Risk Assessment on Nano- and Macro-Scale Emerging Contaminants in Freshwater Systems Using Experimental and Modelling Techniques

Final Report – Volume I

Report to the
WATER RESEARCH COMMISSION

by

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The outcome of this project is presented in two volumes. This is Volume I. The other report is WRC Report No. 2509/2/22).

EXECUTIVE SUMMARY

BACKGROUND

The increasing presence of different classes of emerging contaminants (ECs) in the environmental systems together with largely their unknown impacts to the environment and human health is of great concern. For example, these ECs may adversely affect the ecological lifeforms as well as the quality of drinking water. Currently, among the emerging contaminants of concern include engineered nanomaterials (ENMs), pesticides, and personal care products (PCPs), amongst others. To date, there is limited data on the fate and effects of ECs particularly the categories, namely ENMs, pesticides and PCPs with specific reference to their mixtures in the ecological systems. Although it is challenging to address the impacts of individual chemicals in the selected classes, particularly in the environment, their co-existence as mixtures, their interactions with each other as well as the influence of abiotic factors render evaluation of their fate and toxicity highly complex. Of importance to note is the fact that such studies in South Africa were lacking for both individual and their mixtures.

Thus, in order to effectively manage these chemicals motivates the need to carry out a systematic evaluation of their exposure and hazard both for their individual chemicals as well as their resultant mixtures. This project aimed to investigate the effects, distribution, fate and behaviour of emerging contaminant mixtures using experimental and modelling tools. In the first phase of the project, the findings are reported herein, in Volume I. More specifically, we outline findings on the individual and mixtures of ENMs and PCPs associated with their exposure and hazards in the aquatic systems. The reported fate and toxicity results of these contaminants are useful for exposure- as and hazard-assessments, respectively, which are the key component in undertaking systematic risk assessment paradigm.

AIMS

The key objective of the project was to investigate implications of both individual and mixtures of ECs in the ecological systems. Consequently, the project's outcomes have the potential to contribute towards long-term minimization, elimination, and/or improve management of water quality impacts from individual and mixtures of ECs of focus in this project. This was to be achieved by undertaking the following objectives, viz.:

1. Conduct a systematic review on the fate and effects of ECs mixtures in aquatic systems with specific reference to part or whole of constituent chemicals.
2. Investigate the fate, behaviour, and effects of ECs mixtures in freshwater linked to their chemical compound specific properties and reactivity as influenced by abiotic and biotic factors.

3. Apply modelling techniques and prioritization approaches to predict the distribution, exposure pathways and risk profiles of selected ECs in water, sediments, and soils compartments.

4. Develop decision-support models to aid presentation of data in a format useful to different users, e.g. decision- and policy- makers and water professionals.

The results presented in Volume I are envisaged to achieve objectives 2 to 4. Objective 1 will be addressed comprehensively in Volume 2 since the endeavour to review the current literature on the subject was found to be characterised by huge data gaps, and in this field is increasing at very slow pace though the usefulness of such data is apparent as the results presented in this report indicates. In addition, Volume 2 will address aspects related to pesticides and their mixtures with ENMs.

FATE AND TRANSFORMATION OF ECs IN FRESHWATER SYSTEMS

To fully account for chemicals exposure in the environment, motivates the need to understand their transformation as well as their interactions among themselves within the same classes or with other classes of chemicals as influenced by the water chemistry. Herein, we investigated the fate and behaviour of ECs of individual and chemical mixtures in freshwater sourced from two rivers in South Africa. The focus in this first volume was on PCPs (TCS-triclosan) and ENPs (aluminium oxide (nAl_2O_3) and copper oxide (nCuO) nanoparticles).

Data on fate and transformations of ECs is useful as it offers insights on the degree to which individual chemical contaminants or their mixtures interacts with aquatic organisms and, in turn, induce observable effects. Such effects, for example, are dependent on the chemical's stability and likely final compound metabolites formed linked to their transformation. This is because, bioavailability of toxicants depends on their fate in aquatic systems which in turn influences their toxicity potential within a given set of water chemistry characteristics. Therefore, in this project fate and transformation of the constituent chemicals and their mixtures (binary) were determined. Since the classes of ECs of focus are diverse; viz. ENPs (metal oxides), and organic pollutant (TCS in this case) a suite of tools and techniques were used to elucidate their transformation. For ENPs, key environmental processes considered in this work were limited to dissolution, adsorption, and aggregation/de-aggregation. First, this was done for the individual ENPs, and then as their binary mixtures of ENPs-ENPs and finally a mixture of ENPs-organic pollutant.

From dissolution studies, it was found that nAl_2O_3 in freshwater (sourced from two rivers) exhibited very high dissolution (almost 100%) within the first 2 h at lower concentration of 0.1 mg/L in Elands River; but only increased from *ca* 50% after 2 h to *ca* 70% at 48 h in the Bloubank River. Additionally, as the nAl_2O_3 concentration increased, a decrease in dissolution was apparent. For nCuO, similarly higher dissolution was found at lower concentration of 0.1 mg/L than at higher concentration of 1 mg/L, and no observable change in dissolution at 0.1 mg/L nCuO was apparent over 48 h. However, at 1 mg/L dissolution increased from 2 to 48h, and the detected concentration of copper ions were $\leq$ 1% irrespective of the exposure concentrations (0.1 and 1 mg/L). Thus, dissolution was found to be dependent on the type of ENPs (based on their inherent physicochemical properties), water chemistry, and exposure concentration for the individual ENPs. Results on the aggregation were found to indicate that aggregation of ENPs were concentration dependent with 10 mg/L having the highest hydrodynamic diameter (HDD), and least at 0.1 mg/L regardless of the ENPs (nAl_2O_3 or nCuO). Overall, the low HDD observed in this work were linked to their adsorption of NOM onto the ENPs, which in turn, induced a net negative surface charge thus increasing interparticle repulsions leading to stabilization of ENPs via electrostatic and/or steric repulsion mechanisms. This implies likely longer residence of ENPs in the aquatic systems, and therefore, increases the possibility to interact with water dwelling organisms, e.g. *Daphnia magna* and fish.

Findings on the aggregation of binary mixtures for ENPs simulating variation of nCuO concentration at constant concentration of nAl_2O_3 indicated that HDD of the binary mixtures were greater to that of individual ENPs at corresponding concentrations, and decreased as the concentration of nCuO increased. Conversely, at constant concentration of nCuO under variant concentrations of nAl_2O_3, similarly, HDD increased with the concentration of nAl_2O_3; but the values were slightly higher especially at highest the concentration. Thus, the findings indicate that the fate of ENPs mixtures in a given exposure media (particularly freshwater environments) are distinctive from those of individual ENPs. ENPs mixtures may agglomerate (evidence of higher HDD) and sediment in the aquatic system and interact with benthic organism or maybe stable and interact with organisms within the water column as influenced by the exposure media chemistry as the results in this study illustrates.

Consideration of binary mixtures of ENPs and organic contaminant (in this case TCS), for the case of nAl_2O_3/TCS mixtures, irrespective of the constituent mixture ratios, and exposure media water chemistry, the resultant ENPs were highly stabilized as indicated by lower HDD values, and being similar across all cases investigated. Thus, TCS was found to stabilize the nAl_2O_3. For the nCuO/TCS mixtures, the role of exposure media was evident where in one river water media, the mixture HDD was higher that of the nCuO. This phenomenon was attributed to the competition of the ENPs surfaces essential for adsorption; which in turn, led to stabilized ENPs. However, in another media, irrespective of the constituent mixture ratios, HDD decreased, and the nCuO were highly stabilized. In light of these findings, the interactions of ENPs and the organic pollutant (in this case TCS) were dependent on water chemistry of the exposure media.

EFFECTS OF ECs TO MICROBIAL ORGANISMS IN FRESHWATER SYSTEMS

Optimal functioning of microbial communities in soil, sediment, and aquatic systems is highly essential, for example, it sustains ecological integrity through processes like organic matter decomposition, nutrient cycling, and bioremediation of environmental pollutants. Therefore, the toxic effects of individual and mixtures of ECs to microorganisms, and, in turn, the microbial responses were investigated. Here, the key is to establish whether individual and mixtures of nanoparticles and an organic can alter the functioning of microbial communities, and thus, likely associated implications to ecology.

In brief, bacterial susceptibility to zinc nanoparticles (nZnO) and iron oxide nanoparticles (γ-nFe_2O_3) were investigated in two natural water samples (ER and BR) to aid elucidate ENPs toxicity in environmentally relevant media. Cell viability, cell membrane integrity, adenosine triphosphate levels and reactive oxygen species (ROS) production end points were evaluated to determine the effects of individual ENPs on the bacteria. In addition, dose-response curves of ENPs mixtures as well as with a TCS were used for predictive modelling to determine respective binary mixture toxicity.

Results of individual ENPs exposures indicated diminished cell viability, cell membrane integrity, and ATP production in ER compared with BR water for nZnO exposures. However, γ-nFe_2O_3 showed very low or no cytotoxicity to microorganisms at exposure concentrations used in this work for any of these end-points considered. ENPs toxicity was also found to be influenced by the water chemistry linked to the source. Primarily, the ionic strength and natural organic matter (NOM) in both river systems influenced the aggregation and dissolution of ENMs, and in turn: either (i) mitigated the toxicity of nZnO as was observed in BR and for γ-nFe_2O_3 in both water samples, or (ii) enhanced the toxicity of nZnO in ER water. ROS production was observed to be negligible for both ENPs; thus ruling out oxidative stress as the cause of observed toxicity. Since no interactions of nZnO and bacteria were observed from microscopic techniques, suggested the observed toxicity of nZnO likely to be driven by the release of Zn^{2+}, which in turn, the water chemistry was the key determinant as evidenced by the differences in dissolution results between the two river water samples. And, by using several endpoints to assess the toxicity of ENPs showed insights on discrete effects even in cases where there are no apparent responses, for example, at whole organism such as cell viability.

With respect to the mixtures investigations, nZnO/TCS mixtures were established to exhibit either synergistic or simple addition effects in both river water systems, at higher concentrations with reference to the estimated toxic units (TU) based on the mixture effective concentration (EC) values derived from experimental studies. However, at lower exposure concentrations, the mixtures results pointed to antagonistic effects. The differences in the mixture effects exhibited by the nZnO/TCS mixtures were linked to the concentration-combination of the constituent components and the inherent properties of water chemistry under the mixture conditions considered.

RISK ASSESSMENT ESTIMATION USING MODELLING APPROACHES

To estimate the potential risks of individual and mixtures of ECs, modelling tools were applied to aid undertake first screening exercise especially where data gaps were identified. Choice of the modelling tool used in each study was problem specific in order to derive meaningful findings for the targeted audience. In Volume 1, modelling techniques and prioritization approaches were applied to predict the distribution, exposure pathways and risk profiles of selected ECs, namely several ENPs (e.g. nZnO, nAl_2O_3, $nTiO_2$, etc.), and two commonly used antibacterials in PCPs (triclosan (TCS) and triclocarban (TCC)) in water, sediments, and soils compartments. In particular, the first two studies entailed the development of inventory of products in South Africa, viz.: for the antibacterials and nanoproducts. Subsequently, the data in a specific inventory were used to quantify amounts of likely releases for each specific ECs into different environmental compartments, and potential risks under different scenarios. Scenarios were defined by a set of factors linked to use(s) pattern of a given products containing certain EC(s), market penetration, compartment of focus (freshwater, soils, etc.), removal efficacy in wastewater treatment plants (WWTPs), among other factors. And finally, a decision-support systems (DSS) was developed to aid present data in a format usable to different users, e.g. decision- and policy-makers; and water professionals.

