Journal of Materials Science and Engineering A 11 (10-12) (2021) 107-113
doi: 10.17265/2161-6213/2021.10-12.001

Aquatic Toxicity Studies of Titanium Dioxide and Silver Nanoparticles Using *Artemia franciscana* Nauplii and *Daphnia magna*

Jocelyn P. Reyes[1], John Darryl B. Lagdameo[2], Josefina R. Celorico[1], Ronaniel A. Almeda[2,3], Milagros M. Peralta[2,4] and Blessie A. Basilia[1]

1. Department of Science and Technology, Industrial Technology Development Institute (DOST-ITDI), Gen. Santos Avenue, Taguig City 1631, Philippines

2. Nanotechnology Program, University of the Philippines Los Baños, College, Laguna 4031, Philippines

3. Agricultural Bio-Process Division, Institute of Agro-Industrial Engineering, College of Engineering and Agro-Industrial Technology, University of the Philippines Los Baños, College, Laguna 4031, Philippines

4. Institute of Chemistry, College of Arts and Sciences, University of the Philippines, Los Baños (UPLB), Laguna 4031, Philippines

Abstract: Manufactured nanomaterials are expected to enter the environment due to the increasing number of productions which results in anthropological discharges coming from different effluents and seepages. This event poses potential threat to the environment especially in the aquatic systems. TiO_2 (titanium dioxide) and AgNPs (silver nanoparticles) have significant potential in antibacterial and antiparasitic applications, but despite their significant potential, the toxicity of metal oxide nanoparticles such as TiO_2 and AgNPs restricts their use especially in humans due to their toxicity. In this study, the behavior and toxicity of TiO_2 and AgNPs were investigated in aquatic system using *Artemia franciscana* nauplii and *Daphnia magna*. Nauplii and Daphnia were exposed to TiO_2 and AgNP dispersions at different concentrations. The mortality rates of the nauplii and daphnia were monitored at 6, 24, and 48 h after its exposure. Saltwater results showed that AgNP is highly toxic to the test organisms while TiO_2 was non-toxic after 48 h of exposure. For freshwater, 100% mortality rate on neonates was obtained from the AgNPs dispersion during the first 6 h of exposure while the mortality rate in TiO_2 dispersion was 85% at 100 ppm after 48 h of exposure.

Key words: Toxicity, risk-assessment, saltwater, freshwater, *Artemia franciscana* nauplii, *Daphnia magna.*

1. Introduction

Nanotechnology is the science of controlling the size and structure up to the nanometer scale. It is an emerging field of applied science and technology which has been used in the discoveries and innovations in agriculture, pharmaceuticals, cosmetics, textile industry, water treatment, and electronics [1, 2]. Manufactured nanomaterials have been extensively employed in a number of products because of its unique dimension and their physicochemical properties that enhance the strength and performance of the material [3]. Due to their nanometer size, nanomaterials behave differently from the bulk material counterparts in terms of their electronic states, magnetic and optical properties, as well as their catalytic reactivities. They have high specific surface area which gives rise to high surface reactivity that gives them advantage over the bulk materials [4].

Over the years, the global production of nanomaterials rose continuously and is expected to grow over a half a million tons in the coming years. With the increasing production of nanomaterials, there are growing concerns on its implications when they enter the environment. The human health and the

Corresponding author: Jocelyn P. Reyes, M.Sc., engineer, science research specialist, research fields: nanotechnology and nanosafety.

environment are possibly at risk. Information on the toxicity, risk assessment, and safety of nanomaterials is still limited. Upon its release in the environment, the fate and behavior of nanomaterials in various conditions (i.e., salinity, pH, temperature, etc.) must be deeply understood in order to promote the nanomaterials' sustainable application.

The aquatic system serves as a reservoir of anthropogenic discharges coming from different effluent seepages. It is the most probable area that will be greatly affected by the release of nanomaterials in the environment. The presence of nanomaterials and its effect on the aquatic organisms remain largely undocumented, although it can be considered as a potential threat in the aquatic environment. To better understand the effect of nanomaterials in aquatic systems, toxicity assessments must be conducted. This will help provide information on the toxic effect of a nanomaterial in aquatic systems. *Artemianauplii* sp. and *Daphnia magna* sp. are a useful model in investigating the toxicity of nanomaterials in salt and fresh water environment. These organisms are non-selective filter-feeders that can be easily cultured but are highly vulnerable to many test materials [5].

