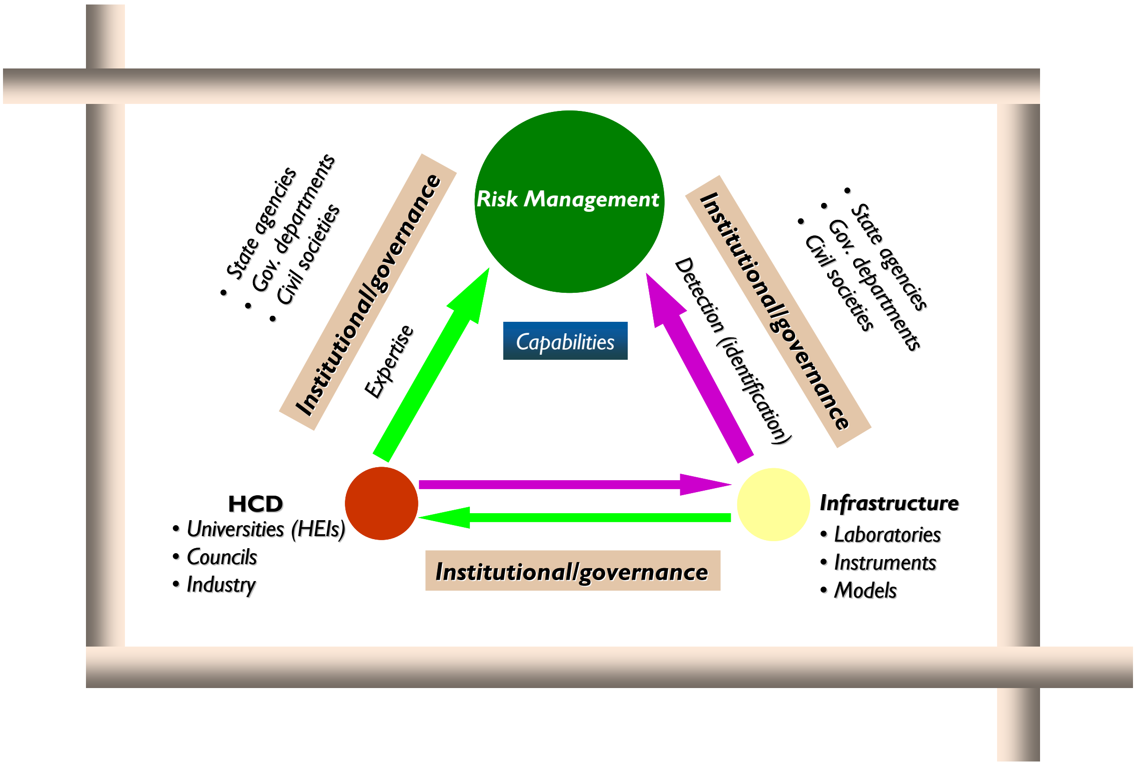
Nano HSE HCD Workshop, 2013



First Human Capital Development Workshop for Nanotechnologies and Nanosciences Risk Assessment

August 13, 2013

Knowledge Commons, Building 50
CSIR Campus, Pretoria

Invited speakers:
Joseph Molapisi (DST), Pete Ashton (CSIR), Lucky Sikwhivhilu (MINTEK), Harrison Piennaar (CSIR), Valelie Naidoo (WRC)

Workshop organizing committee:
Ndeke Musee (CSIR), Melusi Thwala (CSIR) and Chantelle Macrow (CSIR)

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Department: Science and Technology
REPUBLIC OF SOUTH AFRICA
WATER RESEARCH COMMISSION
NRF National Research Foundation