The inventory database developed in this study had 128 and 52 products that contained TCS and TCC, respectively, in the South African market. Results indicated that TCS-containing products were in three broad categories, and distributed as follows: 115 were PCPs (89.8%), 8 were kitchenware (6.3%), and 5 were classified as others (pet care, office accessories, and first aid) (3.8%); whereas for the TCC-containing products, all were in PCPs category. To estimate the quantities of TCS and TCC likely to be released into different environmental systems; only 88 (68.8%) and 45 (86.5%), respectively, of the products in the inventory were used majorly due to inaccessibility of data to determine their market penetration in South Africa, and or were non-PCPs products, e.g. office accessories.

The distribution of TCS and TCC in the environmental compartments (e.g. wastewater, landfills, sediments, freshwater, etc.) were quantified using the mass flow analysis approach (MFA) through the estimation of predicted environmental concentrations (PECs). The PECs were estimated to aid risk characterization by calculating risk quotients (RQ) owing to lack of reported measured environmental concentrations (MECs) of TCS and TCC in South Africa. TCS and TCC in freshwater had RQs > 1 based on estimated PECs with wide variations of *ca* 2 to 232 as performed across three dilutions factors (1, 3, and 10) considered in this study; an indicator of their likely adverse effects on freshwater organisms. In untreated and treated wastewater, TCS RQs values for bacteria were > 1, but < 1 for TCC, implying the former may adversely affect the functioning of wastewater treatment plants (WWTPs); but with no plausible impacts from the latter. In terrestrial systems, RQ results for neither TCS nor TCC revealed no, or limited risks; therefore, additional investigations are required on their toxicity, as effects data was very limited and characterised by wide variations. Hence, future national monitoring programs in developing countries such as South Africa should consider including TCS and TCC as the results suggest both chemicals are of concern to freshwater, and TCS in WWTPs. Potential risks of their metabolites remain unquantified to date although some are known to be more lethal to aquatic organisms compared to their parent chemicals.

Similar to the approach adopted for the development of inventory for products containing TCS and TCC in the South African market, development of an inventory was extended to nanoproducts (products containing ENPs). Inventory analysis revealed that a total of 2002 nanoproducts were likely to be present in the South African market; where some products had confirmed status of ENMs inclusion, whereas others due to data inadequacy were of intermediate status, and the last category was of unknown status. Moreover, these products were in six major nanoproduct categories in the South African retail market, namely: automobile (AUTO); electronics, appliances and accessories (EAA); food and beverages (FB); fitness and textiles (FT); medical (MED), and personal care products (PCP). The

PCP, for example, accounted for 54% of all products in the inventory consisting of nine product types; whereas EAA accounted for 26% under 13 different types, and AUTO contributing 9.6% of total products in the inventory. The remaining 9.9% were made of FB, FT and MED product categories but none had confirmed ENMs. EAA category had most products with ENMs where over 65% of all products in this category were confirmed to contain ENMs, for example, in cameras, personal computers, laptops, mobile phones, printer ink, printer toner, rechargeable batteries, televisions, and washing machines. Only 21% of products found in the PCP category contained ENMs, for example, in bar soaps, conditioners, shower gels, sunscreens, toothpastes, etc.

Based on consumer nanoproducts categories in the South Africa market, quantities of different ENMs likely to be released into the environment were estimated. In this case, income per capita and the concomitant quantities from a particular income group were estimated. Of the four income groups (very low, low, medium and high) considered herein, main contributor of ENMs into the environment were the *medium* income group through consumption of nanoproducts accounting on average 61.5% of all ENMs considered in this study. More specifically, estimated quantities of ENMs linked to usage of nanoproducts by the *medium* income group were: 82.8% of Ag, 74.8% of SiO_2, 61.9% of ZnO, 60.6% of TiO_2 and 57.7% of Al_2O_3; yet this group accounts for 15% of Gauteng Province (GP) population; and 44% on average shop market share. Based on the estimated ENMs releases into the environment, the dominant ENMs quantities in descending order (based on most likely mass) as TiO_2 > SiO_2 > ZnO > Al_2O_3 > Ag > CNT. Thus, these findings can serve as valuable source of information required to estimate the potential risks of ENMs, and in turn, identified ENMs of concern to the South African environment. Secondly, the data can aid to determine the level of human exposure of ENMs in South Africa; and likely implications to human health, an aspect yet remain to be quantified in this country.

In an endeavour to support decision making, accessible data and information on exposure potential of ENMs was solicited from the published literature, and used to develop a decision support systems (DSS) based on fuzzy algorithm. In general, the DSS are a specific class of computerized information system that supports decision-making activities at institutional or organizational level (private or public). The proposed and developed DSS aided to assess current information/data on aspects related to fate and transformation of ENMs in the aquatic systems. The fuzzy based system results derived in this study were an attempt to mimic closely human experts' reasoning approach in domains characterized by high degrees of data uncertainly and ambiguity particularly defined by both qualitative and quantitative attributes such as the exposure potential of ENMs. The developed fuzzy logic systems is deemed to be robust, transparent, and suitable as a computational tool to aid first-level screening tool to estimate the exposure potential of ENPs in the aquatic systems. Moreover, the modular development approach (without the need to re-configure the entire model) used in this work render the model to be flexible and transparent where new data can be easily incorporated in the knowledge-rule base, and can allow additional input parameters be added as new data becomes more accessible. Given the costly and time-consuming nature of experiments on fate of ENPs in the environment, the DSS can provide early warning system of likely adverse implications of ENPs, and in turn, can provide science-evidence to trigger the development of proactive measures as means to protect the aquatic lifeforms.

HUMAN CAPITAL DEVELOPMENT

In terms of human capital development, 3 PhDs and 2 MSc postgraduate students have been recruited, and directly works in this project. Moreover, two PhDs are expected to complete and submit their thesis during 2019/20 academic year, and one of the MSc students is considered for conversion into a PhD due to the quality and quantity of data generated during the course of his Masters project. Moreover, to widen the scope of human capital development and create a health pipeline of future students, and equip future workforce on aspect on ECs, 22 undergraduate students have been recruited and assigned short projects within this project over the last three academic years. Another six undergraduate students have been recruited in 2019/20 academic year, and their titles, and contributions will be included in Volume 2. Details of the human capital development are provided in Appendix table 7-1 to 7-3.

KNOWLEDGE GENERATION AND DISSEMINATION

Due to dearth of knowledge and numerous data gaps regarding ECs in the environmental systems, raises the need for generate of new knowledge. Thus, using relevant platforms, data and knowledge were disseminated to various stakeholders. To date, 11 papers have been drafted where one has been published, currently two are under review, and eight are under preparation phase. The published article in Environmental Pollution Journal is among the few that have sought to estimate the risks of TCS and TCC especially in the developing countries, and for the first one in the world for TCC to the author's knowledge. Notably, in this report, among the results generated are included in various chapters of this report. Also presentations were made in international and national conferences and/or symposiums. A full list of knowledge and dissemination activities are listed in Appendix table 7-3 and 7-4.

CONCLUSIONS

From this study several conclusions regarding the fate, effects and implications of emerging contaminants in the environmental systems based on modelling and experimental approaches. These include:

- There is evidence of nanoproducts in the South African commerce, and are likely to be higher as many of them are of unknown status ENMs. These include products in our everyday lives and show no signs of slowing down regarding market penetration in light of projected world production predictions.

- With the MFA model, data on nanoproducts and other sources were used to estimate likely releases of ENMs from the nanoproducts into the environment and the largest quantities of ENMs were in descending order (based on most likely mass) as TiO_2 > SiO_2 > ZnO > Al_2O_3 > Ag > CNT likely to be discarded into the GP environment.

- Using MFA models, the potential risks of TCS and TCC were estimated by calculating risk quotient (RQs) where in freshwater for both chemicals, the RQs values were greater than 1 based on estimated PECs with wide variations (≈2-232) at different dilutions factors (1, 3, and 10); thus pointing to likely adverse effect of both chemicals in freshwater systems. However, in untreated and treated wastewater, the RQs for TCS to bacteria were greater than 1, but less than 1 for TCC. Thus, TCS is likely adversely effects of TCS to WWTPs. No effects were likely to be induced in terrestrial systems, since RQ results for individual chemicals revealed no or limited risks since RQ << 1.

- For the first time worldwide, TCC risks in the environment were quantified using bottom-up approach in this study; and the level of market penetration of TCS and TCC in a region (in this case Gauteng Province) has significance on the likely levels of risk.

- Given scientific concern on the implications of ENMs and triclo-based chemicals to the environmental systems, databases on products containing these chemicals were established. These databases to estimate the plausible quantities of these nanoscale- and macro-based chemicals in the environmental systems.

- Studies on the fate and transformation of individual and mixtures ENMs (between two ENMs, and one ENMs and organic pollutant (TCS)) revealed high variability as influenced by water chemistry. Thus, individual ENMs were found to be more stable in freshwater; however, binary ENMs mixtures were unstable compared individual ENMs. In addition, the organic pollutant (TCS) was found to exert stabilizing effect on the ENMs, and the degree of stabilization was water chemistry dependent.

- Study findings indicated the influence of water chemistry of freshwater (from different river systems) on the stability and toxicity of metal-based ENPs (nZnO and nFe2O3) to bacteria where enhanced and mitigated effects were observed for nZnO and nFe2O3, respectively.

- Use of both whole body and sub-lethal end-points revealed effects on the latter for nZnO but none to former on bacteria.

- Mixtures effects on bacteria were either synergistic, additive, or antagonistic associated with marked differences in water chemistry properties, and concentrations ratios in a given mixture matrix.

RECOMMENDATIONS

- A temporal and spatial MFA is key in effectively estimating the potential risks of chemicals in specific regions as shown in this study for triclo- and nanoscale-based chemicals. Therefore, it is essential to consider the development of dynamic material flow analysis (DMFA) model coupled with together spatial analysis which incorporates water flow, water bodies, population densities, income densities, etc. to refine the flows, and in turn, the potential of contaminants as those reported in this study.

- Using the results reported herein based on material flow, the scientific community together with other stakeholders, for example, in the case of ECs considered make a case with regards to regulation, and monitoring programmes in the environment especially in regions suspected and/or confirmed as likely hot-spots for these pollutants

- MFA results offer insights that can be used to develop future national monitoring programs in South Africa, by considering the inclusion of TCS and TCC as well as ENPs as a measure to undertake systematic risk analysis at different aquatic systems, e.g. freshwater, wastewater treatment plants and sediments.

- Future studies on the fate and behaviour of mixtures between ENPs and organic pollutants needed to others not covered in this work in an endeavour to gain a better understanding on the mechanisms and wider implications in the ecological systems.