This study aims to determine the possible behavior of TiO_2 (titanium dioxide) and AgNPs (silver nanoparticles) in the aquatic environment and to investigate its toxicity using the test organism *Artemia franciscana* nauplii and *Daphnia magna*.

2. Methodology

2.1 Materials

TiO_2 with diameter of 21 nm, minimum assay of 99.5%, Product CAS No. 13463-67-7 purchased from Sigma Aldrich and AgNPs with diameter of 60 nm, minimum assay of 99.9%, Product CAS No. 7440-22-4 purchased from Sisco Research Laboratories Pvt., Ltd. were used to measure the size dependent aquatic toxicity.

The reagents used for making the artificial seawater were provided by the UPLB (University of the Philippines, Los Banos) Nanoscience and Technology Faculty Analytical and Instrumentation Service Laboratory.

2.2 Characterization of TiO_2 and AgNP

2.2.1 XRD (X-Ray Diffraction)

XRD of nanomaterials was analyzed using Shimadzu XRD-6000 X-ray diffractometer with a copper target, X-ray tube at 40 kW with a scan rate of 2° per minute and scan range of 2° to 90°. X' Pert High Score Plus software with crystalline phase International Center for Diffraction Data Powder Diffraction File (ICDD PDF4$^+$) database for inorganic analysis was used for the post-run analysis of the data collected.

2.2.2 DLS (Dynamic Light Scattering)

The hydrodynamic diameter of the manufactured nanomaterials was determined using Malvern Zetasizer Nano-ZS90 and the analysis was based on ISO 22142:2017. The dispersion medium used was 0.5% PEG (polyethylene glycol) in ethanol. The nanomaterial concentration used in this study was 0.05% *w/v*. Prior to the analysis, the nanomaterial dispersions were bath-sonicated for 15 min.

2.2.3 TEM (Transmission Electron Microscopy)

Particle size, shape and morphology were determined using TEM JEOL JEM-2100F Field Emission Electron Microscope. This is a very powerful tool for the determination of the mean particle diameter of the nanomaterials.

2.2.4 Aggregation Studies

The aggregation of the nanomaterial dispersion was conducted in this study. The concentration used in the aggregation analysis of the manufactured nanomaterials was 50 ppm concentration. Each nanomaterial was dispersed in artificial water. The nanomaterial dispersion was then bath-sonicated for 15 min. The hydrodynamic diameter and count rate of the nanomaterial dispersion were monitored for 0, 6, 24, and 48 h.

2.3 Aquatic Toxicity Studies

2.3.1 Saltwater/Seawater

The toxicity of nanomaterials in *Artemia franciscana* nauplii was studied in this experiment.

Artemia franciscana cysts were first washed, incubated, and aerated for 24 h in artificial seawater. Once the cysts had hatched, the *nauplii* were transferred to exposure wells that contained nanomaterials with different concentrations. Each exposure well holds ten freshly hatched *nauplii*. The concentrations used for this experiment were as follows: 0.5, 1, 5, 10, 20, 50, and 100 ppm. For each nanomaterial concentration, three test organisms necessary to conduct this study were less than 24-h old *Artemia franciscana* nauplii. First, approximately 100 mg of *Artemia franciscana* cysts were decapsulated for 30 min using sodium hypochlorite solution. It was then washed with distilled water. After decapsulation, the cysts were incubated in 100 mL artificial seawater and were aerated overnight. When the cysts had hatched, the *Artemia franciscana* nauplii were transferred to exposure wells containing the nanomaterial dispersion at different concentrations (0.5, 1, 5, 10, 20, 50, and 100 ppm). For each exposure well, ten *nauplii* were held. For the nanomaterial dispersion concentration, three exposure wells were prepared. The mortality rate of the *nauplii* in each exposure well was monitored at 6, 24, and 48 h of exposure. The mortality rate was calculated using the formula presented in Eq. (1). The *nauplii* were not fed for the whole duration of the experiment.

$$\text{Percent mortality} = \frac{\text{number of dead nauplii}}{\text{initial number of live nauplii}} \times 100 \quad (1)$$

2.3.2 Freshwater

Stock solutions of the sample materials (potassium dichromate, TiO_2 and AgNP) were first prepared and each of the sample was sonicated for 15 min to allow dispersion. After sonication, the sample material was placed in the test vessels in preparation for the toxicity assay. Four exposure wells were prepared for this experiment. For each exposure well, five neonates of *Daphnia magna* were exposed in different concentrations (0.5-100 ppm) of the sample materials. The mortality of the neonates was monitored for 6, 24, and 48 h of exposure. Neonates were not fed on the duration of the experiment. Potassium dichromate was used as a positive control in the experiment.

