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"Protective" Effects of Titanium Dioxide Nano-particles on *Daphnia magna* Exposed to UV Radiation

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Authors' contributions

This work was carried out in collaboration between both authors. Author GGR designed the study, the experimental procedures and protocol, performed the statistical analysis and wrote the first draft of the manuscript. Author GRGR collaborated in the experimental procedures, reviewed the draft and managed the literature searches. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Titanium dioxide nano-particles (nano-TiO_2) have been used in sunscreen creams to protect skin againstultra violet (UV) radiation; other applications are polarized glasses, wall paints, etc., however, the ecological risks when nano-TiO_2 residues reach aquatic ecosystems is not well documented; therefore, the objective of this study is to give some insight on the attenuating effects of nano-TiO_2 against UV radiation on *Daphnia magna* and the toxicity of nano-TiO_2 on this organism.
Study Design: Exposing Daphnis to UV radiation in presence and absence of nano-TiO_2.
Place and Duration of Study: Biotechnology Institute, CNR, Canada and Toxicology laboratory, Faculty of Marine Sciences, University of Sinaloa Mexico; between April and July 2012.
Methodology: Daphnis (adults and neonates) were exposed to UV light for varying periods in the absence and presence of nano-TiO_2. After 48 h incubation, mortalities were recorded for each experiment. Similar experiments were performed using bulk TiO_2 instead of nano-TiO_2, to know if protective effect is related to particles size. Finally, to

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know if protective effect is outer (blocking UV radiation) or interior (by cellular processes), Daphnis were pre-treated with nano-TiO_2, then exposed to UV radiation, and mortalities recorded.
Results: The mortalities were significantly lower in presence than in absence of nano-TiO_2. Alsomortalities were significantly higher in bulk TiO_2 than in nano-TiO_2, indicating that nano-TiO_2has a protective effect against UV radiation on Daphnis. Also, results indicate that protective effect is exterior, by blocking of UV rather than cellular repair mechanisms (cellular processes). However, at 100mg/L nanoTiO_2 concentration, 15% mortality was observed.
Conclusion: Nano-TiO_2 is a nanomaterial blocking UV radiation; however if residues reach aquatic ecosystems, could be a risk (toxic) for aquatic organisms, because the highest concentration used in this work caused mortalities in *Daphnia magna*.

Keywords: Nano–TiO_2; UV radiation; Daphnia magna; aquatic ecosystem.

1. INTRODUCTION

Many studies have reported that UV radiation can cause severe damage to living organisms, particularly to aquatic organisms [1-3]. The component of UV radiation most dangerous to biological organism is the short wave UV-B radiation (280-320 nm) which has been reported to cause DNA damage, photosynthesis inhibition, reduced reproduction and finally mortality [2,3]. Also, the effects of UV radiation on aquatic organisms can change behavior, survival and respiration rates. In fact, several aquatic organisms have developed the ability to avoid harmful UV radiation by migrating to more shaded areas [4]. For example, the cladoceran Daphnia exhibit a strong behavioral response to UV radiation; downward migration at lower UV radiation levels has been demonstrated in *Daphnia pulex* [5]. Decreased feeding activity and reproduction rates were observed in *Daphnia magna* exposed to UV radiation, principally to high doses of UV light [6]. The damage caused by UV radiation, can range from eye and skin irritation to DNA strand breakage and matrix protein degradation, and finally death. In many cases, the damage results from the generation of aggressive free radicals, or "reactive oxygen species"(ROS) such as hydrogen peroxide (H_2O_2), hydroxyl (•OH) and superoxide (•O_2) radicals [7,8].

On the other hand, nano-scale compounds, such as titanium dioxide (TiO_2), Zinc oxide (ZnO), silver nano-particles (AgNPs) and others, have broad spectrum UV attenuating properties. Therefore, some of these chemicals are used in personal care products such as sunscreens to protect the human skin against UV radiation [9]. Also, they have other well-known applications such as polarized glasses, wall paints, sunscreen in windows houses, and so on [10-12]. However, other studies report that toxicity is increased when nano-particles such as TiO_2 are present at concentrations that can lead to lipid peroxidation and other chemical reactions in the cells of exposed organisms [13,14]. Contrarily, other studies report that TiO_2 does not lead to significant alterations in gene or ecotoxic parameters in aquatic organisms such as *Daphnia magna* [15]. Due to these contradictions, the objective of this study was to give some insight on the UV attenuating effects of nano-TiO_2 on *Daphnia magna,* and the toxicity of nano-TiO_2 on this organism. This is particularly important because ozone layer reduction in the atmosphere, so intensity of UV radiation reaching aquatic organisms has increased over the last decades and also because the pollution of aquatic environment is a major concern in industrial societies, with the impact of wastewater discharge from agricultural, industrial and domestic sources representing a particular challenge.