TUESDAY 13, AUGUST 2013

Session 1: Official Opening, HCD Nanotechnology Risk Assessment Workshop Session Chair: **Ndeke Musee**, NRE, CSIR	
08:30 – 09:00	Coffee and Delegates Registration
09:00 – 09:10	Welcome, introductions and group Photo – **Ndeke Musee**, NRE, CSIR
09:10 – 09:20	Opening Remarks: **May Hermanus,** NRE, CSIR
09:20 – 09:35	Nanotech risk assessment research: CSIR perspective – **Harrison Piennaar,** NRE, CSIR
09:45 – 10:00	Nanotech risk assessment research: DST perspective – **Joseph Molapisi**, DST
10:00 – 10:15	Nanotech risk assessment research: WRC perspective – **Valerie Naidoo**, WRC
10:15 – 10:30	*Morning Tea Break*
Session 2: Technical presentations on toxicity and applications of modeling tools Session Chair: **Nontombi Marule,** DTI	
10:30 –11:00	A life Time in Science by **Peter Ashton**, NRE, CSIR
11:00 –11:20	Fate and behaviour of ZnO ENPs in a simulated domestic wastewater treatment plant, **Eutilerio Chauque,** MSc, UJ
11:20 –11:40	Application of BN modeling on the stability and toxicity of engineered nanomaterials in aquatic ecosystems, **Mary Ondiaka**, PhD, US
11:40 –12:00	Statistical modelling approach to derive quantitative nanowastes classification index: estimation of nanomaterials exposure, **Loyiso Ntaka**, MSc, UJ
12:00 –12:20	The oxidative toxicity of Ag and ZnO NPs towards aquatic plant *Spirodela punctuta,* and role of testing media parameters, **Melusi Thwala**, PhD, UJ
12:20– 13:10	*Lunch Break*
Session 3: Technical presentations on toxicity, fate, and behavior of nanomaterials Chair**: Joseph Molapisi**, DST	
13:10 – 13:40	Nanoscience and nanotechnology: context in South Africa, and the role of prudent risk management from an industrial perspective, **Lucky Sikhwivhilu,** NIC, MINTEK
13:40 – 14:00	Potential susceptibility inhibitory effect of Ag and ZnO ENPs on bacterial strains: effect of pH and temperature, **Anza Mboyi,** MSc, TUT
14:00 – 14:20	The toxicity of oxidised DWCNTs to the aquatic organisms, and related causing mechanisms, **Lungile Lukhele,** PhD, UJ
14:20 – 14:40	The stability of Ag and ZnO ENPs in Hoagland's nutrient water: the role of particle size and morphology, **Ntsiki Radebe**, MSc, TUT
14:40 – 15:00	Effect of CNSs on algal growth: the influence of physicochemical properties and environmental parameters, **Dumisile Nyembe**, PhD, UJ
15:00 – 15:15	*Afternoon Tea Break*
Session 4: Reflections, discussions, and resolutions on the way forward Session Chair: **Pete Ashton**, NRE, CSIR	
15:15 – 16:00	Feedback and discussions (panel discussions)
16:00 – 16:15	Closure/Vote of Thanks: **Harrison Piennaar**

WORKSHOP

ABSTRACTS

Preface

South Africa's national vision on risk assessment of nanotechnology-based products and materials is profoundly hinged on the National Nanotechnology Strategy (2005) published by the Department of Science and Technology (DST), and recently articulated in the Nanotechnology Implementation Plan of 2010. To ensure nanotechnology is responsibly exploited, the DST has constituted two technical committees; namely; the Nanotechnology Ethics Committee (Nano Ethics Comm.) and Nanotechnology Health, Safety and Environment Committee (Nano HSE Comm.) to address all aspects related but not limited to: safety, ethics, effects, and impacts of nanotechnologies and nanosciences in South Africa. Specifically the Nano HSE Comm. is tasked to develop a research platform to support systematic investigations on the negative effects of nanotechnologies to the humans and the natural environment. Secondly, a policy framework to govern research, manufacturing and application of nanomaterials as well as monitor its implementation.

The research platform on risk assessment of nanotechnologies developed by the Nano HSE Comm. consists of four objectives aimed to: develop critical mass of human capital with requisite competences to conduct cutting edge research on nanotechnology risk assessment, to develop appropriate infrastructure to support world-class nanotechnology risk assessment research; thirdly provide a platform appropriate for the generation of knowledge through targeted research; and finally to build and maintain a database of research outcomes in this field.