- For mixture toxicity studies, further works is desirable using other model organisms such as fish and *Daphnia magna* found in freshwater, and the effect of water chemistry and use of multiple endpoints to ascertain the veracity of findings derived for bacteria

- Other factors such as illumination should be considered for future work to provide meaningful outcomes of possible transformations ecological effects of the ENMs and triclosan under such conditions, for example, triclosan generally transforms to methyl triclosan; where the latter’s effects are poorly accounted both as individual and mixture with ENPs.

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ACRONYMS & ABBREVIATIONS

BR	Bloubank River
CA	Concentration addition
DI	Deionised water
DOC	Dissolved organic carbon
EC	Effective concentration
ENMs	Engineered nanomaterials
ENPs	Engineered nanoparticles
ER	Elands River
GDP	Gross domestic product
HA	Humic acid
HCl	Hydrochloric acid
HDD	Hydrodynamic diameter
IA	Independent action
IS	Ionic strength
MECs	Measured environmental concentrations
MFA	Material flow analysis
MgL^{-1}	Milligrams per litre
mM	Millimolar
mV	millivolts
nAl_2O_3	Aluminium oxide nanoparticles
NaOH	Sodium hydroxide
nCuO	Copper oxide nanoparticles
nFe_2O_3	Iron (III) oxide nanoparticles
Nm	Nanometre
NOM	Natural organic matter
nZnO	Zinc oxide nanoparticles
OD	Optical density
PCPs	personal care products
PECs	Predicted environmental concentrations
pH	Potential of hydrogen
PXRD	powder X-ray diffractometer
PZC	Point of zero charge
Rpm	Revolutions per minute
SD	Standard deviation
SEM	Scanning electron microscope
sp-ICP-MS	Single Particle Inductively Coupled Plasma Mass spectrometry
TCC	Triclocarban

TEM	Transmission electron microscope
TU	Toxic unit
XRD	X-ray diffraction
ZP (ζ)	Zeta potential
γ-Fe_2O_3	Gama Iron (III) oxide nanoparticles

CHAPTER 1 : BACKGROUND

Ndeke Musee

1.1. PROJECT BACKGROUND

Development, distribution and use of chemicals is an integral part of our daily lives. This is to meet diverse societal needs. Examples of the societal needs include: first, meet food exponential demand due to rapidly burgeoning population through improved yield per unit area by use of pesticides, fungicides, etc. in the agricultural sector. Secondly, cure multiple forms of diseases using pharmaceuticals and drugs as is evident in the medical field. Thirdly, maintain personal hygiene, e.g. by use of personal care products; and finally, clean-up polluted ecological systems by employing remediation chemical agents just to mention a few among numerous applications of chemicals in modern society. Besides these well-known beneficial uses of chemicals to society, both their intended and incidental releases to the environment have had, and continue to exert unintended consequences to ecological lifeforms at different trophic levels. This aspect, has raised concerns to various stakeholders, and particularly for chemicals which are not routinely monitored in the environment; but are also outside the preview of present legislative jurisdictions globally (Petrović et al., 2003).

Therefore, to balance scales of chemicals benefits use to society vs their potential risks to the environment has motivated the need to examine the latter aspects. Whereas most studies on chemicals effects and fate have focussed on individual chemicals at a time, however, following an entry of a given chemical into in the environment (Heys et al., 2016) – leads to interactions with other chemicals but also are transformed due to influence of numerous abiotic conditions. Therefore, to generate information useful to support effective management of chemicals in the environment; make it important to focus on the effects, fate and transformation of chemical mixtures as opposed to individual chemicals. Herein, chemical mixtures' refers to combined exposure to multiple chemicals (EFSA, 2013) in a given environmental systems such as freshwaters, sediments, soil, among others.

For the purposes of this project, the chemicals of interest are what generically regarded as emerging contaminants (ECs). The US Environmental Protection Agency (USEPA) defines an emerging contaminant as a chemical or material with perceived, potential, or real threat to the human health or the environment or for which published health standards are lacking (USEPA, 2008). Emerging contaminants, to date, are numerous and Table 1 lists some examples under different classes, which by no means are not deemed exhaustive. Of significance to note is that the list of ECs grows rapidly yearly primarily due to scientific and technological advances where new chemical formulations are introduced into commerce. Thus, the challenge is to elucidate the effects and fate of ECs within the context of mixtures; in addition to the extent how such data can be utilized for prioritization purposes. For example, with respect to establishing monitoring and assessing programmes in real environmental systems, for example, by regulatory authorities in South Africa such as Department of Water and Sanitation (DWS) and Department of Environmental Affairs (DEA).

Table 1-1: Examples of emerging contaminants under different classes.

Pharm.	**PCPs**	**Pesticides**	**ENMs**	**Surfactants**
Anticonvulsants/ Anti-epileptics	*Antimicrobials*	*Acaricides*	*Metal-based*	*Non ionic*
Carbamazepine	Triclosan Triclocarban Biphenyl	Azobenzene Benzoximate Benzyl benzoate	Ag Au Cu Pt Pd	Dodecyl dimethylamine oxide Coco diethanol amide alcohol ethoxylates
Beta-blockers	*Insect Repellent*	*Avicides*	*Metal oxides-based*	*Cationic*
Atenolol Propanolol Metoprolol	N,N-diethyl-m-toluamide (DEET) 1,4-dichlorobenzene	4-aminopyridine Endrin chloralose	TiO_2 CuO ZnO CeO_2 Al_2O_3 Fe_2O_3	Stearalkonium chloride Benzalkonium chloride.
Analgesics and anti-inflammatories	*Nitro musk*	*Herbicide*	*Carbon based*	*Anionic*
Ketoprofen Naproxen Ibuprofen Diclofenac Acetaminophen	Musk ambrette Musk ketone Musk moskene	Atrazine Allidochlor Benzadox amicarbazone	Fullerene SWCNT MWCNT DWCNT	Sodium linear alkylbenzene sulphonate (LABS). Sodium lauryl sulphate. Sodium luaryl et her sulphates
Lipid-lowering drugs	*Polycyclic Musk*	*Bactericides*	*Dendrimers*	*Amphoteric*
Bezafibrate Clofibrate Gemfibrozil	Celestolide Galaxolide Tonalide	Formaldehyde Hexachlorophene	Peptide Chiral Multilingual Frechet-type	Cocamidopropylbetaine Betaines imadazolines

Antibiotics	*Preservative*	*Fungicides*	*Quantum Dots*
Azithromycin Metronidazole Sulfamethoxazole	Isobutylparaben Methylparaben Benzylparaben	Butylamine Cymoxanil Dodicin	Group II and VI CdSe-QDs CdTe-QDs ZnO-QDs Group III and V GaAs-QDs InP-QDs

Abbreviations: Ag: silver, Au: gold, Cu: Copper, Pt: platinum, Pd: palladium, SWCNT: single-walled carbon nanotubes, MWCNT: multi-walled carbon nanotubes, DWCNT: double-walled carbon nanotubes, CdSe: cadmium selenide, CdTe: cadmium telluride, GaAs: gallium arsenide, InP: indium phosphide, ZnO: zinc oxide, PCPs: personal care products, ENMs: engineered nanomaterials; Pharm: pharmaceuticals

Among the features that define emerging mixtures of contaminants in the environmental systems, include: (i) unknown effects to organisms in different trophic levels, (ii) their fate and behaviour are poorly understood, or largely unknown, (iii) are generally found at fairly low level concentrations in the environmental matrixes, (iv) the exact composition of chemicals in the mixture is often unknown, and (v) not currently addressed by legislative frameworks as their potential joint actions are not easy to take into account. For example, the concentrations of emerging contaminants in different environmental compartments can be so low in the order of microgram (µg) and nanogram (ng) per litre (Yang et al., 2014; Sun et al., 2014).

For instance, we know that effect-based tools, environmental monitoring, and surveillance are among essential tools to accurately assess individual chemicals in the environment. The tools are used to generate data on occurrence, distribution, uptake, and consequences of exposure for individual chemicals to organisms in the environment. Despite that such data has been shown limited to predict the implications of consequent of well-studied chemicals; yet chemicals of focus in this project such data is chronically lacking even for the individual chemicals – although it is crucial for estimation of their potential impacts to the environment. Moreover, the agency of such data is more profound when they co-exist in the environment as mixtures.

1.2. SELECTION OF STUDY CHEMICALS FOR INVESTIGATION IN THE PROJECT

According to examples listed in Table 1, there is a large number of ECs. This implies, it is not practically feasible to predict that once the individual toxicity of each chemical is known, and falls within a certain concentration range, therefore, the overall mixture under question can be termed as “safe”. Conversely, this raises the possibility of numerous combinations of chemical mixtures, which, in turn, necessitates toxicity testing for such mixtures – which from a practical view point is impossible. As an example, an attempt to carry out toxicity testing or fate and transformation for each possible combination of mixtures (just based on based on chemicals in Table 1) is not practically feasible in a single project even in the next decade.

Therefore, in light of such constraints among other, three classes of ECs, in this project were selected for investigation, namely; personal care products (PCPs), pesticides, and engineered nanomaterials (ENMs). Three criteria were applied to select these classes of ECs. First, the extent of studies done for a given chemical class in the context of chemical mixtures. Here the PCPs were compared to pharmaceuticals (Brausch and Rans, 2011), and the former was found least studied. Secondly, wide use of a given class of chemicals in the South Africa as is the case for the selected classes, and whether they are classified as of significant environmental concern. Here in this project, we selected pesticides based on recent Water Research Commission (WRC, 2015) where pesticides were among the key emerging contaminants in ecological systems. ENMs were selected as they constitute recent post-2000 class of emerging contaminants (Yan et al., 2010; Kunhikrishnan et al., 2015; Musee, 2017), and, are broadly incorporated into other product, e.g. pesticides (e.g. nanopesticides) and PCPs which also constitute some of the classes of ECs, and are particularly poorly investigated in developing countries such as South Africa (Ebele et al., 2017) although are widely used and ubiquitously present in different environmental compartments as recently highlighted by Musee (2018).

The selection of chemicals from each specific class of ECs for inclusion or exclusion in this project was further refined by a set of additional sub-criteria. The sub-criteria consisted of (i) the breadth of chemical use or chemicals compared to other chemicals in the same class, (ii) individual toxicity of a given chemical in relation to other chemicals in the same class, and (iii) relevance of a given chemical based on published data within the South African context. For sake of clarity, the next sections outline how each of these specific sub-criteria were applied to select the specific chemicals to be investigated into this study. It should be noted that each chemical was not envisaged to meet all the three criteria but following satisfaction of one or more criteria for inclusion.

1.2.1. Engineered nanomaterials

For ENMs, titanium dioxide (TiO_2), silicon dioxide (SiO_2), cerium oxide (CeO_2), silver (Ag), and zinc oxide (ZnO) are among the most produced engineered nanomaterials worldwide and highly used in numerous consumer and industrial applications (Lee et. al. 2010; Piccinno et. al., 2012; Keller et. al., 2013; The Danish Environmental Protection Agency, 2014; PEN, 2016; The Nanodatabase, 2016). For this reason, the Organization for Economic Cooperation (OECD, 2010) highlighted ENMs that requires their potential risks to the human health and the environment be determined. Nano Ag, TiO_2 and nano-ZnO are widely used in cosmetics and sunscreens, paints, and coatings and electronic industries (Hall et. al., 2009; Yang and Westerhoff, 2014), and also widely incorporated in sunscreens, for instance, to protect human skin against UV radiation due to their UV radiation attenuation properties (Wiench et. al., 2009; Shi et al., 2012), or for bacterial protection (in the case of Ag). However, their continued use in PCPs such as cosmetic products had led to their release into the aquatic environments (Wiench et. al., 2009; Wiechers and Musee, 2010); though their impacts are yet to be fully quantified.