2. MATERIALS AND METHODS

Daphnia magna were obtained from Applied Ecotoxicology Laboratory of the Biotechnology Research Institute, CNR, Montreal, Canada. They were cultured in 4000 mL aquaria at a density of 20-30 organisms per 1000 ml of M4 culture media as recommended in the Organization for Economic Cooperation and Development (OECD) [16]. Continuous aeration was supplied and maintained at 20±1°C. Diurnal cycle was 16 h light /8 h dark (1500 – 2000 lx) for a minimum of 24 h before use, using an incubation chamber. *Daphnia magna* were fed daily with Pseudokirchneriella subcapitata (supplying 0.5-1 mg carbon per day with 100% extra given on weekends) supplemented with 2 ml of baker's yeast (100 mg/l). All cultures were initiated with third or fourth brood neonates <24h old. These conditions maintained the Daphnia in the parthenogenesis reproductive cycle. P. subcapitata were maintained in Bold's Basal Medium in a vessel and held at 20°C±1°C, under a light and dark normal day period. To provide a stock for food, algae were collected by centrifugation (2250 x g, 30 min, 20°C), and the pellets re-suspended in a volume of water that gave an optical density (1:10) of 0.8 at 440 nm.

2.1 Exposure of *Daphnia magna* to UV Light With and Without Nano-TiO_2.

Groups of five Daphnia adults (3 to 3.5 weeks old) were transferred to a 100 ml beakers with fresh M4 media (50 ml), and were exposed to an UV light lamp (Sylvania® 30 Watts, 253.7 nm wave length) at increasing durations (0, 5, 15, and 35 min). Each test was done in triplicate. Another group of Daphnia were transferred to a 100 mL beakers containing 50 ml of nano-TiO_2 (Aeroxide® P25, particles size 25-50 nm, Evonik Industries, USA) dispersed in the M4 media to get a final concentration (50 mg/L) using an IKA T25 Ultra-Turrax® homogenizer. Daphnia were then exposed to UV light at the same intensity and durations as described above. The exposed Daphnia with TiO_2 and without TiO_2 were incubated under the same culture conditions as described previously, but without feeding or supplements for 48 h, using the same incubation chamber. At the same time, groups of five Daphnia neonates (< 24 h old) were transferred to beakers containing 25 mL M4 media, and were exposed to same UV light intensity and conditions as adults, but at exposure times 0, 1.0, 2.5, and 5 min. The treatment groups of neonates (with nano-TiO_2) dispersed in M4 media (50 mg/L) were exposed at the same times and UV intensities, and then incubated for 48 h under the same conditions as adults.

2.2 Exposure of Daphnia to Macro-Sized TiO_2 (bulk).

To determine if macro-sized TiO_2 (bulk), has the same effect on Daphnia when exposed to UV as nano-TIO_2, a series of similar experiments were carried out as described above, but using bulk TiO_2 (Titanium IV oxide 99.8% anatase, size ≥ 45 μm. Sigma-Aldrich, USA), instead of nano-TiO_2. The bulk TiO_2 concentration, UV intensities, exposure times, and incubation conditions were the same used in the previous experiments. At the end of the 48 h incubation periods, the Daphnis (adults and neonates) were observed using a binocular stereoscopy (Leica Wild, M3Z0) to record the number of live organisms, or survival of each treatment. Other observations in the Daphnia, such as abnormal or slow motion, heart beat changes, molt increase, etc., were recorded (data not reported).

2.3 Toxic Effect of Nano-TiO_2 on *Daphnia magna*

In order to investigate if nano-TiO_2 is toxic to *Daphnia magna*, five groups of Daphnia per beaker were exposed to different concentrations (0, 25, 50, 75, and 100 mg/L) of nano-TiO_2 dissolved in M4 media, then incubated at the same conditions as in previous experiments. The survival (1/mortality) for each TiO_2 concentration was recorded after 48 h, then percentages of average mortality relative to control were calculated and data were plotted as survival vs. nano-TiO_2 concentration.

