UNIVERSITI PUTRA MALAYSIA

TOXICITY ASSESSMENT OF REDUCED GRAPHENE OXIDE AND TITANIUM DIOXIDE NANOPARTICLES ON GROWTH OF MICROORGANISMS

NURUL SHAHIDAH AHMAD

FK 2019 141

TOXICITY ASSESSMENT OF REDUCED GRAPHENE OXIDE AND TITANIUM DIOXIDE NANOPARTICLES ON GROWTH OF MICROORGANISMS

By

NURUL SHAHIDAH BINTI AHMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science

June 2018

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

TOXICITY ASSESSMENT OF REDUCED GRAPHENE OXIDE AND TITANIUM DIOXIDE NANOPARTICLES ON GROWTH OF MICROORGANISMS

By

NURUL SHAHIDAH BINTI AHMAD

June 2018

Chairman : Norhafizah binti Abdullah, PhD
Faculty : Engineering

Increasing use of nanoparticles (NPs) for several purposes including cosmetics, paints, plastics, and textiles led to their released into environment. This scenario raises a concern toward potential of toxic effects. To date, access to the toxicity data for most manufactured NPs are limited. Hence, the aim of this study is to investigate the toxicity of NPs on living microbial culture. Prior to that, a simple and fast technique of microbial cell viability quantification was developed. This technique was used in assessing toxicity effect of microbial culture when they are exposed to NPs. The study was focused on reduced graphene oxide (rGO) and titanium dioxide (TiO_2) in anatase and rutile forms. *Escherichia coli*, *Bacillus subtilis*, and *Candida albican* were used as the test models to represent Gram-negative, Gram-positive, and yeast culture, respectively. Three microbial quantification techniques were assessed, which are turbidimetric measurement using spectrophotometer, plate count method to enumerate the colony forming units, and direct microscopic count using trypan blue dye that differentiate between viable and dead cells. The latter technique was found to be ideal for fast, easy, non-destructive, economical method and can be used for on-site measurement on viable cell count and thus was used for the subsequent part of this work. Anatase TiO_2 gave the highest toxicity effect among other NPs towards all test models, followed by rGO and rutile TiO_2. At 100 µg/mL of anatase exposure for 96 hours of incubation time, it inhibits the growth of *E. coli*, *B. subtilis*, and *C. albican* by 75%, 73%, and 65%, respectively. All microbial cells were inhibited and *E. coli* was found to be the most sensitive towards NPs. In brief, exposure to NPs not only alter the growth rate (µ) value and cause the loss in cell viability, but it affect the onset and length of growth phases such as shorten the log phase and accelerate the onset of deceleration phase, to name a few. Higher dosage and incubation time of NPs increases their toxicity. Cells were suffered from morphological changes

as it was exposed to NPs and this correlates well with the results showing a culture with altered growth phase. NPs did not penetrate into cell membrane, but only deposited at the cell surface.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENILAIAN TERHADAP KETOKSIKAN NANOZARAH KEKURANGAN GRAPHENE OKSIDA DAN TITANIUM DIOKSIDA KEPADA PERTUMBUHAN MIKROORGANISMA

Oleh

NURUL SHAHIDAH BINTI AHMAD

Jun 2018

Chairman : Norhafizah binti Abdullah, PhD
Faculty : Kejuruteraan

Peningkatan terhadap penggunaan nanopartikel (NP) untuk beberapa tujuan termasuk kosmetik, cat, plastik, dan tekstil membawa kepada pembebasan mereka kepada alam sekitar. Senario ini menimbulkan kebimbangan terhadap potensi kesan toksik terhadap alam sekitar. Sehingga kini, akses kepada data ketoksikan bagi kebanyakan NP yang dihasilkan adalah terhad. Oleh itu, matlamat kajian ini adalah untuk mengkaji ketoksikan NP terhadap mikrobiologi hidup. Sebelum itu, teknik yang mudah dan pantas untuk kuantiti pemantauan mikrobiologi hidup telah dibangunkan. Teknik ini digunakan dalam menilai kesan ketoksikan mikribiologi hidup apabila mereka terdedah kepada NP. Kajian ini difokuskan kepada graphene kekurangan oksida (rGO) dan titanium dioksida (TiO_2) dalam bentuk anatase dan rutil. *Escherichia coli*, *Bacillus subtilis*, dan *Candida albican* masing-masing digunakan sebagai model ujian untuk mewakili Gram-negatif, Gram-positif, dan yis. Tiga teknik pengiraan mikrobiologi hidup telah dinilai, iaitu pengukuran kekeruhan menggunakan spektrofotometer, kaedah pengiraan plat untuk menghitung unit pembentukan jajahan, dan kiraan mikroskopik langsung menggunakan pewarna biru trypan yang membezakan antara sel hidup dan mati. Teknik yang terakhir ini didapati sesuai kerana ianya merupakan kaedah yang cepat, mudah, tidak merosakkan, dan ekonomik dan seterusnya akan digunakan sepanjang kajian ini dijalankan. Anatase TiO_2 memberi kesan ketoksikan tertinggi dalam kalangan NP yang lain ke atas semua model ujian, diikuti oleh rGO dan rutil TiO_2. Pada 100 µg / mL pendedahan anatase selama 96 jam masa pengeraman, ia menghalang pertumbuhan *E. coli*, *B. subtilis*, dan *C. albican* masing-masing sebanyak 75%, 73%, dan 65%. Semua mikrobioligi hidup telah terjejas dan *E. coli* didapati paling sensitif terhadap NP. Ringkasnya, pendedahan kepada NPs bukan sahaja mengubah nilai pertumbuhan (μ) dan menyebabkan kehilangan dalam daya tahan sel, tetapi ia memberi kesan

kepada fasa pertumbuhan sel seperti memendekkan fasa kehidupan dan mempercepatkan fasa kematian. Dos dan masa pengeraman yang lebih tinggi akan meningkatkan kesan ketoksikannya. Sel-sel telah mengalami perubahan morfologi kerana ia terdedah kepada NP dan ini bertepatan dengan hasil yang menunjukkan sel dan fasa pertumbuhan yang berubah. NP tidak berjaya menembusi sel membran, tetapi hanya melekat di permukaan sel.