Moreover, production and applications of other metal oxide-based ENMs are on the increase both in consumer products and industrial applications. However, from the published literature there is limited data on these other types of metal-oxides ENMs potential hazards and exposure in the environmental systems. Hence, in order to contribute and address knowledge gaps, three additional ENMs were considered in this study, viz.: aluminium oxide (Al_2O_3), copper oxide (CuO), iron oxide (Fe_2O_3).

1.2.2. Personal care products

The PCPs are high volume produced antimicrobial agents (Simon et al., 2014) with triclosan (TCS) and triclocarban (TCC) being among the most widely used antimicrobial agents found in diverse consumer products categories (Clarke and smith, 2011). For example, TCS and TCC are widely used polychlorinated, binuclear, and aromatic antimicrobials (Halden and Paull, 2005; Carey and McNamara, 2015) estimated to be contained in over 2 000 different personal care products (PCPs), household, and medical products in the USA market (Young, 2013; Smith, 2013), and perhaps even higher (Halden, 2014).In addition, they are among the 10 top most commonly detected organic wastewater compounds ranked based on frequency and concentration (Kolpin et al., 2002; Halden and Paull, 2005).

Moreover, according to recent review of PCPs concentrations and toxic effects in the aquatic system – both TCS and TCS were ranked as most toxic (Brausch and Rand, 2011). For example, their no observed effect concentrations (NOECs) values were < 1 mg/L (Brausch and Rand, 2011, and numerous references cited therein); which makes them to be of concern since their toxicity can be ranked as very toxic based on Globally Harmonized System (GHS) of Classification and Labelling of Chemicals framework (UN, 2013). TCS and TCC ecotoxicological data show they can induce adverse effects including antibiotics resistance (Oggioni et al., 2013), alterations to microbial community structures (Carey and McNamara, 2015), and induction of high toxicity to the aquatic organisms compared to other disinfectants (Brausch and Rand, 2011).

1.2.3. Pesticides

Pesticides entails a wide breadth of any substance or mixture of substances used to prevent, destroy, repel or mitigate pests, or intended for use as plant regulators, among other uses (USEPA, 2006, 2008). Currently, the most common types of pesticides include herbicides, insecticides, fungicides, and bactericides. In the South African context, pesticides are among the major chemical pollutants in surface water (Ansara-Ross et al., 2012) owing to their extensive use and application in domains of agriculture, forestry, transportation (through control of flora and fauna), and various other industries. Recent findings on the prioritization of pesticides used in South Africa ranked atrazine as among the top 25 (Debrowski et al., 2014; WRC, 2015), and widely detected in the South African freshwater systems (Preez et al., 2005). Among the wide uses of atrazine as a herbicide in agricultural fields include control unwanted of weeds mainly in maize, sorghum, sugarcane and pineapple plantations

Atrazine is of concern to the ecological systems since it inhibits photosynthesis and interferes with enzymatic processes (Roberts, 1998); and yet its use has led to continuous release into the environment. Recently, nanopesticides (pesticides containing ENMs as an active ingredients) have been developed and are rapidly been into the rise in commerce (Kookana et al., 2014). Thus, the increasing interest in use of nanopesticides has also raised concerns in terms of how their potential environmental risks for regulatory purposes (Kah, 2015). Thus, in light of these developments, the choice of pesticides for inclusion in this project in freshwater systems became apparently clear. Studies on the implications of pesticides to the ecological systems will be addressed in Volume II report.

1.3. PROJECT AIMS

This project entails the use of both experimental and modelling techniques to achieve its objectives. The aims of the projects are;

1. Conduct a systematic review on the fate and effects of ECs mixtures in aquatic systems with specific reference to part or whole of constituent chemicals.
2. Investigate the fate, behaviour, and effects of ECs mixtures in freshwater linked to their chemical compound specific properties and reactivity as influenced by abiotic and biotic factors.
3. Apply modelling techniques and prioritization approaches to predict the distribution, exposure pathways and risk profiles of selected ECs in water, sediments, and soils compartments.
4. Develop decision-support models to aid presentation of data in a format useful to different users, e.g. decision- and policy-makers and water professionals.

1.4. SCOPE AND LIMITATIONS

Effects and fate of emerging contaminants are a subject of both national and global interest due to their potential to cause advance impacts to the environment. However, the combinatorial possibilities of chemical mixtures from the wide breadth of emerging contaminant classes make it impossible to study all the possible chemical mixtures. Therefore, for illustrative purposes, we selected four ENMs, two PCPs and one pesticide as chemicals of focus in this project. To date, there is increasing evidence that numerous different types of toxicity and fate studies can be done for any chemical in a given environmental system(s). Taking into consideration this aspect, the duration of the project together with the project budget, the scope of the studies to be carried are limited to the chemicals outlined in section 1.2. Toxicity and fate data reported herein are together with published literature are envisaged to inform systematic risk assessment of mixtures in the environment, and secondly, toadied in the development of skills urgently needed in this domain especially in South Africa through training of undergraduate and postgraduate students, as well as emerging scientists working in the project, and stakeholders. Whereas researchers acknowledge the importance of undertaking risk assessment of chemical mixtures in actual environmental compartments; however, these aspects were outside the scope of the current project.

1.5. REPORT OUTLINE

In this report, each individual chapter seeks to address certain objective of the project. Thus, an outline of specific chapters and the objective they contributed are outline.

Chapter 2 explores the use of data collection methods to develop an inventory of personal care products (PCPs), and their distribution in the South African commerce according to income categories. Next, the developed inventory was used to quantify the flows of TCS and TCC into the environment following their release during the use and disposal phases. The determined concentrations were then used as model inputs to assess the likely risks of TCS and TCC in different environmental compartments. Therefore, this chapter aided to map the distribution, exposure pathways of TCS and TCC as selected macro-scale ECs under the PCPs category. And finally, reports findings where compartments most likely to be adversely affected were identified, and thus can be prioritised as envisaged in objective 3 of the project.

Chapter 3 is concerned with the development of a conceptual framework on exposure assessment of ENPs in the aquatic systems; where key influencing parameters on ENPs exposure potential in aquatic systems were identified. Next, the chapter highlights on the development of a knowledge base and the inference mechanism based on fuzzy set theory. A case study is presented for the design and development of a fuzzy expert system where data for nZnO and $nTiO_2$ was used to illustrate the functionality of the developed model. And finally, the prototype was tested where the derived results are presented and discussed envisaged to reflect several plausible likely exposure scenarios of ENPs in the aquatic systems. The developed DSS in this chapter addresses the fourth project objective in support of management decisions by, for example, policy- and decision-makers.

Chapter 4, results on the development of a database for nanoproducts currently in the South African market are summarised. Data in the database was analysed where three products categories were identified, namely; nano-based, intermediate and unknown. Next, the developed inventory was used to quantify the flows of ENMs into the environment following their release during the use and disposal phases using the material flow analysis (MFA). From this approach the distribution and pathways of ENMs were identified and discussed. The determined concentrations in different environmental compartments were then compared to results developed using other techniques in the published literature, and hence, veracity was verified by way of discussing specific case studies. Thus, the findings in this chapter seeks to address partly project objective 3.

Chapter 5 examines the bacterial susceptibility to nZnO and nFe_2O_3 in two natural water samples to aid elucidate ENPs toxicity in environmentally relevant media. Whole body and sub-lethal effects such as cell viability, cell membrane integrity, adenosine triphosphate levels, and reactive oxygen species production were evaluated to determine the effects of ENPs on the bacteria. Additionally, dose-response curves of the two ENMs as well as a common antimicrobial, triclosan (TCS) were investigated to predict their mixture toxicity using concentration addition (CA), independent action (IA), and toxic unit (TU) models to bacteria. These findings address partly the third objective specifically parts 1 and 3 with respect to the effects of individual and mixtures of ECs to the bacterial community in freshwater systems.

Chapter 6 presents the aggregation and dissolution of individual, and binary mixtures of ENPs (ENPs/ENPs mixture matrixes) and/or ENPs with an organic contaminant (in this case TCS) as influenced by the water chemistry, and concentrations ratios in a given matrix. Findings in this chapter addresses project objective 2.

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CHAPTER 2 : MODELLING ENVIRONMENTAL RISKS OF TRICLO-BASED CHEMICALS FROM PERSONAL CARE PRODUCTS

Ndeke Musee

2.1. INTRODUCTION

Personal care products (PCPs) are widely used, and contains active polychlorinated, binuclear, and aromatic antimicrobials, namely: triclosan (2,4,4 -trichloro-2 -hydroxydiphenyl ether; TCS) and (non-phenolic) carbanilide triclocarban (1-(4-chlorophenyl)-3-(3,4-dichlorophenyl) urea; TCC) (Halden and Paull, 2005; Carey and McNamara, 2015). These additives are used in products such as deodorants, toothpastes, textiles, medical devices, etc. (Young, 2013; Smith, 2013). Both TCS and TCC are high volume chemicals (Richardson, 2009) as are produced annually in large quantities – although reported production quantities vary significantly – and in recent years they have received increasing attention as emerging contaminants of concern (ECC) owing to their ubiquitous presence in different environmental compartments, and adverse impacts to ecological biological lifeforms (Halden and Paull, 2005; Chen and Ying, 2015, Brausch and Rand, 2011; Musee, 2018). For example, estimates suggest that total annual usage of TCS and TCC was approximately 1 220 000 kg based on the consumption statistics of commodities in 2011 in the USA (Zhao et al., 2013) in about 2 000 different PCPs, household as well as medical products in the USA market (Young, 2013; Smith, 2013; Halden, 2014). In addition, similar high usage trends for these antimicrobials have been observed in other markets globally (APUA, 2011; Bedoux et al., 2012). In other studies, estimated annual global production volumes of TCC of up to 10 000 000 kg (TCC Consortium, 2002; TSCA, 2003; Miller et al., 2008); and about 1 500 000 kg for the TCS were estimated to enter into consumer markets globally (Singer et al., 2002).

Following the use of PCPs, both TCS and TCC are released into natural (e.g. freshwater, soils, sediments, etc.), and technical (e.g. wastewater, dams, etc.) systems. For example, TCS and TCC have been detected and measured (reported as measured environmental concentrations (MECs)) in different environmental systems like soils, treated wastewater, freshwater, and sediments at varied concentrations which are region or country dependent (as illustrated in section 2.1.1). As previously observed (Ebele et al., 2017; Musee, 2018), however, most MECs studies were done in developed countries, for example, USA (Yu and Chu, 2009), Switzerland (Lindström et al., 2002), etc.), UK (Sabaliunas et al., 2003). However, TCS and TCC are widely used in developing countries such as South Africa both in over-over the counter antiseptic wash products, and other daily use products as the inventory of products reported in section 2.3.2 – yet MECs remain largely lacking in such countries – although evidence indicate continued existence of these chemicals in commerce (Halden et al., 2017; Musee, 2018).