2.4 "Protective" Effect of Nano-TiO_2, Against UV Radiation

Daphnia (adults and neonates) were exposed to TiO_2 (50 mg/L) for 7 h, then rinsed three times with fresh M4 media to wash off the nano-TiO_2 and exposed to the UV light as described earlier when Daphnia (adults and neonates) were exposed to UV light in the absence of nano-TiO_2. After 48 h of incubation, the mortality was recorded, and then average mortalities were calculated for adults and neonates. The results were compared with those obtained in the experiments without TiO_2 to determine if TiO_2 protection is by exterior UV blocking or by internal cellular processes.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of nano-TiO_2 on adult and neonatal daphnia exposed to UV light

The mortality differences between organisms exposed to UV light with and without nano-TiO_2 are shown in (Fig. 1). As can be observed, the percent mortality is higher in the organisms without TiO_2 than those with TiO_2 at all exposure times. The statistic of data gave significant differences between treatments (Daphnia adults exposed to UV radiation in the presence and in absence of TiO_2). The following P values were obtained (P=.02, P=.00 and P=.01) for 5, 15 and 35 minutes respectively.

Similar results were obtained in the experiments with Daphnia neonates (Fig. 2). Data indicate that UV light is lethal to neonates in the absence of nano-TiO_2, with 100 % mortality in neonates exposed for 5 minutes to UV, whereas for those exposed for 2.5 minutes and 1 minute the mortalities were around 80 and 50 %, respectively. In contrast, in the presence of nano-TiO_2, at 5, 2.5 and 1 minutes of UV light exposure, percent mortalities were 87, 60 and 20% respectively. Significant differences were P =.00, P=.01 and P=.04 for treatment groups of 1, 2.5 and 5 minutes respectively.

3.1.2 Mortality Vs. nano-TiO_2 concentration

The *Daphnia magna* survival (1/mortality) data at different nano-TiO_2 concentrations is shown in (Fig. 3). As can be observed, the % of mortality was only 15% at 100 mg/L of nano-TiO_2; the highest exposure concentration assayed, and at this concentration, some nanoTiO_2 particles were observed into daphnia's bodies. No significant differences were observed into the range of concentration (P= .09, .11 and .08, for 25, 50 and 100 mg/L respectively).

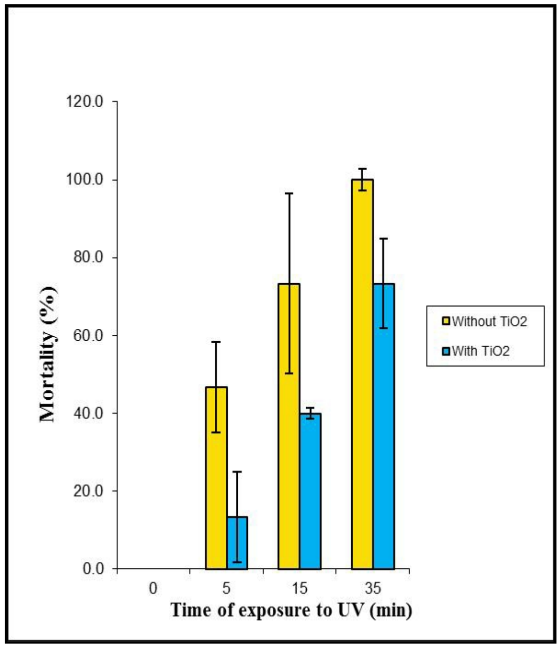

Fig. 1. Mortality differences between adult Daphnia exposed to UV light in presence and absence of nano-TiO_2 (50 mg/L)