ACKNOWLEDGEMENT

First of all, I am grateful to The Almighty Allah for letting me to complete this research. I would like to express my deepest gratitude and appreciation to my project supervisor and committee, Assoc. Prof. Dr. Norhafizah binti Abdullah and Dr. Faizah binti Md Yasin for the guidance, supervision, and also encouragement throughout this research in term of experimental work as well as thesis writing. Their constructive critism and suggestion provide me strength to complete this work despite several obstacles encountered throughout this research. I also would like to thanks to staffs and technician in Biochemical and Material Characterization Laboratories, Universiti Putra Malaysia as well as Bioscience Institute for their guidance and help when I am conducting my research. I am extremely indebted to them for their expert, sincere and valuable guidance and encouragement extended to me.

My thanks and appreciations also go to my parents, Hj Ahmad bin Hj Mohd Nor and Hjh Rahimah binti Hj Jahari for their constant moral support, prayers, understanding, and concern which helped me to achieve success in every sphere of life and without their kind devotion, this thesis would have been a sheer dream. Also, thank you mom and dad for providing me financial support throughout this journey. I am also thankful to my siblings for their encouragement during this research work. Their criticism and advices at the same time made me stronger and eager to complete my Master journey. Also, thanks to my late brother Allahyarham Mohd Kamal Ariffin bin Hj Ahmad who become my inspiration to continue my study. I will try my very best to fulfill your dreams. Our dreams. May Allah grant you the best place in Jannah. You will always in my heart, bro. YNWA.

I also want to take this opportunity to express my most profound gratitude to all my dearest friends that gave me moral support and encouragement during the research period. Deepest thank you to my lovely cockroaches, Nor Shaerah binti Abd Rasid and Amanina Amani binti Kamarul Zaman for lending their shoulders for me to cry on, their ears to listen to, their hands for me to hold, their wings for me to fly, and their feet to walk with me whenever my research went wrong and driven me to the right path whenever I lost in my own world. My special thanks also go to my lab mates who are willing to share knowledge as well as apparatus and chemical with me. Also, thanks to my roommates who are always hear to my story about my research even though we are in different fields. Thank you for spending your precious time with me. Hopefully they will be able to complete their research too. Keep up your great work, mates.

I sincerely acknowledge the efforts of all those who have directly and indirectly helped me in completing my thesis successfully. This thesis would not be possible without the support from all of them. THANK YOU!

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Norhafizah binti Abdullah, PhD
Associate Professor
Faculty of Engineering
University Putra Malaysia
(Chairman)

Faizah binti Md Yasin, PhD
Senior Lecturer
Faculty of Engineering
University Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 17 October 2019

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotation, illustration and citation have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceeding, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: ______________________________ Date: _______________

Name and Matric No.: Nurul Shahidah binti Ahmad (GS43807)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of the thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhere to.

Signature: ____________________________

Name of Chairman of Supervisory Committee: Assoc. Prof. Dr. Norhafizah binti Abdullah

Signature: ____________________________

Name of Member of Supervisory Committee: Dr. Faizah binti Md Yasin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CFU	Colony forming unit
DNA	Deoxyribonucleic acid
EDX	Electron dispersive x-ray
GFN	Graphene family nanoparticles
GO	Graphene oxide
LB	Luria-Bertani
NA	Nutrient agar
NPs	Nanoparticles
OD	Optical density
rGO	Reduced graphene oxide
ROS	Reactive oxygen species
SEM	Scanning electron microscope
TEM	Transmission electron microscope
TiO_2	Titanium dioxide
τ_d	Doubling time
k_d	Specific death rate
μ_g	Specific growth rate

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nanoparticles (NPs) exist in various structures and shapes for instance in needles, whiskers, spheres, plates, tubes, and sheets with size ranging between 1 to 100 nm. From the toxicological perspective, surface area and particle size are very important. The shape and size of NPs can contribute to the commencement of cytotoxicity, for instance multi-wall nanotubes are less toxic than single-wall nanotubes (Jia *et al*., 2005; Kang *et al*., 2008). NPs are unique and have many interesting properties that are useful in a diverse biomedical and biological system and find its way into environment (Seetharam and Sridhar, 2007). Some NPs form agglomerates and aggregates under ambient condition by fusing and deposition from their bulk component. By interparticle interaction, primary free NPs form agglomerated particles which form a collection of particles that are attached together by strong and weak forces including sintered bonds, van der Waals forces, and electrostatic forces. NPs suspended in liquid have fewer tendencies to stick to each other as compared in the solid or gas form (Oberdörster *et al*., 2005).

NPs can be classified into four types which are inorganic (all metals and metal oxide NPs), organic (polymeric and biologically compatible NPs), carbon-based (CNTs, graphene, carbon black, carbon rod, etc), and organic-inorganic hybrid NPs. The behavior of NPs are based on the susceptibility and solubility to degradation and neither the particle size nor chemical composition to remain constant over time. The increase entry of NPs into living cells membrane may cause cellular toxicities at various levels including damage to deoxyribonucleic acid (DNA), protein, and lipid. Researchers have confirmed that metallic NPs can pass through to the cell membrane or remain attached on it (Feng *et al*., 2000; Sondi and Salopek, 2004). Cell lost their cellular integrity, with cell membrane being severely destroyed (Hu *et al*., 2010). NPs can exhibit different toxicities based on the chemical nature, morphology, reactivity, stability, surface chemistry, mobility, and size.

Many researchers reported on the probable mechanism of NP toxicity which includes its involvement in the disruption of cell membrane integrity, genotoxicity towards cells causing oxidative stress to the cell as evidenced by reactive oxygen species (ROS) formation, and organic radicals' generation. The type of interaction between cell membrane and NPs was via electrostatic or adsorption. Since NPs are smaller than bacterial pores, there are possibility that they may cross into cell membrane (Thill *et al*., 2006).

1.2 Problem Statement

NPs have been increasingly used for various field in past decade for example in technology and medicine (Jing *et al*., 2010). The increasing use of NPs in industrial and domestic sectors led to their release to environment. Despite of their distinctive application and advantages in industrial and domestic sectors, the use of materials with nanometers dimension has raised the issue of safety for consumers and environment. Research on the exposure effect of discrete NPs and their toxicity is very important as their small size cause more inflammation than bulk counterparts when delivered at the same mass dose. Because of their small size and their unique characteristic, NPs have the ability to harm human, microorganisms, and other wildlife by interacting through various mechanisms.