It is within this context that the Human Capital Development Workshop is hosted by the CSIR to provide an opportunity for stakeholders to assess progress achieved in building requisite competences for evaluating the potential impacts of nanomaterials in the ecosystems. The workshop proceedings comprise of invited presentations from organizations that support the development of policy framework to govern nanotechnology in South Africa, fund research and/or undertake research in this domain. Secondly, plenary presentations from experts aimed to equip and challenge the emerging breed of scientists pursuing doctoral and masters degrees to consider careers in this domain. Finally, presentations of scientific papers by doctoral and masters students. The technical papers cover three broad targeted research areas, viz.: ecotoxicity of nanomaterials (aimed to understand their potential toxic effects to biological life forms), fate and behavior of nanomaterials in the ecosystems, and application of modeling approaches to estimate nanomaterials effects and exposure to aquatic ecosystems.

I would like to acknowledge several organizations and individuals for their support: The DST for setting a national vision on risks associated with nanotechnology, and funding research in this domain. The CSIR for the financial support of nanotechnology risk assessment research in aquatic ecosystems, and as the organization hosting this workshop. The Water Research Commission and National Research Foundation for funding research, and supporting postgraduate students. The Department of Trade and Industry is acknowledged for the attendance but also because it holds key in the commercialization of nanotechnology-based products and materials in South Africa. This makes DTI an end user of data generated from this research. Sincere thanks to the organizing committee for the efforts of providing logistics to make the workshop a success. And finally, the delegates and presenters are acknowledged for collectively making this workshop truly special and unique as South Africa sets into a new terrain to develop skills critical in assessing the risks of nanomaterials in the ecosystems.

Warm welcome to all!

Ndeke Musee
First HCD Workshop Convener (Ecosystems), 2013

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Fate and behaviour of ZnO engineered nanoparticles in a simulated domestic wastewater treatment plant

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Abstract

Wastewater treatment plants (WWTPs) employ activated sludge processes to treat domestic wastewater using a consortium of bacteria essentially to degrade organic matter. However, bacteria activity is inhibited by toxic substances; thus, potentially adversely impacting on the wastewater biological treatment processes. Among the increasing emerging contaminants into wastewater are engineered nanoparticles (ENPs). However, the impacts of these contaminants including metal oxides ENPs on the treatment efficiency of WWTPs are largely non-quantified. In addition, the fate and behaviour of ENPs from influent point to the effluent discharge point is not well established. Therefore, here findings on the fate and behaviour of ZnO ENPs derived using simulated (model) wastewater treatment plant developed following prescribed Organization for Economic Co-operation and Development (OECD) specifications are described. Preliminary results from our study using ICP-OES – for the analysis of zinc – suggest the release of low levels of zinc (about 50–200 ppb) in the effluent compared to concentrations in the sludge (about 2000 ppb). Our findings indicate efficiency removal of ZnO ENPs from domestic wastewater exceeding 90%. The findings suggest ZnO ENPs were removed from influent through abiotic, biosorption, and biosolid settling mechanisms. This phenomenon was confirmed using X-ray fluorescence spectroscopy (XRF) analysis showing the presence of zinc on the surface of the sludge. Overall, our results indicate that, after release of ENPs, for example ZnO, into WWTPs only low levels are likely to be released into the environment, but the sludge may require additional treatment steps due to elevated metallic concentrations.

Keywords: Wastewater, activated sludge, engineered nanoparticles, zinc oxide, bacteria

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Application of Bayesian Network modeling on the stability and toxicity of engineered nanomaterials in aquatic ecosystems