3. Results and Discussion

3.1 Characterization Studies

3.1.1 XRD

Fig. 1 shows the XRD pattern of TiO_2 and AgNP. Distinct diffraction patterns were observed on each manufactured nanomaterial. Based on the results, TiO_2 presented a sharp and well-defined peak. Peaks with sharp and well-defined peaks mostly are indicative of materials with high crystallinity. AgNP also possessed characteristic peaks similar to its crystalline forms.

The XRD pattern of AgNP is almost similar to the pattern seen in literature [7]. AgNP has a face-centered cubic structure.

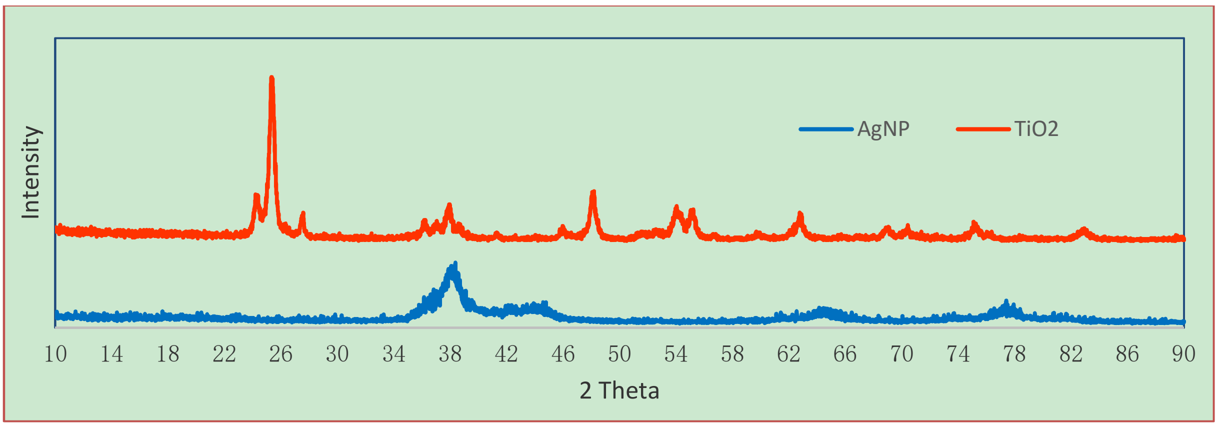


Fig. 1 XRD pattern of TiO_2 and AgNP.

The characteristic peaks of AgNP can be seen at 38.19, 44.37, 64.56, and 77.47 which corresponds to (111), (200), (220), and (311) crystallographic planes, respectively [6]. Thus, AgNP has a crystalline nature.

Sharp peaks were observed on the XRD pattern of TiO_2. It was found that the nanomaterial has a tetragonal structure and has anatase phase. Peaks found at 25° and 48° confirm the anatase phase of TiO_2 [7]. Sharp peak in TiO_2 diffractogram is an indication that the nanomaterial is crystalline.

3.1.2 DLS

Table 1 presents a summary of the hydrodynamic diameter and polydispersity index of TiO_2 and AgNP. Based on Malvern Instruments, the hydrodynamic diameter is defined as "the size of a hypothetical hard sphere that diffuses in the same fashion as that of the particle being measured." The value of the hydrodynamic diameter that is being measured in DLS refers to the diffusion of particles in the fluid.