An evaluation of the potential toxicity of nanomaterials is highly essential due to expanding use of NPs and commercialization of nanotechnology product that cause an increase in the exposure of NPs to human and environment. Previous toxicological studies on NPs were focused on same group of NPs (organic, inorganic, carbon-based and organic-inorganic hybrid NPs) but the studies on different class of NPs are limited. Preliminary work was done on manganese oxide, graphene oxide, and carbon black showed that it exhibited toxicity on microbial culture at different magnitude. Because rGO and TiO_2 have many potential in industrial and domestic sector, their toxicity effect must be take into account. The usage of higher organism such as animal and human as a model in toxicity study may lead to the ethical issues. Bacteria are the good models to investigate the NPs toxicity as it appear as a single cell organism. Their interaction with NPs gives overviews about the effect of NPs when released into ecosystem. Bacteria perform crucial roles in the ecosystem, and therefore it will be used for toxicity assessment in term of fate of NPs upon adsorption into the organism as well as within food chain cycle.

Previous researchers used various techniques in assessing the toxicity of NPs. The most popular techniques are turbidimetric measurement (Gurunathan *et al*., 2012; Kasemets *et al*., 2009) and plate count (Pal *et al*., 2007; Sondi-Salopek, 2004). The use of turbidimetric measurement technique to monitor the growth rates has pros and cons. This technique did not measure the true value of viable cells, as the optical density values represent the number of viable and non-viable cells in the suspension. When NPs were introduced to the cell culture, it is difficult to separate the cells and NPs, making this technique did not reliable (Liu *et al*., 2009). Another commonly used technique is plate count method. Although this technique represents true value of viable cell, it is a time consuming. Besides, some microorganisms are unable to produce visible colonies on agar plate (Jung *et al*., 2008). Therefore, a new quantification method for cell viability assessment needs to be developed.

1.3 Objectives of Study

The aims of this research are:

1. To select a suitable fast and real-time staining method for quantification of microbial growth profile for use in toxicity assessment of NPs.
2. To investigate the effect of two different types of NPs (carbon based NP of rGO and inorganic NP of TiO_2) on three types of microbial growth which are *E. coli, B. subtilis,* and *C. albican*.
3. To assess the concentration- and time-dependent effect of NPs towards cell growth.
4. To characterize the morphology of cells exposed to NPs and predict the deposition mechanism of the NPs using electron microscopy and EDX methods.

1.4 Scope of work

Gram-negative (*E. coli*), Gram-positive (*B. subtilis*) bacteria and fungi (*C. albican*) were used as model system. Two different types of NPs were chosen, inorganic NPs (TiO_2) and carbon-based NPs (rGO). Turbidimetric measurement, plate counts, and direct microscopic count using dyes were used as quantification methods to assess the viability of cells. Trypan blue was used as a dye to differentiate between living and dead cells. The efficiency of the methods was compared and contrasts, and the best technique was used in the subsequent of this work. The growth curve of bacteria was constructed. Different concentration of NPs was used to compare the toxicity effect between NPs. The numbers of viable and non-viable cells were counted and the percentage of viable cells was calculated. The effect of NPs on morphology of microbial culture was analyzed using Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). Electron Dispersive X-ray (EDX) was used to investigate whether NPs was adsorbed on the surface microbial cells.

1.5 Thesis Layout

The layout of this dissertation is illustrated in Figure 1.1.

CHAPTER 1: INTRODUCTION

- Background of study
- Problem statement
- Objectives
- Scope of work

↓

CHAPTER 2: LITERATURE REVIEW

- Factors affecting toxicity
- In vitro and in vivo studies
- Cell viability assay

↓

CHAPTER 3: MATERIALS AND METHODS

- List of materials
- Preparation of growth media, stock culture and NPs suspension
- Development of quantification method
- Cell viability assay and morphological observation

↓

CHAPTER 4: RESULTS AND DISCUSSIONS

- Development of quantification method
- Effect of NPs on growth profile and cell morphology
- Concentration- and time-dependent antibacterial activity of NPs

↓

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

Figure 1.1: Layout of dissertation

REFERENCES

Adams, L. K., Lyon, D. Y., & Alvarez, P. J. J. (2006). Comparative eco-toxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions. *Water Research*, *40*(19), 3527–3532.

Akhavan, O., Ghaderi, E., & Akhavan, A. (2012). Size-dependent genotoxicity of graphene nanoplatelets in human stem cells. *Biomaterials*, *33*(32), 8017–8025.

Akhavan, O., Ghaderi, E., Emamy, H., & Akhavan, F. (2013). Genotoxicity of graphene nanoribbons in human mesenchymal stem cells. *Carbon*, *54*, 419–431.

Akhavan, O., Ghaderi, E., & Esfandiar, A. (2011). Wrapping bacteria by graphene nanosheets for isolation from environment, reactivation by sonication, and inactivation by near-infrared irradiation. *Journal of Physical Chemistry B*, *115*(19), 6279–6288.

Akhavan, O., Ghaderi, E., Hashemi, E., & Akbari, E. (2015). Dose-dependent effects of nanoscale graphene oxide on reproduction capability of mammals. *Carbon*, *95*, 309–317.

Aquino, A., Chan, J., Giolma, K., & Loh, M. (2010). The effect of a fullerene water suspension on the growth, cell viability, and membrane integrity of *Escherichia coli* B23. *Journal of Experimental Microbiology and Immunology*, *14*, 13–20.

Azam, A., Ahmed, A. S., Oves, M., Khan, M. S., Habib, S. S., & Memic, A. (2012a). Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: A comparative study. *International Journal of Nanomedicine*, *7*, 6003–6009.

Azam, A., Ahmed, A. S., Oves, M., Khan, M. S., & Memic, A. (2012b). Size - dependent antimicrobial properties of CuO nanoparticles against Gram - positive and - negative bacterial strains. *International Journal of Nanomedicine*, *7*, 3527–3535.

Balusamy, B., Kandhasamy, Y. G., Senthamizhan, A., Chandrasekaran, G., Subramanian, M. S., & Kumaravel, T. S. (2012). Characterization and bacterial toxicity of lanthanum oxide bulk and nanoparticles. *Journal of Rare Earths*, *30*(12), 1298–1302.

Bao, Q., Zhang, D., & Qi, P. (2011). Synthesis and characterization of silver nanoparticle and graphene oxide nanosheet composites as a bactericidal agent for water disinfection. *Journal of Colloid and Interface Science*, *360*(2), 463–470.

Bianco, A. (2013). Graphene: Safe or toxic? The two faces of the medal. *Angewandte Chemie - International Edition*, *52*(19), 4986–4997.

Boulos, L., Prévost, M., Barbeau, B., Coallier, J., & Desjardins, R. (1999). LIVE/DEAD® BacLight™: Application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. *Journal of Microbiological Methods*, *37*(1), 77–86.

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M. F., & Fiévet, F. (2006). Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano Letters*, *6*(4), 866–870.