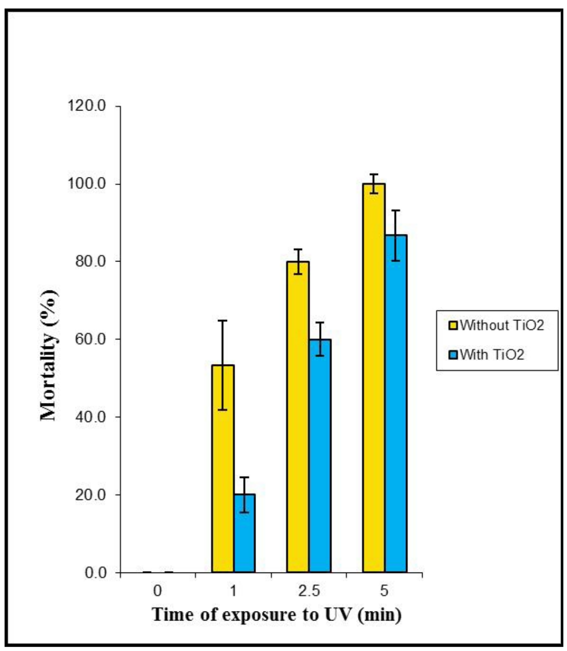

Fig. 2. Mortality differences between Daphnia neonates exposed to UV light in presence and absence of nano-TiO_2 (50 mg/L)

3.1.3 Effect of nano-TiO_2 against UV light on daphnia

In Figs. 4 and 5 are shown the results obtained from the experiments when Daphnia (adults and neonates) were pre-treated in nano-TiO_2 (50 mg/L) for 7 h, then rinsed three times with fresh M4 media to wash off the nano-TiO_2 and finally exposed to UV light. As can be observed, the mortalities are similar to those obtained in experiments without TiO_2. The statistic of data, gave not significant differences between pre-treated and without nano-TiO_2. The *P* values ranged from .09 to .17 at all exposure times.

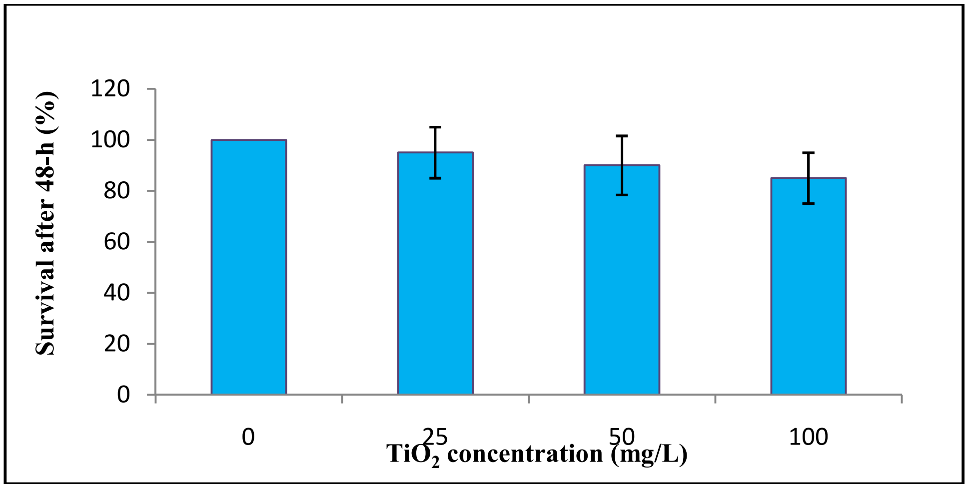

Fig. 3. Survival, (1/ Mortality) of Daphnis adults, exposed to different Concentrations of nano-TiO_2

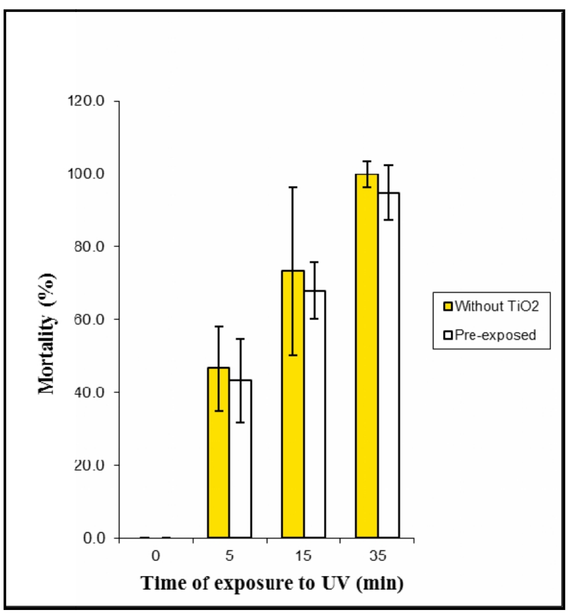

Fig. 4. Mortality differences between Daphnia adults exposed to UV light vs. adults pre-treated with nano-TiO_2 for 7h, then washing off the TiO_2 and exposed to UV light