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Abstract

The stability of engineered nanomaterials (ENMs) in the aquatic systems influences their eventual interactions with aquatic biota – and subsequently the observed toxic effects. Increasing data suggests that physicochemical properties of ENMs such as size, surface charge, chemical composition etc. can be enhanced, modified, or neutralized owing to biotic and water chemistry factors such as organic and inorganic substrates, and ionic strength. When ENMs enter aquatic systems, they undergo transformation, and acquire dynamic properties such as dispersion, agglomeration/aggregation, and sedimentation which control their interactions with organisms in the water column and sediments. In this study, stability and toxicity data of ENMs solicited from published scientific reports was used to develop a Bayesian Network (BN) model to predict their potential risks to the aquatic organisms. The suitability of the BN modeling tool was exploited to investigate the different plausible scenarios of complex interactions of multiple causal and effect variables. Likely states of each variable had a predictive evidence of ≤ 1 probability. The model was designed in a modular version where each sub-model evaluated a single dynamic property of ENMs in a stepwise manner. Here, the functionality of the developed model is illustrated using data for different types (e.g. anatase, rutile) and forms (bare, coated, pristine, and formulated) of $nTiO_2$. Both the prior and posterior models of the BN were developed using collected and collated data obtained from scientific published literature. Our findings demonstrate predictive reasoning capability to link causes and effects of ENMs in the aquatic systems. Depending on the degree of influence of interacting variables, high probability outcomes from our results indicate high likelihood of the causal variables influencing the succeeding effects. The BN tool applied here illustrates its suitability to evaluate and predict the potential the risks of ENMs in aquatic systems using probability methods.

Keywords: Modeling, stability, aquatic systems, Bayesian Network, risks, probability

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Statistical modelling approach to derive quantitative nanowastes classification index: estimation of nanomaterials exposure

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Abstract

The widespread use of engineered nanomaterials (ENMs) in consumer products and industrial applications has raised concerns because of their potential impacts in the environmental systems. These concerns are due to dramatically increasing reports on the toxicity of ENMs to biological life forms in the ecosystems, and secondly, owing to rapid generation of new waste streams previously unknown and generically regarded as nanowastes. Currently, nanowastes classification remains poorly studied despite the large diversity of nanoproducts (e.g. cosmetics, textiles, etc.) being introduced in the global markets. Systematic classification of nanowastes is one of the useful tools to support efficient and effective nanowastes management by stakeholders, for example, governments, regulatory agencies, industries producing nanoproducts and ENMs, waste management industry, among others. In this paper, we present preliminary results on quantitative nanowastes classification through estimation of exposure parameter. Risk is a function of hazard and exposure; here results on the potential exposure of ENMs from nanowastes are presented. The estimation was done using statistical modelling to model the dynamics of aggregation as a surrogate parameter for exposure. In this work, statistical inference approach specifically the non-parametric bootstrapping and linear model were applied. Data used to develop the model were sourced from the literature. 104 data points with information on aggregation, natural organic matter (NOM), pH (including pH_{pze}), ionic strength (IS), and size were analysed. Results from linear regression analysis of aggregation as a function of four variables (NOM, IS, pH, size) indicated IS as the single most significant variable (at 5% level). Notably, IS accounted for aggregation with a linear coefficient and standard error 4.466 and 1.543, respectively. In addition, 5000 boot-strap estimates were performed to obtain the sampling distribution for the linear regression coefficient of aggregation on IS. The boot-strapping results were consistent as they yielded a linear coefficient of 4.466 and standard error of 1.754. Thus, our results support the hypothesis that IS is an adequate abiotic parameter to account for the aggregation of ENMs in the aquatic systems.

Keywords: Nanowastes classification, statistical modelling, exposure, abiotic factors

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The oxidative toxicity of Ag and ZnO nanoparticles towards the aquatic plant *Spirodela punctuta* and the role of testing media parameters

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Abstract

The toxicity effects of silver (nAg) and zinc oxide (nZnO) engineered nanoparticles (ENPs) on the duckweed *Spirodela punctuta* were studied to investigate the potential risks posed by these ENPs towards higher aquatic plants. The influence of media abiotic factors on the stability of the ENPs was also evaluated. Marked agglomeration of ENPs was observed after introduction into testing media whereby large particles settled out of suspension and accumulated at the bottom of testing vessels. The high ionic strength (IS) promoted agglomeration of ENPs because it reduced the inter-particle repulsion caused by a reduction in their surface charge. Low dissolution was observed for nAg, reaching only 0.015% at 1000 mg/L, whilst improved dissolution was observed for nZnO, only falling below analytical quantification at 0.1 mg/L and lower. The quantification of free radicals namely, reactive oxygen and nitrogen species (ROS/RNS) and hydrogen peroxide (H_2O_2), indicated the induction of oxidative stress in plants exposed to the ENPs. A definite dose influence was observed for ROS/RNS volumes in plants exposed to nZnO for 14 days, a response not always observed. The total antioxidant capacity (TAC) and superoxide dismutase (SOD) activity in plants indicated varying degrees of oxidative toxicity caused by exposure to ENPs. This toxicity was driven mainly by particulates in plants exposed to nAg, whilst dissolved Zn^{2+} was the main driver for toxicity in plants exposed to nZnO. Our findings suggest that the toxicity of nAg and nZnO could be caused by both the particulates and ionic forms, as modified by media properties.