Chang, Y., Yang, S.-T., Liu, J.-H., Dong, E., Wang, Y., Cao, A., Liu, Y,. & Wang, H. (2011). In vitro toxicity evaluation of graphene oxide on A549 cells. *Toxicology Letters*, *200*(3), 201–210.

Chen, M., Yin, J., Liang, Y., Yuan, S., Wang, F., Song, M., & Wang, H. (2016). Oxidative stress and immunotoxicity induced by graphene oxide in zebrafish. *Aquatic Toxicology*, *174*, 54–60.

Cho, K. H., Park, J. E., Osaka, T., & Park, S. G. (2005). The study of antimicrobial activity and preservative effects of nanosilver ingredient. *Electrochimica Acta*, *51*(5), 956–960.

Cho, M., Chung, H., Choi, W., & Yoon, J. (2004). Linear correlation between inactivation of *E. coli* and OH radical concentration in TiO_2 photocatalytic disinfection. *Water Research*, *38*(4), 1069–1077.

Churg, A., Stevens, B., & Wright, J. L. (1998). Comparison of the uptake of fine and ultrafine TiO_2 in a tracheal explant system. *The American Journal of Physiology*, *274*(1), 81–86.

Cliff, J. B., Jarman, K. H., Valentine, N. B., Golledge, S. L., Gaspar, D. J., Wunschel, D. S., & Wahl, K. L. (2005). Differentiation of spores of *Bacillus subtilis* grown in different media by elemental characterization using time-of-flight secondary ion mass spectrometry. *Applied and Environmental Microbiology*, *71*(11), 6524–6530.

Combarros, R. G., Collado, S., & Díaz, M. (2016). Toxicity of graphene oxide on growth and metabolism of *Pseudomonas putida*. *Journal of Hazardous Materials*, *310*, 246–252.

Dalgaard, P., & Koutsoumanis, K. (2001). Comparison of maximum specific growth rates and lag times estimated from absorbance and viable count data by different mathematical models. *Journal of Microbiological Methods*, *43*(3), 183–196.

Das, M. R., Sarma, R. K., Saikia, R., Kale, V. S., Shelke, M. V., & Sengupta, P. (2011). Synthesis of silver nanoparticles in an aqueous suspension of graphene oxide sheets and its antimicrobial activity. *Colloids and Surfaces B: Biointerfaces*, *83*(1), 16–22.

Diebold, U. (2003). The surface science of titanium dioxide. *Surface Science Reports*, *48*(5–8), 53–229.

Ding, L., Stilwell, J., Zhang, T., Elboudwarej, O., Jiang, H., Selegue, J. P., Cooke, P. A., Gray, J. W., & Chen, F. F. (2005). Molecular characterization of the cytotoxic mechanism of multiwall carbon nanotubes and nano-onions on human skin fibroblast. *Nano Letters*, *5*(12), 2448–2464.

Donaldson, K., Beswick, P. H., & Gilmour, P. S. (1996). Free radical activity associated with the surface of particles: a unifying factor in determining biological activity? *Toxicology Letters*, *88*, 293–298.

Donaldson, K., Tran, L., Jimenez, L. A., Duffin, R., Newby, D. E., Mills, N., MacNee, W., & Stone, V. (2005). Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. *Particle and Fibre Toxicology*, *2*(10), 1–14.

Du, J., Wang, S., You, H., & Zhao, X. (2013). Understanding the toxicity of carbon nanotubes in the environment is crucial to the control of nanomaterials in producing and processing and the assessment of health risk for human: A review. *Environmental Toxicology and Pharmacology*, *36*(2), 451–462.

Elechiguerra, J. L., Burt, J. L., Morones, J. R., Camacho-Bragado, A., Gao, X., Lara, H. H., & Yacaman, M. J. (2005). Interaction of silver nanoparticles with HIV-1. *Journal of Nanobiotechnology*, *3*(6), 1–10.

Feng, Q. L., Chen, G., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., & Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *Journal of Biomedical Materials Research*, *52*(4), 662–668.

Fu, G., Vary, P. S., & Lin, C.-T. (2005). Anatase TiO_2 nanocomposites for antimicrobial coatings. *The Journal of Physical Chemistry B*, *109*(18), 8889–8898.

Geim, A. K., & Novoselov, K. S. (2007). The rise of graphene. *Nature Materials*, *6*(3), 183–191.

Ghafari, P., St-Denis, C. H., Power, M. E., Jin, X., Tsou, V., Mandal, H. S., Bols, C. N., & Tang, X. S. (2008). Impact of carbon nanotubes on the ingestion and digestion of bacteria by ciliated protozoa. *Nature Nanotechnology*, *3*(6), 347–351.

Gopal, M., Moberly Chan, W. J., & De Jonghe, L. C. (1997). Room temperature synthesis of crystalline metal oxides. *Journal of Materials Science*, *32*(22), 6001–6008.

Gordon, R. E., haynes, W. C., & Pang, C. H. N. (1973). The genus bacillus. *US Department of Agriculture Handbook*, 427, 109-135.

Gurr, J. R., Wang, A. S. S., Chen, C. H., & Jan, K. Y. (2005). Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology*, *213*(1–2), 66–73.

Gurunathan, S., Han, J. W., Dayem, A. A., Eppakayala, V., & Kim, J. H. (2012). Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in *Pseudomonas aeruginosa*. *International Journal of Nanomedicine*, *7*, 5901–5914.

Gurunathan, S., Han, J. W., Dayem, A. A., Eppakayala, V., Park, M. R., Kwon, D. N., & Kim, J. H. (2013). Antibacterial activity of dithiothreitol reduced graphene oxide. *Journal of Industrial and Engineering Chemistry*, *19*(4), 1280–1288.

Hajipour, M. J., Fromm, K. M., Akbar Ashkarran, A., Jimenez de Aberasturi, D., Larramendi, I. R. de, Rojo, T., Serpooshan, V., Parak, W. J., & Mahmoudi, M. (2012). Antibacterial properties of nanoparticles. *Trends in Biotechnology*, *30*(10), 499–511.

Hancock-Chen, T., & Scaiano, J. C. (2000). Enzyme inactivation by TiO_2 photosensitization. *Journal of Photochemistry and Photobiology B: Biology*, *57*(2–3), 193–196.

Hart, G. A., Kathman, L. M., & Hesterberg, T. W. (1994). In vitro cytotoxicity of asbestos and man-made vitreous fibers: Roles of fiber length, diameter and composition. *Carcinogenesis*, *15*(5), 971–977.