According to Bhaatcharjee [8], the intensity of light scattered by particles with different sizes is described by the polydispersity index. This can be calculated by $(\text{width/mean})^2$ for each peak presented in the intensity distribution plot. As reported also by Bhaatcharjee [8], a sample is said to be highly monodisperse if its polydispersity index is less than 0.1. Meanwhile, samples which are categorized as moderately polydisperse have a polydispersity index value ranging from 0.1-0.4. Lastly, samples classified as highly polydisperse have a polydispersity index greater than 0.4. Results showed that the TiO_2 and AgNP are moderately dispersed based on their relative polydispersity indices.

3.1.3 TEM

Fig. 2 presents the TEM images of TiO_2 and AgNP. Based on the results of TEM, nano TiO_2 and AgNP have a spherical structure with diameter of 30 nm and 25 nm respectively.

3.1.3 Aggregation Studies

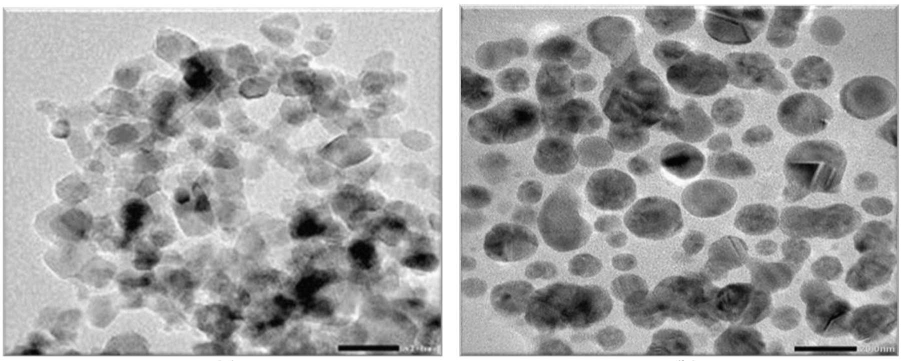
The behavior of TiO_2 and AgNP in artificial seawater was studied. Table 2 presents the hydrodynamic diameter of TiO_2 and AgNP. It was observed that these nanomaterials have high values of hydrodynamic diameter in artificial seawater. Given the ionic nature of artificial seawater, nanoparticles tend to aggregate faster. The aggregation and agglomeration of the nanomaterials can be expected from the saline environment since surface interactions between the nanomaterials are affected by the ions present in the dispersion medium.

Table 1 Hydrodynamic size and polydispersity index.

Sample	Hydrodynamic size (nm)	Polydispersity index
TiO_2	158.3 ± 11.3	0.185 ± 0.051
AgNP	153.7 ± 1.5	0.399 ± 0.054

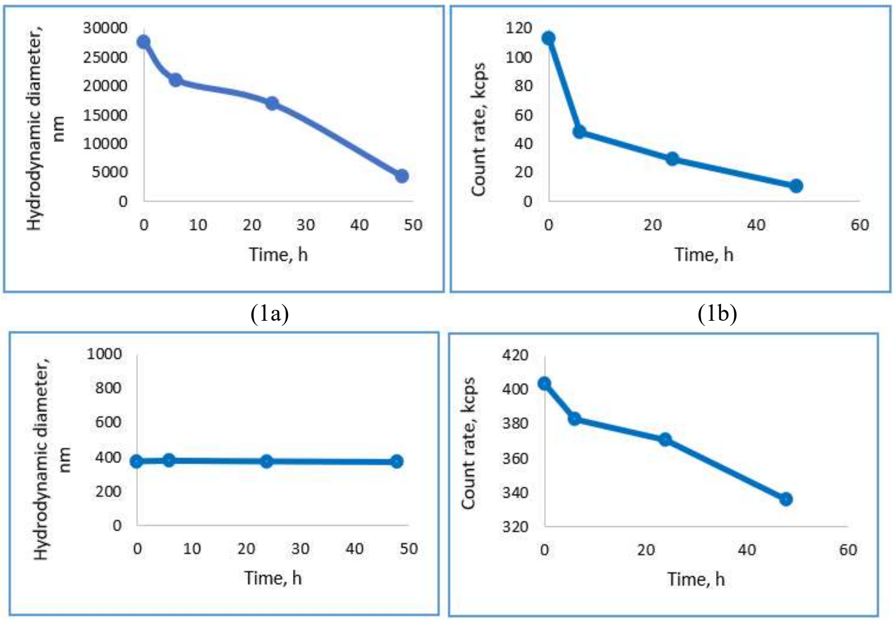
Table 2 Hydrodynamic diameter of TiO_2 and AgNP monitored for 0, 6, 24, and 48 h.

