리고 치약의 성분이 그것이다. 이와 같은 TiO_2의 높은 이용성에 때문에 의도하지 않게 환경 중으로 배출이 발생된다. 또한 배출된 TiO_2는 오존층의 감소로 인해 증가한 자외선과 반응하여 독성영향을 변화시킬 수 있다. 하지만 TiO_2 물질의 수중생물에 대한 광독성에 대한 연구는 많이 진행되지 않았다.

연구목적

본 연구의 목적은 입자의 크기와 광조건이 TiO_2 의 독성을 변화시켜 어떻게 *Daphnia magna* (*D. magna*) 에 영향을 미치는지를 평가하기 위함이다.

연구방법

급성독성 실험과 항산화효소분석 실험을 진행하였다. 실험 물질은 표면을 pyrophosphate로 코팅한 물질($^{pyro-}TiO_2^{P25-70}$)과 표면을 코팅 처리 하지 않은 물질(TiO_2^{P25-70})을 이용하여 세가지 다른 조건의 광조건인 형광등 조건, 자외선-B(ultraviolet-B)조건, 태양광 조건에 노출을 시켰다. 또한 TiO_2의 독성 기전을 알아보기 위해 항산화효소인 catalase (CAT), glutathione s-transferase (GST), superoxide

dismutase (SOD)와 lipid peroxidation (MDA)를 분석하였다.

연구결과

TiO_2의 독성은 광조건에 따라 달라졌다. UV-B 조건과 태양광 조건에서 독성의 세기가 증가하였다. TiO_2^{P25-70}의 노출이 $^{pyro-}TiO_2^{P25-70}$의 노출보다 *D. magna*에 더 영향을 미쳤다. 항산화효소인 CAT와 SOD는 *D. magna*의 치사(mortality) 경향과 비슷하게 발현이 되었으나 MDA는 CAT와 SOD의 발현과 반대 양상으로 발현이 되었다.

결론

TiO_2는 광독성을 일으킬 수 있기 때문에 태양광이 존재하는 환경으로 노출이 된다면 TiO_2의 독성은 증가할 것이다. 또한 나노크기(nm)의 물질의 독성이 마이크로크기(μm)의 독성보다 항상 강하지 않았으며 이것은 물질의 특성과 생물의 특성에 따라 달라지므로 독성평가를 할 경우 이 두 가지 요소를 고려해야 할 것이다. TiO_2의 독성은 주로 산화손상기전을 통해 발현이 되나 생물학적 표면코팅(biological surface coating)의 기전을 통해서 또한 독성 기전을 설명할 수 있을 것이다. 이번 연구는 실험실 조건에서 진행한 다른 실험들과는 달리 실제 태양광에 물질을 노출시켜 실제 환경에 노출된 TiO_2의 조건을 가정한 실험이므로 기존에 진행한 실험들 보다 더욱 가치 있는 연구이며, TiO_2의 실제 독성을 설명하기에 적합한 연구임에 틀림없을 것이다.

주요어: Titanium dioxide; *Daphnia magna*; 광독성; 자외선; 항산화효소

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