He, H., Klinowski, J., Forster, M., & Lerf, A. (1998). A new structural model for graphite oxide. *Chemical Physics Letters*, *287*(1–2), 53–56.

He, L., Liu, Y., Mustapha, A., & Lin, M. (2011). Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. *Microbiological Research*, *166*(3), 207–215.

Helland, A., Wick, P., Koehler, A., Schmid, K., & Som, C. (2007). Reviewing the environmental and human health knowledge base of carbon nanotubes. *Environmental Health Perspectives*, *115*(8), 1125–1131.

Henriques, A O., Glaser, P., Piggot, P. J., & Moran, C. P. (1998). Control of cell shape and elongation by the rodA gene in *Bacillus subtilis*. *Molecular Microbiology*, *28*(2), 235–247.
Hirakawa, K.., Mori, M., Yoshida, M., Oikawa, S., & Kawanishi, S. (2004). Photo-irradiated titanium dioxide catalyzes site specific DNA damage via generation of hydrogen peroxide. *Free radical research*, *38*(5), 439-447.
Houghton Mifflin Harcourt. (2016). Introduction to Prokaryotes, Eukaryotes.. Retrieved from https://www.cliffsnotes.com/ at 7th September 2017.
Horváth, L., Magrez, A., Burghard, M., Kern, K., Forró, L., & Schwaller, B. (2013). Evaluation of the toxicity of graphene derivatives on cells of the lung luminal surface. *Carbon*, *64*, 45–60.
Hu, W., Peng, C., Luo, W., Lv, M., Li, X., Li, D., Huang, Q., & Fan, C. (2010). Graphene-based antibacterial paper. *ACS Nano*, *4*(7), 4317–4323.
Hu, X., Cook, S., Wang, P., & Hwang, H.-M. (2009). In vitro evaluation of cytotoxicity of engineered metal oxide nanoparticles. *Science of the Total Environment*, *407*(8), 3070–3072.
Hussain, S. M., Hess, K. L., Gearhart, J. M., Geiss, K. T., & Schlager, J. J. (2005). In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicology in Vitro*, *19*(7), 975–983.
Imlay, J. A. (2003). Pathways of oxidative damage. *Annual Review of Microbiology*, *57*(1), 395–418.
Ji, H., Sun, H., & Qu, X. (2016). Antibacterial applications of graphene-based nanomaterials: Recent achievements and challenges. *Advanced Drug Delivery Reviews*, *105*, 176–189.
Jia, G., Wang, H., Yan, L., Wang, X., Pei, R., Yan, T., Zhao, Y., & Guo, X. (2005). Cytotoxicity of carbon nanomaterials: Single-wall nanotube, multi-wall nanotube, and fullerene. *Environmental Science and Technology*, *39*(5), 1378–1383.
Jing, H., Wang, J., Yang, P., Ke, X., Xia, G., & Chen, B. (2010). Magnetic Fe_3O_4 nanoparticles and chemotherapy agents interact synergistically to induce apoptosis in lymphoma cells. *International Journal of Nanomedicine*, *5*(1), 999–1004.
Jones, N., Ray, B., Ranjit, K. T., & Manna, A. C. (2008). Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS Microbiology Letters*, *279*(1), 71–76.
Ju-Nam, Y., & Lead, J. R. (2008). Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications. *Science of the Total Environment*, *400*(1–3), 396–414.
Jung, W. K., Koo, H. C., Kim, K. W., Shin, S., Kim, S. H., & Park, Y. H. (2008). Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. *Applied and Environmental Microbiology*, *74*(7), 2171–2178.
Kakinoki, K., Yamane, K., Teraoka, R., Otsuka, M., & Matsuda, Y. (2004). Effect of relative humidity on the photocatalytic activity of titanium dioxide and photostability of famotidine. *Journal of Pharmaceutical Sciences*, *93*(3), 582–589.
Kang, S., Herzberg, M., Rodrigues, D. F., & Elimelech, M. (2008). Antibacterial effects of carbon nanotubes: Size does matter! *Langmuir*, *24*(13), 6409–6413.

Kang, S., Pinault, M., Pfefferle, L. D., & Elimelech, M. (2007). Single-walled carbon nanotubes exhibit strong antimicrobial activity. *Langmuir*, *23*(17), 8670–8673.

Kasemets, K., Ivask, A., Dubourguier, H. C., & Kahru, A. (2009). Toxicity of nanoparticles of ZnO, CuO and TiO_2 to yeast *Saccharomyces cerevisiae*. *Toxicology in Vitro*, *23*(6), 1116–1122.

Kikuchi, Y., Sunada, K., Iyoda, T., Hashimoto, K., & Fujishima, A. (1997). Photocatalytic bactericidal effect of TiO_2 thin films: dynamic view of the active oxygen species responsible for the effect. *Journal of Photochemistry and Photobiology A: Chemistry*, *106*(1–3), 51–56.

Kilgour, J. D., Alexander, D. J., & Reed, C. J. (1998). A rat nasal epithelial model for predicting upper respiratory tract toxicity: In vivo–in vitro correlations. *Toxicology Methods*, *8*(4), 301–317.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., Hwang, C.-Y., Kim, Y.-K., Lee, Y.-S., Jeong, D. H., & Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *3*(1), 95–101.

Kim, K.-J., Sung, W. S., Suh, B. K., Moon, S.-K., Choi, J.-S., Kim, J. G., & Lee, D. G. (2009). Antifungal activity and mode of action of silver nano-particles on *Candida albicans*. *Biometals*, *22*(2), 235–242.

Koper, O. B., Klabunde, J. S., Marchin, G. L., Klabunde, K. J., Stoimenov, P., & Bohra, L. (2002). Nanoscale powders and formulations with biocidal activity toward spores and vegetative cells of Bacillus species, viruses, and toxins. *Current Microbiology*, *44*(1), 49–55.

Kovtyukhova, N. I., Ollivier, P. J., Martin, B. R., Mallouk, T. E., Chizhik, S. A., Buzaneva, E. V., & Gorchinskiy, A. D. (1999). Layer-by-layer assembly of ultrathin composite films from micron-sized graphite oxide sheets and polycations. *Chemistry of Materials*, *11*(3), 771–778.

Koyama, S., Kim, Y. A., Hayashi, T., Takeuchi, K., Fujii, C., Kuroiwa, N., Koyama, H., Tsukahara, T., & Endo, M. (2009). In vivo immunological toxicity in mice of carbon nanotubes with impurities. *Carbon*, *47*(5), 1365–1372.