Time (h)	Hydrodynamic diameter (nm)	
	TiO_2	AgNP
0	27,510 ± 1,638	374.4 ± 8.4
6	21,030 ± 1,694	377.3 ± 4.7
24	16,870 ± 3,744	374.3 ± 6.2
48	4,356 ± 625.6	370.6 ± 11.1



(a) (b)

Fig. 2 TEM image of (a) TiO_2 and (b) AgNP.



(2a) (2b)

Fig. 3 Aggregation profile of (1) TiO_2 and (2) AgNP in terms of (a) hydrodynamic size and (b) count rate against time.

Fig. 3 illustrates the behavior of TiO_2 and AgNP in artificial seawater. Results showed that there is a decrease in the hydrodynamic diameter of TiO_2 during the first 6 h of observation which continued until 48 h of monitoring. The sedimentation of nanoparticles down the test vessel is a possible reason for the decreased hydrodynamic size. Larger particles settled at the bottom of the vessel while smaller particles remained suspended in the aqueous environment. Thus, it is possible that the observed values on the hydrodynamic size were attributed to the small particles suspended in the dispersion medium.

However, based on the count rate of the nanomaterials under observation, a gradual decrease in number of suspended nanomaterials was observed until the end of the experiment. The progression of the sedimentation of nanomaterial can be observed for the next 48 h of monitoring the behavior of the nanoparticles in artificial seawater. Since monovalent and divalent ions are present in artificial seawater, partial saturation of the nanoparticle surface occurs. There is a compression in the electrical double layer on the nanoparticle surface, leading to the aggregation and further sedimentation of the nanoparticles in the highly ionic nature of the dispersion medium [9-11].

It is important to thoroughly characterize the behavior of the nanomaterial dispersion in conducting ecotoxicity studies to be able to determine the toxic effect of the nanomaterial on the test organisms. Based on the data collected, it can be speculated that the sedimentation of nanomaterial may lessen the level of exposure on the test organisms. The number of suspended nanomaterials on the dispersion medium has reduced gradually resulting in a limited interaction between the dispersed nanomaterial and the test organism.

3.2 Aquatic Toxicity Studies

3.2.1 Salt/Sea Water

Fig. 4 shows the graphical representation of the mortality rate and concentration of *Artemia franciscana* nauplii exposed to TiO_2 and AgNP dispersion for 6, 24 and 48 h. A high mortality rate was observed for AgNPs especially at high concentrations. Results showed that the maximum mortality rate of *nauplii* in the AgNP dispersion is 100% at 50 ppm concentration after 48 h of exposure. The second highest mortality

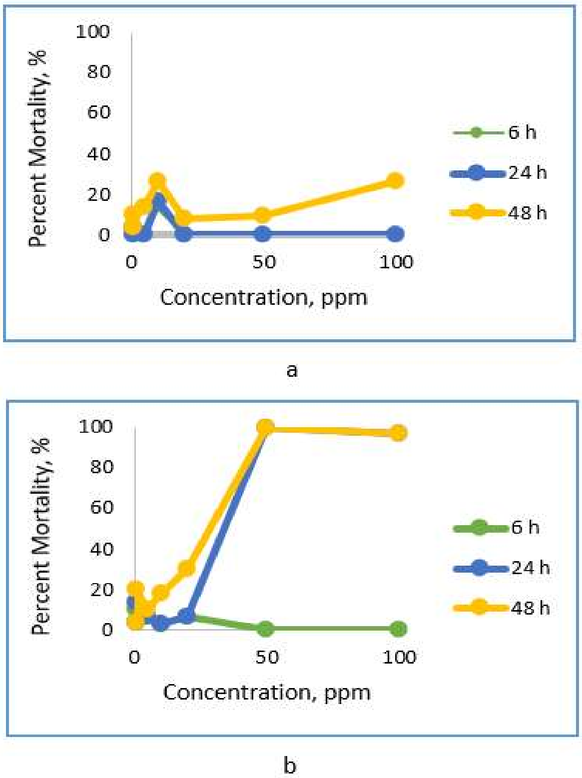


Fig. 4 The graphical representation of the mortality rate vs. concentration of *Artemia franciscana* nauplii exposed to (a) TiO_2 and (b) AgNP dispersion for 6, 24, and 48 h.