In developing countries, however, use of TCS and TCC (Halden et al., 2017) is on the rise despite both chemicals have been banned in the US effective from September 2016 (FDA, 2016). Moreover, a recommendation by the European Chemicals Agency (ECHA) formed the basis for the EU not to approve TCS for use in human hygiene biocidal products (ECHA, 2015; EC, 2016), and subsequently, no TCS has been incorporated in such products since February 2017 in the EU. The ban was motivated by the hazards of, and lack of sufficient evidence demonstrating the safety for long-term daily use with respect to reduction and/or prevention of spread of illness and infection by TCS and TCC (FDA, 2016; Halden et al., 2017). And, to date several international manufacturers of PCPs category have started to phase out TCS from certain products in jurisdictions they operate. However, similar ban or better regulation on these chemicals remain yet to be promulgated in countries like South Africa. Therefore, lack of data on the chemical quantities or and use information impedes the plausible quantification of their potential risks in the environmental systems.

The question that arises is then why TCS and TCC are of concern in the ecological and human systems to merit urgent attention particularly in South Africa? The underpinning reasons are numerous but not limited to: their wide use as antibacterials in consumer products (Young, 2013; Smith, 2013; Halden, 2014; Musee, 2018) with high concentration per article (w/w %) ranging between 0.3 and 1.5% (Musee, 2018), ubiquitously presence in aquatic systems (Hua et al., 2005; Sabaliunas et al., 2003; Musee, 2018), high toxicity to aquatic organisms (Chalew and Halden, 2009; Brausch and Rand, 2011; Tamura et al., 2013; Xu et al., 2015), biopersistence in the environment (Fiss et al., 2007; Clarke et al., 2016) since they do not biodegrade (Walters et al., 2010) among other factors discussed elsewhere (Halden et al., 2017, Musee, 2018). Moreover, TCS and TCC can potentially induce adverse human health impacts including: endocrine disruption effects (Witorsch and Thomas, 2010; Lee et al., 2014), birth defects (Geer et al., 2016), increased risk to obesity (Lankester et al., 2013), and likelihood for the proliferation of cancer cells (Dinwiddie et al., 2014; Winitthana et al., 2014).

Thus, in light of these facts, in this chapter, the overarching aim is to elucidate and quantify the potential risks of TCS and TCC in the natural and technical systems using a modelling approach owing to lack of MECs. This is because of the lack of environmental monitoring data that provides MECs essential to screen the likely risks of a given contaminant rapidly as to date, such tools are largely lacking for conventional ECs like those in the personal care products (PCPs) (Kolpin et al., 2002; Noguera-Oviedo and Aga, 2017). And the chapter will close by making several recommendations on future directions authorities should consider to adopt in dealing with emerging contaminations.

2.2. LITERATURE REVIEW

Owing to the breadth of the published studies on TCS and TCC both for their hazards and exposure in the environmental systems, literature review herein focuses on the data required to develop a model for their estimation of potential risks to the ecological systems. Thus, the review will seek to examine published studies on MECs and predicted environmental concentrations (PECs) in the environmental systems. Moreover, the toxicity of TCS and TCC reported in literature is reviewed although is not intended to be exhaustive but rather offer basis for hazard characterization in the model.

2.2.1. Environmental concentrations of TCS and TCC

Evidence of ubiquitous presence of TCS and TCC in the environmental systems is illustrated by numerous reported MECs and PECs values, and detailed accounts have recently been published (Montaseri and Forbes, 2016; Ebele et al., 2017), and therefore, will not repeated here. Rather, based on the retrieved scientific literature, only few examples are presented for illustrative purposes. Coogan et al. (2007) detected the presence of TCS and TCC as well as its metabolite methyl-triclosan (M-TCS) in algae samples collected around a wastewater treatment plant (WWTP) in Texas downstream at 1.1 and 3.8 km downstream of sewage downfall. Results indicated that although MECs in water samples were low ranging between 50 and 200 ng/L; but higher levels of 50-400 ng/g (w/w) fresh weight were detected in algae. The bioaccumulation factors (BCFs) in algae, respectively, ranged from (700-1 500), (900-2 100) and (1 600-2 700) for M-TCS, TCS and TCC (Coogan et al., 2007).

Monitoring studies in WWTPs and surface water in Northern Germany for TCS and M-TCS revealed detected concentrations in the ranges of between <3 and 10 ng/L, and between 0.3 and 10 ng/L, respectively (Bester, 2005). Additionally, both in influent and effluent higher concentrations of TCS were detected of 4 800-7 300 ng/L and 10-600 ng/L, respectively; indicating possible 87-95% removal efficiency in WWTPs (Bester, 2005). TCS was detected and reported in three rivers in China with values ranging from below limit of quantification (LOQ) to 478 ng/L whereas in the effluents values of 10.9-241 ng/L were measured. The concentrations of TCS were 15 times higher in the most heavily polluted river compared to the least polluted one; whereas high concentrations of 1 329 ng/L TCS were found in the sediments (Zhao et al., 2010).

Targeted National Sewage Sludge Survey indicated that in 84 sewage samples analysed they contained TCC, in the Greater Baltimore area (USA) with concentrations, respectively, of between 25 and 5 600, and 6 750 ng/L in river water and the wastewater (Halden & Paull, 2004). The TCC concentration for 49 streams were estimated to be in the range of 9-550 ng/L with mean and median values of 213 and 109 ng/L, respectively (Halden & Paull, 2005). TCC was detected and reported in three rivers in China with values ranging from below limit of quantification (LOQ) to 338 ng/L whereas in the effluents values of 23.9-342 ng/L were measured. The concentrations of TCC were nine times higher in the most heavily polluted river compared to the least polluted one; whereas high concentrations of 2 633 ng/L TCS were found in the sediments (Zhao et al., 2010). In another study in China, TCC in a river system was estimated at a concentration range of 32-382 ng/L (Wang et al., 2014).

In South Africa, however, only a handful studies have reported TCS MECs in wastewater, one in freshwater, but none in sediments, or soils (Table 9-5). Amdany et al. (2014) quantified TCS in the Johannesburg area with the concentrations in the influent and effluent found to be extremely high (78 460-127 700 ng/L) compared to other regions globally, and similar findings in the same region have been documented by Lehutso et al. (2017). Also, Madikizela et al. (2014) detected TCS in the influent and effluent in Durban (Table 9-5). The high TCS MECs reported in South Africa were attributed to wide use of PCPs, incorporation of high TCS concentrations in PCPs per article compared to other countries or other regions, and the country's WWTPs' low removal efficiency for chemicals since treatment plants operate above design capacity. However, in South Africa only a single study for TCC MECs values has been published to date (Table 9-5) (Lehutso et al., 2017), although TCC has higher affinity to accumulate in the environmental systems when compared to TCS (Brausch and Rand, 2011).

2.2.2. Toxicity of TCS and TCC to ecological organisms

To gain understanding on the hazard of TCS and TCC in the ecological systems, their toxicity data were collected from the published literature. The relevant data was derived for *Daphnia magna*, fish, bacteria, algae and earthworms (among other terrestrial organisms) to obtain a full view of hazards of these chemicals to organisms in different environmental compartments. Notably, only end-points that reflected the effects of TCS and TCC at population level, namely: survival, hatching, growth, reproduction, and development were included in the database both for acute and chronic toxicity expressed as, no-observed effect concentration (NOECs), lethal concentration (LC_{50}), and effect concentration (EC_{50}) values. Since, none of these data were uniform for all organisms whether acute or chronic; both types of data were used in the model.

The derived hazard in the dataset were used to calculate the predicted no-effect concentrations (PNEC); where if PNEC was found to be above the estimated environmental flows for these chemical concentrations; no action was required; However, in a case where the PNEC was below the emitted concentrations; implied necessity to triggering action as certain taxa may be considered to be at risk. More importantly, this means low PNEC does not imply high risk. Rather, given risk is a function of exposure (estimated or measured chemical concentration(s) in a given ecological system), and hazard (toxicity of a chemical); only when the chemical concentrations were low; then the consequent risk were deemed likely to be low, or insignificant.

2.3. METHODOLOGY: MODEL DEVELOPMENT

To develop a model that can aid to determine the risks of TCS and TCC in the environmental systems; four key aspects were considered, viz.: (i) definition of the problem system and its associated boundaries, (ii) development of an inventory of PCPs containing TCS and TCC in commerce in South African, (iii) estimation of TCS and TCC flows in different environmental systems, and (iv) using information and data in (i) to (iii), carry out risk assessment of both chemicals. In the following sections, each of these steps are summarised in order to provide the reader with insights on the results derived – as well as the suggested recommendations in an endeavour to advance our knowledge and decision making aimed to address the implications of TCS and TCC in the environmental systems.

2.3.1. Problem boundaries and modelling tool

2.3.1.1. Defining problem system boundary

To provide context on the results derived in this study; a well-defined system boundary that considered each chemical contaminant, to: (i) flow from grave to cradle; (ii) plausible several ways in which each waste streams are managed; and (iii) consider probable eventual impacts on natural (e.g. freshwater) and technical systems (WWTPs) was adopted. To achieve this objective, a granular approach was adopted to develop the model reported herein to allow adequate flexibility to make further modifications as new data become accessible, or to achieve a certain purpose and need within the confines of a specific case study. A temporal boundary of one year (2014) was used; which can easily be extended to previous and future years subject to data accessibility and study purpose.

The provincial administrative boundaries in South Africa were considered as system boundaries for the MFA modelling done in this work. The Gauteng Province (GP), one of the nine provincial administrative regions in South Africa was chosen as the system boundary to estimate TCS and TCC risks. The choice of GP was based on four reasons:

(i) having better input data available required for model development compared to other administrative regions in the country,

(ii) being most urbanized region in South Africa, has high income per capita (second to Western Province (WC) (Table 2-1), contributes about 33% of the country's GDP, its inhabitants have high income per capita in South Africa – both as individuals and households (Statistics South Africa, 2015; Musee, 2017),

(iii) aid to focus a specific region to aids to bring into sharp focus on end-of-life (EoL) issues, in this case, for TCS- and TCC-containing products in pursuit of formulating evidence-based approaches to protect the environment where specific aspects unique to a developing world country like South

Africa are into account as well as marked differences concerning PCPs consumption trends driven by income disparities in a specific region,

(iv) the province has the smallest land area (about 1.49% of South Africa total area), home to the largest population per province in South Africa with estimated population density of 726 persons/km^2. It is important to note that although WC have the highest income per capita (Table 2-1), however, its population was considered highly distorted as higher percentage only habits along the coastline with the inland defined with very low population density as most areas are under agricultural land, and

(v) is served by a network of WWTPs and sewage compositing facilities (Musee, 2011, 2017).

Table 2-1: Average household income (HI) per Provinces in South Africa (in Rands) (Source: Statistics South Africa, 2015).