Krishnamoorthy, K., Veerapandian, M., Zhang, L. H., Yun, K., & Kim, S. J. (2012). Antibacterial efficiency of graphene nanosheets against pathogenic bacteria via lipid peroxidation. *Journal of Physical Chemistry C*, *116*(32), 17280–17287.

Kubitschek, H. E. (1990). Cell volume increase in *Escherichia coli* after shifts to richer media. *Journal of Bacteriology*, *172*(1), 94–101.

Kulk, G., van de Poll, W. H., Visser, R. J. W., & Buma, A. G. J. (2011). Distinct differences in photoacclimation potential between prokaryotic and eukaryotic oceanic phytoplankton. *Journal of Experimental Marine Biology and Ecology*, *398*(1–2), 63–72.

Kumari, J., Kumar, D., Mathur, A., Naseer, A., Kumar, R. R., Thanjavur Chandrasekaran, P., Nagarajan, R., & Mukherjee, A. (2014). Cytotoxicity of TiO_2 nanoparticles towards freshwater sediment microorganisms at low exposure concentrations. *Environmental Research*, *135*, 333–345.

Lam, C.-W., James, J. T., Richard McCluskey, R., & Hunter, R. L. (2004). Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicological Sciences*, *77*, 126–134.

Li, C., Wang, X., Chen, F., Zhang, C., Zhi, X., Wang, K., & Cui, D. (2013). The antifungal activity of graphene oxide-silver nanocomposites. *Biomaterials*, *34*(15), 3882–3890.

Li, D., Gilje, Muller, M. B., Gilje, S., Kaner, R. B., & Wallace, G. G. (2008). Processable aqueous dispersions of graphene nanosheets. *Nature Nanotechnology*, *3*(2), 101–105.

Lindqvist, R. (2006). Estimation of *Staphylococcus aureus* growth parameters from turbidity data: Characterization of strain variation and comparison of methods. *Applied and Environmental Microbiology*, *72*(7), 4862–4870.

Liu, S., Hu, M., Zeng, T. H., Wu, R., Jiang, R., Wei, J., Wang, L., Kong, J., & Chen, Y. (2012). Lateral dimension-dependent antibacterial activity of graphene oxide sheets. *Langmuir*, *28*(33), 12364–12372.

Liu, S., Zeng, T. H., Hofmann, M., Burcombe, E., Wei, J., Jiang, R., Kong, J., & Chen, Y. (2011). Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: Membrane and oxidative stress. *ACS Nano*, *5*(9), 6971–6980.

Liu, Y., He, L., Mustapha, A., Li, H., Hu, Z. Q., & Lin, M. (2009). Antibacterial activities of zinc oxide nanoparticles against *Escherichia coli* O157:H7. *Journal of Applied Microbiology*, *107*(4), 1193–1201.

Lok, C.-N., Ho, C. M., Chen, R., He, Q.-Y., Yu, W.-Y., Sun, H., Tam, P. K.-H., Chiu, J.-F., & Che, C. M. (2006). Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *Journal of Proteome Research*, *5*(4), 916–924.

Louis, K. S., & Siegel, A. C. (2011). Cell viability analysis using trypan blue: Manual and automated methods. In *Mammalian Cell Viability: Methods and Protocols* (pp. 7–12), Humana Press

Luttrell, T., Halpegamage, S., Tao, J., Kramer, A., Sutter, E., & Batzill, M. (2015). Why is anatase a better photocatalyst than rutile? - Model studies on epitaxial TiO_2 films. *Scientific Reports*, *4*(1), 4043–4050.

Ma, L., Liu, J., Li, N., Wang, J., Duan, Y., Yan, J., Liu, H., & Hong, F. (2010). Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO_2 delivered to the abdominal cavity. *Biomaterials*, *31*(1), 99–105.

Maness, P., Smolinski, S., Blake, D. M., Huang, Z., Wolfrum, E. J., & Jacoby, W. A. (1999). Bactericidal activity of photocatalytic TiO_2 reaction: Toward an understanding of its killing mechanism. *Applied and Environmental Microbiology*, *65*(9), 4094–4098.

Martins, N., Ferreira, I. C. F. R., Barros, L., Silva, S., & Henriques, M. (2014). Candidiasis: Predisposing factors, prevention, diagnosis and alternative treatment. *Mycopathologia*, *177*(5–6), 223–240.

Monod, J. (1949). The growth of bacterial cultures. *Annual Reviews Microbiology*, *3*(1), 371–394.

Murray, A. R., Kisin, E., Leonard, S. S., Young, S. H., Kommineni, C., Kagan, V. E., Castranova, V., & Shvedova, A. A. (2009). Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes. *Toxicology*, *257*(3), 161–171.

Nair, S., Sasidharan, A., Divya Rani, V. V., Menon, D., Nair, S., Manzoor, K., & Raina, S. (2009). Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. *Journal of Materials Science: Materials in Medicine*, *20*, 235–241.

Nowack, B., & Bucheli, T. D. (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Environmental Pollution*, *150*(1), 5–22.
Oberdörster, G., Maynard, A., Donaldson, K., Castranova, V., Fitzpatrick, J., Ausman, K., Carter, J., Karn, B., Kreyling, W., Lai, D., Olin, S., Monteiro-Riviere, N., Warheit, D., & Yang, H. (2005). Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. *Particle and Fibre Toxicology*, *2*(1), 8.
Pal, S., Tak, Y. K., & Song, J. M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Applied and Environmental Microbiology*, *73*(6), 1712–1720.
Panáček, A., Kolář, M., Večeřová, R., Prucek, R., Soukupová, J., Kryštof, V., Hamal, P., Zboril, R., & Kvítek, L. (2009). Antifungal activity of silver nanoparticles against Candida spp. *Biomaterials*, *30*(31), 6333–6340.
Pandey, P., Merwyn, S., Agarwal, G. S., Tripathi, B. K., & Pant, S. C. (2012). Electrochemical synthesis of multi-armed CuO nanoparticles and their remarkable bactericidal potential against waterborne bacteria. *Journal of Nanoparticle Research*, *14*(1), 709.
Persidsky, D., & Stuart, G. (1977). Fluorometric test of cell membrane integrity. *Cryobiology*, *14*(3), 322–331.
Raghupathi, K. R., Koodali, R. T., & Manna, A. C. (2011). Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. *Langmuir*, *27*(7), 4020–4028.
Ramage, G., Bachmann, S., Patterson, T. F., Wickes, B. L., & Lopez-Ribot, J. L. (2002). Investigation of multidrug efflux pumps in relation to fluconazole resistance in *Candida albicans* biofilms. *Journal of Antimicrobial Chemotherapy*, *49*(6), 973–980.
Reddy, K. M., Feris, K., Bell, J., Wingett, D. G., Hanley, C., & Punnoose, A. (2007). Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. *Applied Physics Letters*, *90*(1), 1–3.
Reyes-Coronado, D., Rodríguez-Gattorno, G., Espinosa-Pesqueira, M. E., Cab, C., de Coss, R., & Oskam, G. (2008). Phase-pure TiO_2 nanoparticles: Anatase, brookite and rutile. *Nanotechnology*, *19*(14), 145605–145614.
Riding, M. J., Martin, F. L., Trevisan, J., Llabjani, V., Patel, I. I., Jones, K. C., & Semple, K. T. (2012). Concentration-dependent effects of carbon nanoparticles in gram-negative bacteria determined by infrared spectroscopy with multivariate analysis. *Environmental Pollution*, *163*, 226–234.
Rincón, A. G., & Pulgarin, C. (2004). Bactericidal action of illuminated TiO_2 on pure *Escherichia coli* and natural bacterial consortia: Post-irradiation events in the dark and assessment of the effective disinfection time. *Applied Catalysis B: Environmental*, *49*(2), 99–112.
Risom, L., Møller, P., & Loft, S. (2005). Oxidative stress-induced DNA damage by particulate air pollution. *Mutation Research*, *592*(1–2), 119–137.
Roduner, E. (2006). Size matters: Why nanomaterials are different. *Chemical Society Reviews*, *35*(7), 583–592.