rate was 96.67% which can be found at 100 ppm concentration. The EC_{50} after 48 h of exposure in AgNP dispersion was 24.287 ppm. The high mortality rate in AgNP dispersion can be due to the toxic property of AgNP itself. According to Morelli et al. [11], AgNP owes its antimicrobial property to the dissolution of the nanoparticle; it is transformed from Ag to Ag^+ upon its dissolution in the aquatic medium. Thus, AgNP can be a potential toxic pollutant when released to the aquatic environment.

3.2.2 Fresh Water

Fig. 5 illustrates the graphical representation of the mortality rate of the neonates against the different concentrations of TiO_2 and AgNP dispersion. A 100% mortality rate on neonates was obtained from the AgNP dispersion during the first 6 h of exposure. This shows that at the given range of concentration of the nanomaterial dispersion, AgNP is lethal to the test organisms. Thus, it is highly recommended to lower down the concentration of nanomaterial dispersion in order to determine the EC_{50} of AgNP and to prolong the observation on the toxicity of AgNPs within the given exposure time.

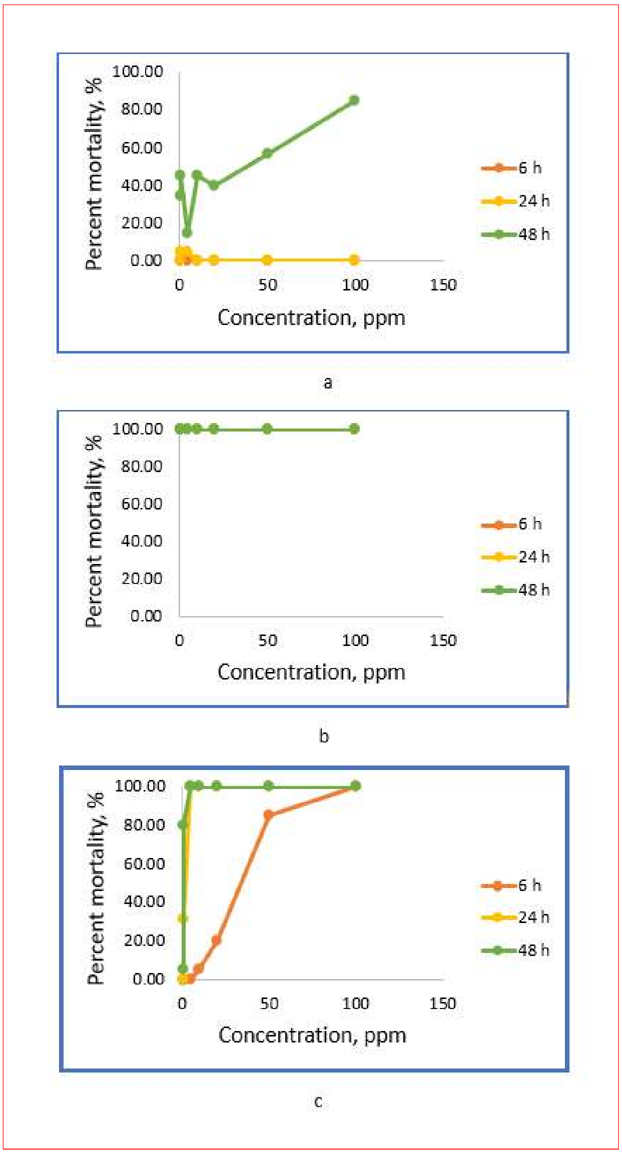


Fig. 5 The mortality rate of *Daphnia magna* in (a) TiO_2, (b) AgNP and (c) potassium dichromate dispersion.

On the other hand, more than 50% of the neonates have died in TiO_2 dispersion. The maximum mortality rate of neonates in TiO_2 dispersion was 85% at 100 ppm after 48 h of exposure.