Province	Area (km^2)	Population	PD (people/km^2)	HI (Rands)	HI/HI$_{(RSA)}$
Gauteng	18 178	13 200 300	726.17	193 771	1.76
Mpumalanga	76 495	4 283 900	56.00	107 561	0.98
KwaZulu-Natal	94 361	10 919 100	115.72	101 088	0.92
North West	104 882	3 707 000	35.34	86 926	0.79
Limpopo	125 755	5 726 800	45.54	79 152	0.72
Western Cape	129 462	6 200 100	47.89	222 959	2.03
Free State	129 825	2 817 900	21.71	98 529	0.90
Eastern Cape	168 966	6 916 200	40.93	90 156	0.82
Northern Cape	372 889	1 185 600	3.18	103 912	0.95
South Africa	1 220 813	54 956 900	45.02	138 168	1.26

HI$_{RSA}$: average household income in South Africa: PD: population density.

2.3.1.2. Modelling tool

Among the widely used approaches to track the mass-flows of contaminants in the environment from production to disposal phases; or final sinks in different environmental compartments is the materials flow analysis (MFA) (Brunner and Rechberger, 2004) – based on the concept of substance flow analysis (SFA) (Baughman and Lassiter, 1978). These deterministic models hinged on the law of mass conservation to estimate temporal influent, matrix and sub-matrix flows, and effluent loads of chemical pollutants in the environment – based on lifecycle analysis concept. A distinctive advantage of MFA models is the ability to track materials from production and manufacturing to use, followed by end-of-life stages and finally disposal. In particular, for any given stage of TCS and TCC in the products, the MFA model were used to aid identify how much each was released into different technical or environmental compartments. However, for the MFA models to yield useful outcomes, datasets on the production, use, and disposal of a given product are required. Therefore, in this case, market information required to estimate the input concentrations of TCS and TCC released from the PCPs was derived based on the inventory described in Section 2.3.3.3 and consequent distribution into different environmental compartments.

2.3.2. Inventory of TCC- and TCS-containing products

As mentioned in Section 2.3.1.2., MFA requires input data, for example, based on products containing certain chemicals that are in commerce in a given region, and secondly, due to paucity of MECs for these chemicals in the environment in South Africa; save two studies for TCS (Amdany et al., 2014; Lehutso et al., 2017) and one for TCC (Lehutso et al., 2017) in the region of focus. To develop an inventory for disinfectants (TCC and TCS)-containing products previously published reports in grey and scientific literature were used to identify such product categories. This was due to lack such data in South Africa to the author's knowledge. Thus, the first step entailed the identification of consumer products likely to containing TCS and TCC based on published literature and other inventories for each specific chemical reported elsewhere globally (Perencevich et al., 2001; SCCS, 2010; QYResearch, 2015).

From the published literature, no publicly accessible production data or imported quantities of TCS and TCC in South Africa, nor quantities released during the use and disposal phases from different products categories (e.g. PCPs, textiles, textiles, medical applications, etc.) were found. In this study, the products considered for the TCS and TCC were limited to PCPs, as the literature suggest that such products constitutes the highest usage of these both chemicals (Perencevich et al., 2001; SCCS, 2010; QYResearch, 2015). In the final analysis, we only report inventory on PCPs as they account for the highest use of TCS and TCC as previously reported (Perencevich et al., 2001; SCCS, 2010; QYResearch, 2015).

This raised question whether all the listed products from various sources were; from a practical view point present in the South Africa retail market. This was achieved through physical inspection of the products on retail outlet shelfs was done to aid exclusion of "dead products" from the inventory. Dead products herein refers to products that may appear online in the retail stores but were no longer in the market. Thus, the next step entailed data solicitation of products in the South African retail market from on-line websites for different trading and manufacturing companies. This was done through targeted online searches, and use of key words (single or multiple). Among the key words used in this study were: antibacterial, triclosan, TCS, sanitizing, triclocarban, TCC, trichlorocarbanilide, antibacterial soaps, antibacterial lotions, different triclosan or triclocarban trade names on the South African online retail shopping sites. From the products identified; the following set of information was coded into the databases, viz.: TCS or TCC, concentrations in the product articles (w/w), product-holding matrix (liquid, gaseous suspensions or solid), product mass, volumes, and product price.

However, the author acknowledges that the exactness and reliability of data obtained has certain degree of uncertainties; and therefore, to ascertain the accuracy of the market information obtained; further information was obtained from South African retail market outlets. The data was obtained from the outlets through visit to major retail stores around Gauteng Province; and this was considered reasonable, and representative of consumer products in the South Africa market. This is for the reason that PCPs retail market in South Africa is dominated by few large companies (PriceWaterhouseCoopers South Africa, 2012) which were visited during data collection phase of this study.

2.3.3. Estimation of TCC and TCS flows in GP

In this study, both TCS and TCC flows were determined using the bottom up-based approach where the use and phase of the PCPs was considered. Thus, the key model input data included: market share of a given sub-product category (%), TCS or TCC concentration incorporated in a given product (w/w %), and daily usage per given product and/or product category (g/capita/d). The following expression was used to estimate TCS or TCC from a specific product type according to the expression (Zhang et al., 2015; Musee, 2017):

$$M_{i,j,totali} = \sum M_{i,j} = \sum (C_{i,j} * DQ_{i,j} * MS_j) \qquad (1)$$

where $M_{i,j}$ is quantity of chemical *i* (*i* = TCS or TCC) from PCP *j* (*j* = deodorants, toothpaste, soaps, etc.) released into GP region annually (kg), $M_{i,j,totali}$ as total mass of chemical *i*, $C_{i,j}$ concentration of active chemical *i* in a PCP *j*, $DQ_{i,j}$ the daily usage per person of a PCP *j* containing chemical *i* (g/ca/d) based on ECB (2003) and USEPA (2011) data, and MS_j represents market penetration of a given PCP *j* in South Africa (dimensionless quantity). Therefore, the total quantity of chemical *i* released into the environment was a summation of masses for a given chemical *i* from different consumer product categories. In the following sections, for brevity and offer clarity to the reader, we describe how each model input parameter, viz.: daily release rate, market share, and concentration were derived.

2.3.3.1. Daily release rate

Due to lack of specific data on daily usage for different categories of PCPs in South Africa, we used both the European Chemicals Bureau data (ECB, 2003) and United States Environmental Protection Agency (USEPA, 2011) databases to estimate daily release rates. This was to take into account that South Africa as a developing country – and given the huge income disparities among South Africa population – it became apparent that the daily usage data for any given PCP category were likely to be lower than the values published by ECB (2003) and USEPA (2011) as listed in Table S4. This is true especially for the middle- and lower-income groups whose consumptions are lower than those of consumers in developed countries. To account for the differences in the usage rates among groups in South Africa (based on income per capita) as well as between South Africa and the developed countries; daily usage rate per each income group was determined using the expression:

$$DQ_{i,j,k,RSA} = DQ_{i,j} * \frac{P_k}{P_4} \tag{2}$$

with $DQ_{i,j,RSA}$ representing the adjusted daily usage for income group k (k - 1, 2, 3, 4) represents: very low, low, medium, and high income groups, correspondingly. P_k is the population income group under consideration, and P_4 is population group with the highest income per capita, and also was deemed as the population group whose daily usage rate of PCPs as equal to that of developed countries. This implied that the $DQ_{i,j,k,RSA} = DQ_{i,j}$ for the higher income group. Thus, the average daily usage of a chemical *i* used as model input for the GP was calculated following the expression:

$$DQ_{i,j,GP} = cf_1 \times X_y \times \left(\frac{\sum_k^4 DQ_{i,j,k,RSA}}{GP_T}\right) \tag{3}$$

where $DQ_{i,j,GP}$ is the average daily usage per product category in GP, GP_T is total population in GP, cf_1 = $\frac{HI_{GP}}{HI_{SA}}$ is the ratio of household income (HI) of GP to that of South Africa, and X_y represents likely percentage of chemical *i* into different environment pathways *y (y* = 1, 2, 3 represents wastewater, landfill, and run-off, respectively). Both demographic and economic data used in the model were for 2014.

2.3.3.2. Concentration of TCC and TCS in products

The PCPs identified containing TCS and TCC as described in Section 2.3.2 had either information on the concertation of TCS or TCC either expressed as w/w %, or weight of TCS or TCC and the total weight of the product (article); where in the latter case the w/w% value was calculated. For PCPs with no information on quantities of TCS or TCC although either of the chemical was listed as an active ingredient; the w/w% was estimated to be equivalent to the allowable maximum concentration of TCS or TCC per specific product based on the applicable allowable South Africa regulatory limits. Here, the allowable maximum values for TSC and TCC were obtained from the South African Foodstuffs, Cosmetics, and Disinfectants Act (FCDA) of 1972 (Act No. 54 of 1972; Amendment Act, No. 39 of 2007). For the TCS and TCC, the allowable maximum values were 0.3% and 1.5%, respectively, according to FCDA regulations (Act No. 54 of 1972; Amendment Act, No. 39 of 2007).

2.3.3.3. Market share

The market share for various PCPs were estimated from the total number of brands identified in the retail stores and the number of products in each of the categories in the South African retail market identified during market survey phase. Thus, market share of a given product was based on market survey data for branded products obtained from the South African Audience Research Foundation (SAARF). SAARF (2015) database provided information on the number of products purchased for each brand included in the survey; and the market share was calculated using the equation:

$$MS_j = \frac{x_b}{x_{total}} * \frac{1}{N_b} \tag{4}$$

where x_b is the number of purchased products in brand b (b = Dettol, Lux, Protex, etc.) and x_{total} is the total number of purchased products within a product category (for example soaps, toothpaste, after shave). N_b is the number of products from a specific brand within the product category under consideration (for example, AquaFresh Fresh and Minty, AquaFresh Ultimate, AquaFresh High Definition, etc.).

Of importance to note is that certain brands were not included in this market survey. For such products the market share was calculated using the expression:

$$MS_j = \frac{x_{unknown\,brand}}{x_{total}} * \frac{1}{N_b} * \frac{1}{N_{brands}} \tag{5}$$

where $x_{unknown\,brand}$ is the number of purchased products within the survey not listed under a specific brand and N_{brands} is the number of known brands not included in the survey. Based on this proposed formalism, the method yielded accurate market share information for brands included in the survey, unlike in the case of those excluded. Furthermore, no differentiation in market share was made between products of the same brand.

2.4. ESTIMATION OF PECS IN ENVIRONMENTAL SYSTEMS

Herein, the approach adopted to estimate the likely concentrations of TCS or TCC in different environmental compartments (WWTPs, soils, and freshwater) is described.

2.4.1. PEC in wastewater treatment plants

Calculation TCS and TCC concentrations in the WWTPs offer quantitative measure of their potential risk(s) to organisms in sewage systems, or downstream following the release of treated wastewater (effluent) into the aquatic or soil environmental compartments. Herein, the concentration of TCS or TCC in the wastewater (C_{WWi}) was assumed to be equivalent to the concentration in the WWTP (C_{WWTPi}) for chemical *i*, and evaluated using the expression:

$$C_{WWi} = C_{WWTPi} = \frac{M_{WW,Totali,WWTP} \times 10^{12}}{POP \times WW_{percapita} \times f_{WWTP}} \tag{6}$$

where: C_{WWTPi} is the concentration of chemical *i* in the WWTP (μgℓ$^{-1}$); C_{WWi} is the concentration of chemical *i* in wastewater (μgℓ$^{-1}$); f_{WWTP} is fraction of wastewater treated in WWTPs; $M_{WW,Totali,WWTP}$ is the amount of chemical *i* entering into the WWTPs (t·a^{-1}) determined as $M_{i,j,totali} \times f_{WWTP}$; and $WW_{percapita}$ is wastewater generated per person per year (m^3·p^{-1}·a^{-1}), and POP is number of inhabitants (dimensionless).