Rolfe, M. D., Rice, C. J., Lucchini, S., Pin, C., Thompson, A., Cameron, A. D. S., Alston, M., Stringer, M. F., Betts, R. P., Peck, M. W., & Hinton, J. C. D. (2012). Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. *Journal of Bacteriology*, *194*(3), 686–701.

Saito, T., Iwase, T., Horie, J., & Morioka, T. (1992). Mode of photocatalytic bactericidal action of powdered semiconductor TiO_2 on mutans streptococci. *Journal of Photochemistry and Photobiology, B: Biology*, *14*(4), 369–379.

Sanchez, V. C., Jachak, A., Hurt, R. H., & Kane, A. B. (2012). Biological interactions of graphene-family nanomaterials – An interdisciplinary review. *Chemical Research in Toxicology, 25* (1), 15-34.

Santos, C. M., Mangadlao, J., Ahmed, F., Leon, A., Advincula, R. C., & Rodrigues, D. F. (2012). Graphene nanocomposite for biomedical applications: Fabrication, antimicrobial and cytotoxic investigations. *Nanotechnology*, *23*, 395101.

Sawangphruk, M., Srimuk, P., Chiochan, P., Sangsri, T., & Siwayaprahm, P. (2012). Synthesis and antifungal activity of reduced graphene oxide nanosheets. *Carbon*, *50*(14), 5156–5161.

Schaechter, M., Williamson, J. P., Hood, J. R., & Koch, A. L. (1962). Growth, cell and nuclear divisions in some bacteria. *Journal of General Microbiology*, *29*(3), 421–34.

Seetharam, R. N., & Sridhar, K. R. (2007). Nanotoxicity: Threat posed by nanoparticles. *Current Science*, *93*(6), 769–770.

Shvedova, A. A., Kisin, E. R., Mercer, R., Murray, A. R., Johnson, V. J., Potapovich, A. I., Tyurina, Y. Y., Gorelik, O., Arepalli, S., Schwegler-Berry, D., Hubbs, A. F., Antonini, J., Evans, D. E., Ku, B.-K., Ramsey, D., Maynard, A., Kagan, V. E., Castranova, V., & Baron, P. (2005). Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *American Journal Physiology-Lung Cellular Molecular Physiology*, *289*(5), 698–708.

Shuler, M. L., & Kargi, F. (2002). How cells grow. *Bioprocess Engineering Basic Concepts. Prentice Hall Upper Saddle River, NJ*, 162-164.

Simon-Deckers, A., Loo, S., Mayne-L'Hermite, M., Herlin-Boime, N., Menguy, N., Reynaud, C., Gouget, B., & Carriere, M. (2009). Size-, composition- and shape-dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes toward bacteria. *Environmental Science and Technology*, *43*(21), 8423–8429.

Sondi, I., & Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science*, *275*(1), 177–182.

Stankovich, S., Dikin, D. A., Piner, R. D., Kohlhaas, K. A., Kleinhammes, A., Jia, Y., Wu, Y., Nguyen, S. T., & Ruoff, R. S. (2007). Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide. *Carbon*, *45*(7), 1558–1565.

Stearns, R. C., Paulauskis, J. D., & Godleski, J. J. (2001). Endocytosis of ultrafine particles by A549 cells. *American Journal of Respiratory Cell and Molecular Biology*, *24*(2), 108–115.

Stoddart, M. J. (2011). Cell viability assay: Introduction. In *Mammalian Cell Viability: Methods and Protocols* (pp. 1–6), Humana Press.