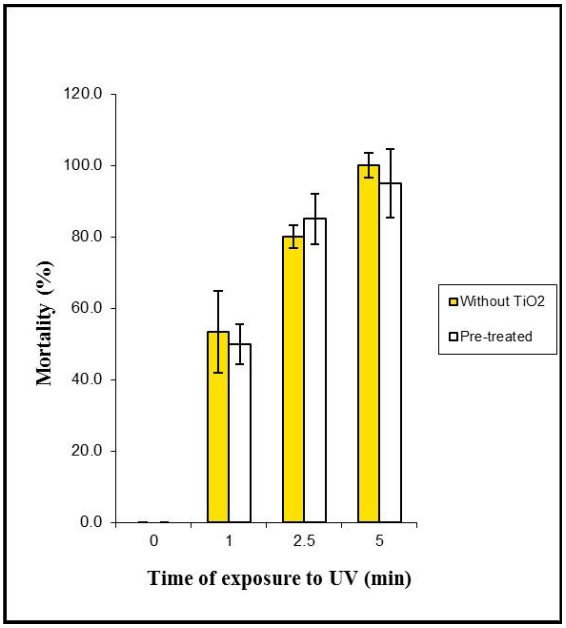

Fig. 5. Mortality differences between Daphnia neonates exposed to UV light vs. neonates pre-treated with nano-TiO_2 for 7 h, then washing off the TiO_2 and exposed to UV light

3.1.4 Particle Size effect of TiO_2 on daphnia

Fig. 6 shows the mortality differences between Daphnis exposed to UV light with TiO_2 bulk (50mg/L) and without TiO_2. As can be observed, the mortalities are very similar, and there are no significant differences (*P*=.09, *P*=.07 *P*=.45 and *P*=.24) at 0, 5, 15 and 35 minutes exposure respectively between Daphnis exposed to UV light with and without TiO_2 bulk.

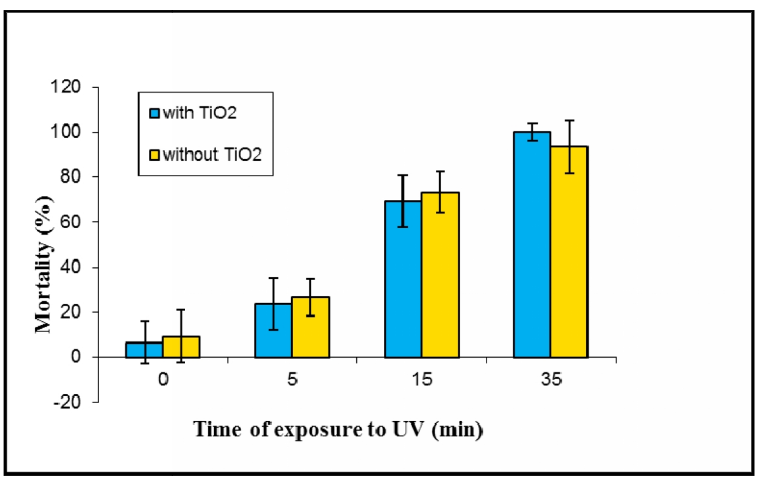

Fig. 6. Mortality of Daphnis exposed to UV light in the presence or absence of bulk TiO_2 (50 mg/L)

3.1.5 Statistical analyses

The SYSTAT software (Version 11, SPSS) was used to calculate average mortalities relative to control, and standard deviation (SD) for each experiment, also ANOVA tests were performed for the comparison between treatments, and a Fisher Least Significant Difference (LSD) with significant level at $P < .05$ was calculated for each experiment. Data are shown as graphs of mortality vs. exposure time, except when otherwise stated.