2.4.2. PEC in wastewater treatment plants

Estimation of TCS and TCC emitted to freshwater systems were considered to be from two sources: (i) untreated run-off wastewater reaching the freshwater and (ii) quantities present in the effluent (un-removed amounts) from the conventional WWTPs. Thus; the total emitted TCS or TCC was computed using the expression:

$$M_{i,j,fw} = [M_{i,j,totali} * (1 - f_{WWTPi})] + [M_{i,j,totali} * (f_{WWTPi} - f_{WWTPi} * f_{removali})] \quad (7)$$

where: $M_{i,j,fw}$ is the quantity of chemical *i* reaching the freshwater systems (kg); f_{WWTPi} is the fraction of wastewater treated in WWTPs; $f_{removali}$ signifies the fraction of a given chemical *i* removed during treatment through the WWTPs, and $M_{i,j,totali}$ is the total chemical *i* for type *j* released from the PCPs (kg). Notably, the first part of Eq. 7 determines the quantity of TCS or TCC into the aquatic environment from run-off sources without treatment, and second part computes the released quantity of chemical *i* from the WWTPs due to the systems inefficiency as $f_{removali} < 1$. A simplified Eq. 7 yields:

$$M_{i,j,fw} = M_{i,j,totali} \times (1 - f_{WWTPi} \times f_{removali}) \quad (8)$$

Thus, the PEC in freshwater systems is calculated as:

$$PEC_{i,fw} = \frac{M_{i,j,fw} \times 10^9}{POP \times WW_{percapita} \times D_k} \quad (9)$$

where $PEC_{i,fw}$ is the concentration of chemical *i* in aquatic environment (μg/ℓ), and D_k is the dilution factor of the treated wastewater in the receiving freshwater system (dimensionless). Here, three dilution factors (D_k) were considered in the calculations of PEC, viz.: 1, 3 and 10 based on values used in Musee (2011). According to Keller et al. (2014), however, the D_f for South Africa was reported to be between $10 < D_f < 40$.

Notably, unlike in Europe where the default dilution factor of 10 is used (ECB, 2003), in South Africa; low precipitation, and seasonal river systems in GP, the D_f was taken to be 1 or 3. During prolonged draught season the likelihood is that $D_f < 1$ due to high evaporation, and increased water from the river systems for irrigation and other purposes (Musee, 2011). Therefore, dilution factors of 1 and 3 were regarded as realistic for the estimation of PEC values of TCS and TCC over the entire year.

2.4.3. PEC in soil

Based on mass balance, TCS and TCC quantities received into the terrestrial systems are through sludge (biosolids) application for agricultural purposes. The quantity entering the terrestrial systems per unit mass of sludge used is the ratio of the difference between the amount released through the effluent and the amount removed by the WWTPs, determined by the expression (assuming all sludge is use for agricultural purposes):

$$M_{i,j,sludge} = \frac{[M_{i,j,totali} \times (1-(f_{WWTPi} \times (1-f_{removali})))]}{M_{total\,sludge}} \quad (10)$$

where $M_{i,j,sludge}$ is concentration of TCS or TCC per unit mass (mg/kg) in the sludge, and $M_{total\,sludge}$ is the total sludge generated in GP, South Africa from the WWTPs.

Therefore, the predicted environmental concentration ($PEC_{soil,}$ mg/kg) in the soil as a result of sludge use for agricultural purposes was determined following the equation (ECB, 2003):

$$PEC_{soil} = \frac{M_{i,j,sludge} \times Appl_{sludge}}{RHO_{soil} \times D_{soil}} \quad (11)$$

where $Appl_{sludge}$ is the dry-sludge application rate (1.0 kg m^{-2} y^{-1} or as the allowable maximum value for South Africa (Snyman and Herselman, 2006)), RHO_{soil} is the bulk density (1 700 kg m^{-3}), and D_{soil} is the agricultural soil mixing depth (0.20 m) (EEC, 1986; ECB, 2003). In this study application rate of 10 000 kg/ha was used. Calculations for $PEC_{soil,}$ signifies the worst-case scenario as the allowable maximum sludge rate was adopted as input in this model.

2.5. RESULTS AND DISCUSSION

2.5.1. Inventory analysis for TCC- and TCS-containing products

Following the approach describe in section 3.2, a total of 128 and 52 products were identified to contain TCS and TCC, respectively, in the South Africa retail market as summarized in Table 2-2 Broadly, the TCS- and TCC-containing products were 16 and 5 sub-categories as shown in Table 2-2 Broadly, the TCS-containing products had three categories, and distributed as follows: 115 were PCPs (89.8%), 8 were kitchenware (6.3%), and 5 were classified as others (pet care, office accessories, and first aid) (3.8%). Results from this study indicate that the distribution of products containing TCS were similar to findings reported in previous inventories (SCCS, 2010; QYResearch (2015) with PCPs being predominant in deodorants (44.5%) and liquid hand soaps (14.8%).

Data analysis further revealed that TCC-containing products were all under the PCPs category – a finding found to be in agreement to use of TCC reported in previous inventories (Perencevich et al., 2001; EC, 2005; Halden and Paul, 2005); where the bar soaps were most dominant (69.2%) followed by hand wash products (13.5%) and liquid soaps (7.7%), and rest 9.6% used in shower gels and after shave. For example, in the US retail market survey, TCC was found predominantly in liquid soaps and bar soaps (Perencevich et al., 2001).

However, in this model, only 88 (68.8%) and 45 (86.5%), respectively, of the TCS- and TCC-containing products were used to estimate their potential risks into different environmental systems. The excluded products from the model were either non-PCPs (e.g. kitchen ware or office accessories), lacked daily usage data per capita (hand wash for TCC-related products), or no data on market penetration in the South Africa was accessible.

Table 2-2: Product categories containing TCS and TCC in South African retail market.

Product	Number of products containing	
	TCS	TCC
Deodorant	57	
Toothpaste	7	
Hand Lotion	3	
Liquid soaps	19	4
Mouthwash	2	
Body Lotion	4	
Shower gel		3
Pet Care	3	
Face Treatment	9	
After shave		2
Foot Products	4	
Bath Additives	2	
Hand wash		7
Hair Products	4	
First Aid	1	
Bar Hand Soaps	2	36
Hygienic Wipes and Sprays	2	
Kitchenware	8	
Office Accessories	1	
Total	128	52

2.5.2. Estimation of TCC and TCS flows in GP

To quantify the TCS and TCC flows, first, the input parameters the use per capita and the market penetration of the PCPs were calculated using Equations 2 to 4 (described in Section 2.3.3). The usage rate per capita for various categories of PCPs in GP was determined by modifying the daily usage data sourced from ECB (2003) and USEPA (2011) (Table 2-5) – as none was available for South Africa – through integration it with population (Table 2-3), and aggregated income per capita for different income groups in on South Africa (Table 2-5). Consequently, the results derived for the usage of different PCPs are for GP are listed are in Table 2-6.

Table 2-3: Data on per capita product usage (Sources: ECB, 2003; USEPA, 2011).

Product category	BS	LS	HW	SG	AS	T	BL	HL	D	FT	HP
Usage (g/p/d)	3.6	3.64	1.36	7.5	1.2	2.1	11.5	0.4	6	3.55	7.55

*T: toothpaste; BL: body lotion; BS: bar soaps; HL: hand lotion; D: deodorant; FT: face treatment; HP: hair product; BA: bath additive; LS: liquid soaps; AS: after shave; SG: shower gel; HW: hand wash.

Table 2-4: Monthly income distribution for GP per household (Source: Statistics South Africa, 2013).

Income Groups	Black	Coloured	Indian/Asian	White	Total
None	39 687	2 446	1 587	15 520	59 240
R1-R400	173 916	3 698	1 111	9 757	188 482
R401-R800	366 078	7 012	2 200	14 785	390 075
R801-R1 600	640 281	17 520	7 513	43 659	708 973
R1 601-R3 200	428 436	29 226	19 155	119 295	596 112
R3 201-R6 400	188 746	26 995	27 211	222 406	465 358
R6 401-R12 800	64 118	12 805	19 061	205 012	300 996
R12 801-R25 600	19 491	3 571	8 245	114 316	145 623
R25 601-R51 200	6 369	885	2 174	42 871	52 299
R51 201-R102 400	2 125	369	701	14 125	17 320
R102 401-R204800	1 121	182	402	6 651	8 356
R204 801 and above	873	86	183	4 174	5 316
Total	1 931 241	104 795	89 543	812 571	2 938 150

Table 2-5: Estimated daily usage rates for different personal care products in GP, South Africa.

Product category	Different income groups usage (g/p/d)			
	Very low	Low	Medium	High*
Bar soaps	0.223	0.282	0.671	3.600
Liquid soaps	0.225	0.286	0.679	3.640
Hand wash	0.084	0.107	0.254	1.360
Shower gel	0.464	0.589	1.399	7.500
Aftershave	0.074	0.094	0.224	1.200
Toothpaste	0.130	0.165	0.392	2.100
Body lotion	0.711	0.902	2.145	11.500
Hand lotion	0.024	0.031	0.075	0.400
Deodorants	0.371	0.471	1.119	6.000
Face treatments	0.251	0.319	0.757	4.060
Hair products	0.464	0.589	1.399	7.550

*Values from ECB (2003) and USEPA (2011).

From the results of each category of PCPs with results listed in Table 2-2, each product's market penetration using Eq. 4 was determined, and the results derived are summarised for each PCPs in Tables 2-6 and 2-7 for TCC and TCS, respectively.

Next, using Eq. 1 the total quantities of TCS and TCC based on input parameter values of usage rates (Table 2-5) as well as concentration and market penetration for each product type (Tables 2-1 and 2-2) were determined. Estimates of TCS and TCC from various PCPs are given in Tables 2-7 and 2-8, respectively.

From the results, estimated total TCS (Table 2-7) and TCC (Table 2-8) under the probable scenario into different environmental systems were 332.2 kg and 1 763.7 kg, respectively. For the TCC, results indicated that, bar soaps accounted for about 68.5% (Table 2-8) of the total released into environment as these products were found to be dominant TCC-containing category in South Africa retail market (account for 69.2% (Table 2-1)), and in addition to being more affordable (price per unit mass) unlike other higher priced product categories, e.g. liquid soaps and shower gel. Findings of per capita consumption of TCS and TCC in GP was estimated, respectively, as 25.2 and 133.6 mg/c/pa. The per capita GP values in comparison to those of other regions or countries globally were found to be lower (Table 2-9).

Table 2-6: Estimated quantities (kg) of TCS from PCP's released into the GP environment (2014).