Stoimenov, P. K., Klinger, R. L., Marchin, G. L., & Klabunde, K. J. (2002). Metal oxide nanoparticles as bactericidal agents. *Langmuir*, *18*(17), 6679–6686.
Sudbery, P., Gow, N., & Berman, J. (2004). The distinct morphogenic states of *Candida albicans*. *Trends in Microbiology*, *12*(7), 317–324.
Sun, X., Liu, Z., Welsher, K., Robinson, J. T., Goodwin, A., Zaric, S., & Dai, H. (2008). Nano-graphene oxide for cellular imaging and drug delivery. *Nano Res*, *1*(3), 203–212.
Sunada, K., Kikuchi, Y., Hashimoto, K., & Fujishima, A. (1998). Bactericidal and detoxification effects of TiO_2 thin film photocatalysts. *Environmental Science and Technology*, *32*(5), 726–728.
Szabó, T., Szeri, A., & Dékány, I. (2005). Composite graphitic nanolayers prepared by self-assembly between finely dispersed graphite oxide and a cationic polymer. *Carbon*, *43*(1), 87–94.
Takenaka, S., Karg, E., Roth, C., Schulz, H., Ziesenis, A., Heinzmann, U., Schramel, P., & Heyder, J. (2001). Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environmental Health Perspectives*, *109*(Suppl 4), 547–551.
Tawakoli, P. N., Al-Ahmad, A., Hoth-Hannig, W., Hannig, M., & Hannig, C. (2013). Comparison of different live/dead stainings for detection and quantification of adherent microorganisms in the initial oral biofilm. *Clinical Oral Investigations*, *17*(3), 841–850.
Thill, A., Zeyons, O., Spalla, O., Chauvat, F., Rose, J., Auffan, M., & Flank, A. M. (2006). Cytotoxicity of CeO_2 nanoparticles physico-chemical insight of the cytotoxicity mechanism. *Environmental Science & Technology*, *40*, 6151–6156.
Tian, F., Cui, D., Schwarz, H., Estrada, G. G., & Kobayashi, H. (2006). Cytotoxicity of single-wall carbon nanotubes on human fibroblasts. *Toxicology in Vitro*, *20*(7), 1202–1212.
Tran, A. N., Mir, A., Mallik, D., Sinha, A., Nayar, S., & Webster, T. J. (2010). Bactericidal effect of iron oxide nanoparticles on *Staphylococcus aureus*. *International Journal of Nanomedicine*, *5*, 277–283.
Tu, Y. S., Lv, M., Xiu, P., Huynh, T., Zhang, M., Castelli, M., Liu, Z., Huang, Q., Fan, C., Fang, H., & Zhou, R. H. (2013). Destructive extraction of phospholipids from *Escherichia coli* membranes by graphene nanosheets. *Nature Nanotechnology*, *8*(8), 594–601.
Uchino, T., Tokunaga, H., Ando, M., & Utsumi, H. (2002). Quantitative determination of OH radical generation and its cytotoxicity induced by TiO_2-UVA treatment. *Toxicology in Vitro*, *16*(5), 629–635.
Upadhyayula, V. K. K., Deng, S., Smith, G. B., & Mitchell, M. C. (2009). Adsorption of *Bacillus subtilis* on single-walled carbon nanotube aggregates, activated carbon and NanoCeram™. *Water Research*, *43*(1), 148–156.
Wang, H., Wick, R. L., & Xing, B. (2009). Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode *Caenorhabditis elegans*. *Environmental Pollution*, *157*(4), 1171–1177.
Wang, K., Ruan, J., Song, H., Zhang, J., Wo, Y., Guo, S., & Cui, D. (2011). Biocompatibility of graphene oxide. *Nanoscale Research Letters*, *6*(1), 1–8.

Warheit, D. B., Webb, T. R., Reed, K. L., Frerichs, S., & Sayes, C. M. (2007). Pulmonary toxicity study in rats with three forms of ultrafine-TiO_2 particles: Differential responses related to surface properties. *Toxicology*, *230*(1), 90–104.

Westmoreland, C., Walker, T., Matthews, J., & Murdock, J. (1999). Preliminary investigations into the use of a human bronchial cell line (16HBE14o-) to screen for respiratory toxins in vitro. *Toxicology in Vitro*, *13*(4–5), 761–764.

Xiong, D., Fang, T., Yu, L., Sima, X., & Zhu, W. (2011). Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. *Science of the Total Environment*, *409*(8), 1444–1452.

Yamamoto, O. (2001). Influence of particle size on the antibacterial activity of zinc oxide. *International Journal of Inorganic Materials*, *3*(7), 643–646.

Yang, C., Mamouni, J., Tang, Y., & Yang, L. (2010). Antimicrobial activity of single-walled carbon nanotubes: Length effect. *Langmuir*, *26*(20), 16013–16019.

Yang, K., Li, Y., Tan, X., Peng, R., & Liu, Z. (2013). Behavior and toxicity of graphene and its functionalized derivatives in biological systems. *Small*, *9*(9–10), 1492–1503.

Yeung, K. L., Leung, W. K., Yao, N., & Cao, S. (2009). Reactivity and antimicrobial properties of nanostructured titanium dioxide. *Catalysis Today*, *143*(3–4), 218–224.

Yoon, K.-Y., Hoon Byeon, J., Park, J.-H., & Hwang, J. (2007). Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. *Science of The Total Environment, 373*(2-3), 572-575.

Yu, A. C. S., Loo, J. F. C., Yu, S., Kong, S. K., & Chan, T. F. (2014). Monitoring bacterial growth using tunable resistive pulse sensing with a pore-based technique. *Applied Microbiology and Biotechnology*, *98*(2), 855–862.

Zhang, H., & Banfield, J. F. (1998). Thermodynamic analysis of phase stability of nanocrystalline titania. *Journal of Materials Chemistry*, *8*(9), 2073–2076.

Zhang, H., Peng, C., Yang, J., Lv, M., Liu, R., He, D., Fan, C., & Huang, Q. (2013). Uniform ultrasmall graphene oxide nanosheets with low cytotoxicity and high cellular uptake. *ACS Applied Materials and Interfaces*, *5*(5), 1761–1767.

Zhang, L., Jiang, Y., Ding, Y., Povey, M., & York, D. (2007). Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). *Journal of Nanoparticle Research*, *9*(3), 479–489.

Zhang, L., Xia, J., Zhao, Q., Liu, L., & Zhang, Z. (2010). Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. *Small*, *6*(4), 537–544.

Zhou, Y., Kong, Y., Kundu, S., Cirillo, J. D., & Liang, H. (2012). Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and bacillus Calmette-Guérin. *Journal of Nanobiotechnology*, *10*(1), 19–27.

BIODATA OF STUDENT

Nurul Shahidah binti Ahmad was born in Muar, Johor on 20th October 1992. She received the Bachelor of Chemical and Environmental Engineering from the Universiti Putra Malaysia in2015. She continues her studies in Master of Science (Biochemical Engineering) in the same university. Her current research interest focused on the toxicological of nanoparticles.

LIST OF PUBLICATIONS

Ahmad, N. S., Abdullah, N., & Yasin, F. M., (2019). Antifungal activity of titanium dioxide nanoparticles against *Candida albican*. *Bioresources,* 14(4), 8866-8878 (**published**).

Ahmad, N. S., Abdullah, N., & Yasin, F. M., (2019). Toxicity assessment of reduced graphene oxide and titanium dioxide nanomaterials on gram-positive and gram-negative bacteria under normal laboratory lighting condition, *Toxicology Reports* (**submitted**).

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : First Semester 2019/2020

TITLE OF THESIS / PROJECT REPORT :

TOXICITY ASSESSMENT OF REDUCED GRAPHENE OXIDE AND TITANIUM DIOXIDE NANOPARTICLES ON GROWTH OF MICROORGANISMS

NAME OF STUDENT: NURUL SHAHIDAH BINTI AHMAD

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from __________ (date) until __________ (date)

Approved by:

(Signature of Student)
New IC No/ Passport No.:

Date :

(Signature of Chairman of Supervisory Committee)
Name:

Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]