Keywords: Nanotoxicity, oxidative stress, antioxidant capacity, nanoparticle characterisation

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Potential susceptibility inhibitory effect of Ag and ZnO engineered nanoparticles on bacterial strains: effect of pH and temperature

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Abstract

The economic and social benefits of metallic silver (*n*-Ag) and zinc oxide (*n*-ZnO) engineered nanoparticles (ENPs) applications in medical, technological, environmental remediation, consumer products, among other have widely been demonstrated. Conversely, concerns have been owing to these ENMs widely unknown environmental and public health effects. Until now, ENMs have been traced into the wastewater treatment plants (WWTPs); however, their impacts to the treatment systems are yet to be fully elucidated. The WWTPs main goal is to reduce the pollution level of urban and industrial wastewaters, prior to discharge into the environment. One way of achieving this objective is by exploiting diverse microbial ecology such as bacteria, fungi and protozoa – which are essential in secondary wastewater treatment processes. However, the benefits of biological treatment processes can be reversed or dramatically diminished when ENMs are introduced into WWTPs. This because certain ENMs can adversely affect the performance of wastewater treatment processes by various mechanisms, for example, inhibition of microorganisms in the secondary treatment process due to their antibacterial properties. Our study investigated the potential impacts of *n*-Ag and *n*-ZnO ENPs (i) to microbial populations, (ii) to the resistance limits of wastewater bacteria after exposure, and (iii) to the nutrient removal efficacy from the effluent. Using the disc diffusion techniques, the wells were impregnated with *n*-Ag and *n*-ZnO at concentrations varying between 1 and 100 mg/L and incubated for 24-h to ascertain the growth inhibition of selected wastewater bacterial strains (*Bacillus licheniformis*, *Brevibacillus laterosporus*, *Pseudomonas putida*). Our findings on bacterial growth rates, lethal limits, and tolerance limits were found to be dependent on abiotic factors such as temperature and pH changes.

Keywords: silver nanoparticles, zinc nanoparticles, bacteria, wastewater treatment plants, biological treatment, inhibitory growth.

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The toxicity of oxidised DWCNTs to the aquatic organisms, and related causing mechanisms

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Abstract

Carbon nanotubes (CNTs) find wide applications as their science and applicability are well understood. According to Lux Research Inc., the market for CNTs increased from $43 million in 2005 to $260 million by 2010. Whilst successful applications of CNTs are important for economic growth globally, there is necessity to understand their potential environmental impacts, and how such effects can be mitigated if established. Therefore, in this paper we present findings on the acute toxicity of double walled carbon nanotubes (DWCNTs) and possible mechanisms in which they cause toxic effects on: *Pseudokirchneriella subcapitata* (algae), *Daphnia pulex* (macro-invertebrates), and *Poecilia reticulata* (fish). The effective concentration resulting in 50% effect (EC_{50}) of DWCNTs to *P. subcapitata* was 25.7 mg/L whilst the lethal concentrations (LC_{50}) of DWCNTs to *D. pulex* and *P. reticulata* were 4.48 mg/L and 113.644 mg/L, respectively. The toxicity mechanism of DWCNTs to *P. subcapitata* was established to be through shading effect and agglomeration processes. Conversely, the toxicity to *D. pulex* and *P. reticulata* was through agglomeration, physical interaction (piting), and oxidative stress mechanisms – which were confirmed to be similar with mechanisms earlier reported for the three aquatic organisms in earlier scientific reports. Finally, the paper discusses the linkage between the toxicity mechanisms and the physicochemical properties of DWCNTs, namely: agglomeration state, surface chemistry, and morphology. Our findings highlight the significance of linking engineered nanomaterials toxicity to their inherent physicochemical properties. Data of this nature is useful in supporting systematic risk assessment of ENMs in the aquatic ecosystems.