3.2 Discussion

The experimental results with Daphnia adults (Fig. 1) and neonates (Fig. 2) exposed to UV light in the presence or absence of nano-TiO_2, indicate that nano-TiO_2 can protect Daphnia to some degree against the lethal effects of UV light. Other studies have reported damage on Daphnia exposed to UV light, such as decreasing in respiration rates [17] and reduction in reproductive capacity [18]. However, this protection is limited to short durations of exposure, because the mortality differences between Daphnia in the presence and absence of nano-TiO_2 are less significant at longer exposure times (particularly in the neonates at 5 minutes). In other words, the nano-TiO_2 capacity to block UV radiation becomes reduced when exposure time increases. This assertion is reinforced with results from the experiments when Daphnia were first treated with nano-TiO_2 for 7 h, then TiO_2 washed off, and finally exposed to UV irradiation. In this case, the mortalities were similar to those obtained for Daphnia without TiO_2 (Figs. 4 and 5).

Previous studies have reported low toxicity of nano-TiO_2 on *Daphnia magna* at low concentration, in the (mg/L) range [19,20]. In this study, nano-TiO_2 caused low toxicity on Daphnia as shown in Fig. 3. When Daphnia were exposed to increasing concentrations (0 to100 mg/L) of nano-TiO_2, a maximum mortality rate of 15% was measured. The 15% mortality in this experiment could be due to nano-TiO_2, particularly when the contact with TiO_2 is long (48 h or more) or to other factors beyond the scope of this study. Other papers have reported nano-TiO_2 toxicity in *Daphnia magna* at the same concentration ranges and under similar experimental conditions [21,22]. They have reported EC_{50} values > 100 mg/L of TiO_2 at 48 h. Therefore, it can be concluded that nano-TiO_2, based on nominal concentrations used, exhibits low concern for this aquatic crustacean.

In all experiments with nano-TiO_2, after 24 h incubation (data not reported), Daphnia were covered with TiO_2 agglomerates, which probably block their gills, thereby reducing the respiration rate. Other authors have reported that *Daphnia magna* might ingest nano-TiO_2 from the aqueous suspension, and that the amount of nano-particles via filtration is higher than surrounding their bodies [19]. The same phenomenon was observed in the present study where TiO_2 was observed in the digestive tract and also in aqueous suspension. Therefore, it can be suggested that Daphnia ingests nano-TiO_2 during the experimental period (48 h). It was also found out that normal reproduction was evidenced because neonates were observed after 48 h incubation (data not reported). This observation reinforces the conclusion that nano-TiO_2 exhibits a low toxicity on *Daphnia magna*.

In a work with African clawed frogs exposed to UV radiation, the authors reported that nano-TiO_2 exhibit stronger photochemical oxidation/reduction capacity compared with their bulk counterparts, i.e., the interaction with UV light strongly depends on particle size [23]. Other authors claim that protection mechanisms against UV radiation could be due to the presence of organic carbon particles in the water or inside the organism's body, by interaction between sensitive molecules e.g. flavins, reduced pyridine nucleotides and photons [24]. In this study, even though nano-TiO_2 was found in the interior and exterior of the body of Daphnia, the principal protection was by the exterior, as could be observed from the experiments when Daphnis were pre-treated with TiO_2 solution for 7 h, then TiO_2 rinsed off, and organisms exposed to UV light (Figs. 4 and 5). Other experiments with the cladoceran Daphnia have shown that organisms react to UV radiation through negative phototaxis, migrating toward deeper water [25], but these UV protection movements often negatively affect Daphnia because they are forced to move to water where food is scarce and physiological functions occur at slower rates. In the present study, a similar behavior was observed in Daphnia. During exposure to UV light, the organisms moved to the bottom of the beakers. Also, slower movements and decreased heart rates were observed (data not reported here), which may indicate a reduction of physiological functions.

4. CONCLUSION

Nano-TiO_2 is a nanomaterial capable to blocking UV radiation; however, if its residues at elevated concentration reach aquatic ecosystems, could be toxic for aquatic organisms, since the highest concentration (100 mg/L) used in this work, caused mortalities (15%) in *Daphnia magna*. Also, the experiment with adult Daphnia exposed to UV light with and without bulk TiO_2, shown no significant differences in mortality; therefore the protection against UV is related to particle size of TiO_2. In abstract, from the results of this work, it is possible to conclude that protective effect of nano-TiO_2 in Daphnis exposed to UV radiation is in function of exposure time, the particle size and concentration of TiO_2.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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