The validity of the test assay can be assessed based on the mortality of the *Daphnia magna* neonates in the control groups. In the blank control group, zero mortality rate was observed. This means that the water did not contribute to the mortality of the neonates. On the other hand, a positive control group was also conducted using potassium dichromate. In Fig. 4c, results showed that potassium dichromate is lethal to the neonates after several hours of exposure. Potassium dichromate is a common reagent used as a positive control in toxicity assay due to its consistent and reliable results in the mortality of the test organisms.

4. Conclusion

Nanomaterials are novel materials that are widely employed in various products in order to enhance its properties and improve its quality. The widespread production of nanomaterials may result in its release in the environment, especially during its production and disposal. Its presence in the environment can lead to potential threat in human health and different organisms. By conducting toxicity and risk assessments on TiO_2 and AgNPs, the sustainability of its application was achieved.

In this study, the behavior and toxicity of TiO_2 and AgNPs were investigated in aquatic system using *Artemia franciscana* nauplii and *Daphnia magna*. *Nauplii* and *Daphnia* were exposed to TiO_2 and AgNP dispersions at different concentrations. The mortality rates of the *nauplii* and *daphnia* were monitored at 6, 24, and 48 h after its exposure. Saltwater results showed that AgNP is highly toxic to the test organisms while TiO_2 was non-toxic after 48 h of exposure. For freshwater, 100% mortality rate on neonates was obtained from the AgNP dispersion during the first 6 h of exposure while the mortality rate in TiO_2 dispersion was 85% at 100 ppm after 48 h of exposure.

Acknowledgement

This research project would not have been possible without the funding from the Department of Science and Technology, Philippine Council for Industry, Energy and Emerging Research and Development Grant in Aid (DOST-PCIEERD-GIA).

References

[1] Arulvasu, C., Jennifer, S. M., Prabhu, D., and Chandhirasekar, D. 2014. “Toxicity Effect of Silver Nanoparticles in Brine Shrimp Artemia.” *Scientific World Journal*. Volume 2014, Article ID 256919. doi: 10.1155/2014/256919.

[2] Schmid, K., and Riediker, M. 2008. “Use of Nanoparticles in Swiss Industry: A Targeted Survey.” *Environmental Science Technology* 42: 2253-60.

[3] Maurer-Jones, M. A., Gunsolus, I. L., Murphy, C. J., and Haynes, C. L. 2013. “Toxicity of Engineered Nanoparticles in the Environment.” *Analytical Chemistry* 85: 3036-49.

[4] Handy, R. D., von der Kammer, F., Lead, J. R., Hassellöv, M., Owen, R., and Crane, M. 2008. “The Ecotoxicology and Chemistry of Manufactured Nanoparticles.” *Ecotoxicology* 17: 287-314.

[5] Rekulapally, R., Chavali, L. N. M., Idris, M. M., and Singh, S. 2018. “Toxicity of TiO_2, SiO_2, ZnO, CuO, Au and Ag Engineered Nanoparticles on Hatching and Early Nauplii of *Artemia* sp.” *Peer J.* 6: e6138. doi: 10.7717/peerj.6138.

[6] Anandalakshmi, K., Venugobal, J., and Ramasamy, V. 2016. “Characterization of Silver Nanoparticles by Green Synthesis Method Using *Pedalium murex* Leaf Extract and Their Antibacterial Activity.” *Appl. Nanosci.* 6: 399-408.

[7] Theivasanthi, T., and Alagar, M. 2013. “Titanium Dioxide (TiO_2) Nanoparticles XRD Analyses: An Insight.” *Chemical Physics* 1307: 1091.

[8] Bhaatcharjee, S. 2016. “DLS and Zeta Potential—What They Are and What They Are Not?” *Journal of Controlled Release* 235: 337-51.

[9] Keller, A. A., Wang, H., Zhou, D., Lenihan, H. S., Cherr, G., Cardinale, B. J., Miller, R., and Ji, Z. 2010. “Stability of Metal Oxide Nanoparticles in Natural Aqueous Matrices.” *Envi. Sci. Technol*. 44: 1962-7.

[10] 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. Nanopart. Res.* 18: 113.

[11] Morelli, E., Gabellieri, E., Bonomini, A., Tognotti, D., Grassi, G., and Corsi, I. 2018. “TiO_2 Nanoparticles in Seawater: Aggregation and Interactions with the Green Alga *Dunaliella tertiolecta*.” *Ecotoxicology and Environmental Safety* 148: 184-193.