Keywords: Double walled carbon nanotubes, toxicity, aquatic organisms, toxicity-causing mechanisms

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The stability of Ag and ZnO engineered nanoparticles in Hoagland's nutrient water: the role of particle size and morphology

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Abstract

The current study seeks to investigate the dissolution dynamics of silver (nAg) and zinc oxide (nZnO) engineered nanoparticles (ENPs), and how this affects their uptake by aquatic higher plants. First, the influence of particle size and morphology on the stability of nAg and nZnO in Hoagland's nutrient medium will be investigated. The nutrient medium will be dosed with ENPs concentrations ranging from 1-1000 µg/L whereby the dissolved metal ions will be quantified using the ICP-OES with a detection limit of 1 µg/L. Dry powder ENPs will be characterised for size, size distribution, and morphology using TEM, surface area using BET, surface charge using Zetasizer, and surface crystallinity using XRD. In Hoagland's media, the ENPs hydrodynamic size and surface charge as well as particle counts will be determined using Zetasizer and NTA, respectively. Secondly, the study will investigate the uptake dynamics of nAg and nZnO by an aquatic higher plant *Spirodela punctuta*, and evaluate their toxicity over a 20 day period. The role of hydrodynamic size, particle morphology as well as media ionic strength on ENP uptake and toxicity will be examined. Uptake investigations will be undertaken through SEM imaging inspection of plant sections whilst OxiSelect assay kits will be used to evaluate catalase activity, protein carbonyls, and free radical activity to ascertain potential ENPs linked induced toxicity. Through timeous analysis of chemical and physical state of ENPs, the proposed study envisages to generate detailed data useful to support sound hazard assessment and ultimately the risk assessment of metallic- and metal oxide-ENPs towards higher aquatic plants.

Abbreviations: engineered nanoparticles (ENPs), Inductively Coupled-Optical Emission Spectroscopy (ICO-OES), transmission electron microscope (TEM), X-ray diffraction (XRD), Brunauer-Emmett-Teller (BET), nanotracking analysis (NTA), scanning electron microscope (SEM).

Keywords: metallic nanoparticles, nanoparticle characterisation, particle stability, risk assessment

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Effect of carbon nanospheres on algal growth: the influence of physicochemical properties and environmental chemistry

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Abstract

Algae (*Pseudokirchnierella subcapitata*) were exposed to pristine (p-) and oxidised (o-) forms of carbon nanospheres (CNSs) at concentrations of 0.1, 0.18, 0.32, 0.46 and 20 mg/L. At each concentration, the CNSs were sonicated for 1-h in three types of water (artificial soft, moderately hard, and hard water) for 72-h at 24-h intervals: (i) in the absence (0 mg/L) of humic acid, and (ii) presence of 5 mg/L humic acid at neutral to slightly alkaline pH. The study findings suggested that, algal growth in the presence of humic acid was visible to the human eye, however, in the absence of the humid acid; the growth could only detected using a light microscope. Additionally, in the absence and presence of humic acid in all concentrations of o-CNSs algal growth was established in soft water, moderately hard water, and hard water as highest, moderate, and inhibited, correspondingly. Similar trend was observed for p-CNSs with a distinctive difference in that the growth inhibition was concentration (dose) dependent. The exposure suspensions were then monitored for CNSs' aggregate size and surface charge changes over the same exposure period under similar environmental conditions. We observed that the p-CNSs dispersed with difficulties unlike the oxidised forms that were easily dispersed. Our findings suggested that the presence of humic acid and water hardness were factors that influenced CNSs dispersion in water. Moreover, the high surface charge (≥ +30 mV) was also established as an additional factor in enhancing the ease of CNSs dispersion in the aquatic environment although for the pristine form re-aggregation was observed as a function of time. In summary, we established that the algal growth inhibition and/or stimulation by CNSs is largely dependent on the presence or absence of humic acid, degree of water hardness as well as the CNSs' concentration, and surface functionalization.

Keywords: carbon nanospheres, water hardness, algae, aggregate size, surface charge

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