Income group	T	BL	BS	HL	D	FT	HP	BA	LS
Very low	2.73	0.15	0.06	0.01	5.62	0.80	0.02	0.00	0.03
Low	14.65	0.80	0.32	0.04	30.15	4.28	0.09	0.01	0.16
Medium	34.82	1.91	0.76	0.09	71.65	10.18	0.22	0.03	0.37
High	44.18	2.42	0.97	0.12	90.91	12.91	0.28	0.04	0.47
Sub-total	96.37	5.28	2.11	0.25	198.33	28.16	0.61	0.09	1.03

*T: toothpaste; BL: body lotion; BS: bar soaps; HL: hand lotion; D: deodorant; FT: face treatment; HP: hair product; BA: bath additive; LS: liquid soaps

Table 2-7: Estimated quantities (kg) of TCC from PCP's released into the GP environment (2014).

Income group	BS	HW	LS	SG	AS
Very low	34.27	2.16	12.01	1.42	0.13
Low	183.74	11.58	64.43	7.64	0.72
Medium	436.68	27.53	153.12	18.15	1.71
High	554.05	34.93	194.27	23.03	2.16
Sub-total	1 208.73	76.20	423.83	50.25	4.72

*BS: bar soaps; LS: liquid soaps; HW: hand wash; SG: shower gel; AS: after shave

Table 2-8: Global production of triclocarban (TCC) and triclosan (TCS), and consumption per capita in several countries.

	PCR (mg/c/a)	Country	Population	Production (t/a)	Reference
TCC	950	USA	306 800 000	291.46	Heidler & Halden (2009)
TCC	2 870	USA	298 400 000	856.41	Heidler et al. (2006)
TCC	3 000	USA	287 600 000	862.8	The Consortium (2002)
TCC	134	GP	13 200 300	–	This study
TCS	299	Canada	34 750 000	10.39	Health and the Environment (2012)
TCS	327	UK	64 130 000	20.97	Sabaliunas et al. (2013)
TCS	229	Germany	82 490 000	18.89	Bester (2003)
TCS	233	Switzerland	7 285 000	1.7	Singer et al. (2002)

	PCR (mg/c/a)	Country	Population	Production (t/a)	Reference
TCS	144	Switzerland	7 285 000	1.05	Lindström et al. (2002)
TCS	1 380	USA	287 600 000	396.89	McAvoy et al. (2002)
TCS	1 100	USA	306 800 000	337.48	Heidler & Halden (2009)
TCS	2 490	USA	298 400 000	743.02	Heidler & Halden (2007)
TCS	598	China	1 364 000 000	815.67	Huang et al. (2014)
TCS	231	UK	61 320 000	14.16	Winkler et al. (2007)
TCS	1 000	UK	61 810 000	61.81	Capdevielle et al. (2008)
TCS	25	GP	13 200 300	–	This study

PCR: Per capita release; USA: United States of America; UK: United Kingdom; GP: Gauteng Province.

In terms of high per capita consumption of TCC compared to TCS; this was attributed to the high allowable concentration of TCC incorporated in PCPs (in South Africa) with a maximum of 1.5% (Table A1) compared to 0.3% for TCS (Table A2) per product article according to FCDA regulations as earlier mentioned in section 2.3.3.2.

Findings revealed that deodorants and toothpastes accounted for 56.7% and 29% of total TCS released into the environment, respectively (Table 2-7). These results revealed quantities of TCS released into the environment were dependent on several factors. Whereas toothpastes had only seven sub-categories of TCS-containing products compared to 19 and 57 for liquid soaps and deodorants, respectively, (Table 2-3); but released considerably very high quantities of TCS into the environment. This points to likelihood of other drivers on the quantities of TCS or TCC released besides the number of products per given sub-category per given product (Tables 2-3, 2-7, and 2-8). The high market penetration, for toothpaste products, for example, when compared to other product categories (Tables 2-3, 2-7, and 2-8), for instance, where liquid soaps had 19 sub-category products yet only released 1.03 kga^{-1} (Table 2-7). Overall, products with high price per unit article, for example, hand lotion, face treatment, hair products, and bath additives, among others accounted for very low releases of TCS releases into the environment as they had with low market penetration (Table A2).

In summary, from this study, estimated quantities of TCS or TCC flows into the environment from various sub-category products were dependent on income per capita, market penetration, and concentration in a given product. TCC, for example, market penetration varied from 0.03% to 1.86% whereas concentration per article ranged from 0.02% to 1.5%, and was dependent on product category in focus (Table A1). As such, liquid soaps with only fewer sub-categories (4) compared to hand wash (7); the former accounted for higher quantities of TCC releases compared to the later (Table2-8) due to average higher concentration of TCC in liquid soaps per article than in hand wash (Table A1).

2.5.3. Calculations of predicted no effect concentrations

Using acute- and chronic-toxicity data collected from the literature PNECs were calculated for three organisms in freshwater (algae, *D magna*, and fish), wastewater (bacteria), and terrestrial (earthworms, etc.) systems. The PNECs results for the acute and chronic toxicity of TCS and TCC, given in Figures 2-1 and 2-2, respectively. For TCS (Figure 2-1), results for chronic- and acute-toxicity, respectively, showed algae as the most sensitive organism as they ranged in 5 and 3 orders of magnitude, and *D magna* as the least sensitive. Thus, NOECs for TCS yielded the least PNEC of 0.0015 *μ*g/L – an indication of highest toxicity. Our findings indicate good agreement, and are comparable to previous works of other researchers (Brausch and Rand, 2012; Tamura et al., 2013). Note that long-terms studies (chronic values) were very limited, and this raise concerns in understanding the long-terms implications as both chemicals are known to bioaccumulate in organisms and different environmental systems. In wastewater, the bacteria exhibited high toxicity variability, with TCS PNEC values spanning over 7 orders of magnitude (acute toxicity: Figure 2-1.b) plausibly due to high diversity of bacteria strains present in WWTPs. Conversely, in the terrestrial systems, organisms with the lowest PNECs were algae and plants species.

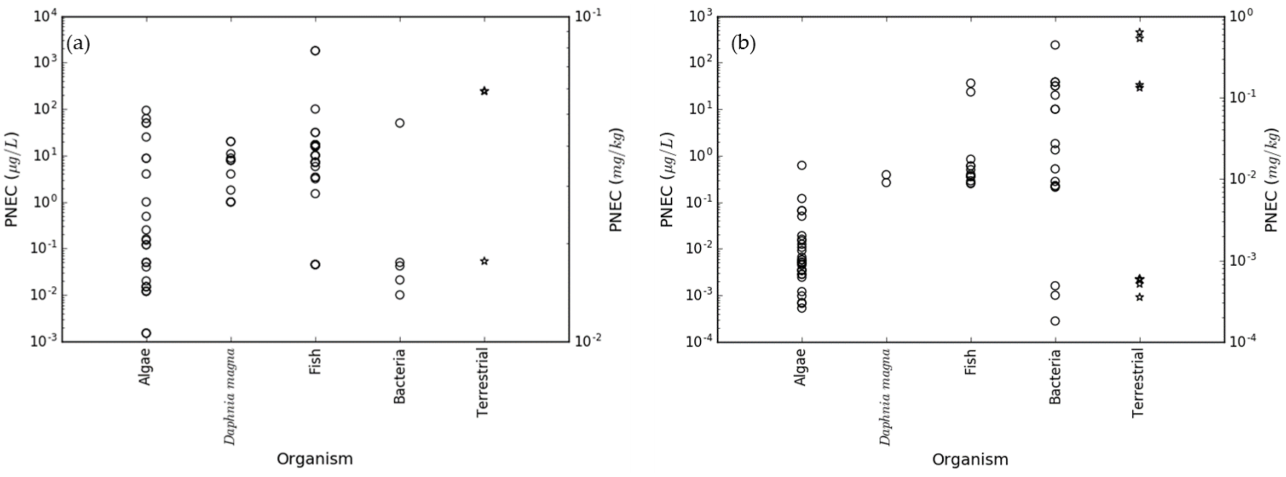

Figure 2-1: Calculated PNECs of TCS in three environmental systems, viz.: freshwater, wastewater, and soil for: (a) chronic, and (b) acute toxicity data. Symbol (o) represents PNECs for freshwater and wastewater organisms, and (☆) for soil organisms.

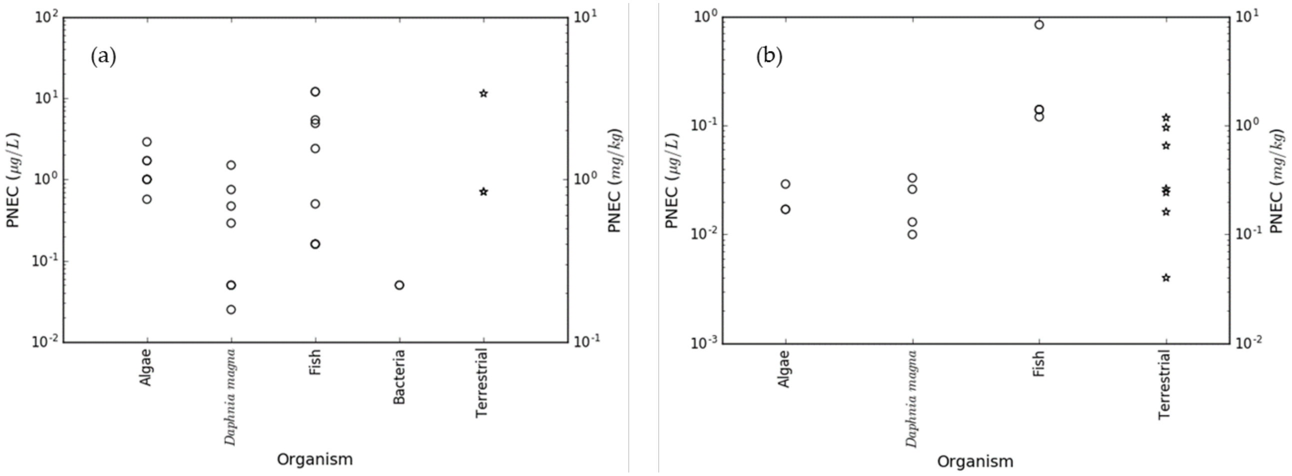

Figure 2-2: Calculated PNECs of TCC in three environmental systems, viz.: freshwater, wastewater, and soil for: (a) chronic, and (b) acute toxicity data. Symbol (o) represents PNECs for freshwater and wastewater organisms, and (☆) for soil organisms.

From the retrieved data, TCC data both for limited acute- and chronic-toxicity data were few as attested by fewer PNECs reported in Figure 2-2. It was also apparent that the observed variability was much less lower compared to TCS (Figure 2-1). For TCC, D magna was the most sensitive organism and algae as the least 1 order of magnitude). From the data collected, one acute toxicity of TCC was found for bacteria but none under chronic exposure conditions. Notably, given the PNECs of TCS and TCC to bacteria in wastewater were low (< 0.1), may suggest likely diminished treatment efficacy and microbial diversity in activated sludge units during wastewater treatment, and in turn, lead to potential environmental and human health concerns due to inadequate treatment. With respect to organisms found in the soil compartment both chronic and acute PNECs values were only calculated for *Eisenia fetida* (red worm) as these were the only ones accessible. In all the calculations reported in this section were done according to ECB (2003) based on the rules summarised in Table 2-10.

Table 2-9: ECB (2003) proposed assessment factors (AFs) applicable for determination of PNECs for environmental contaminations in freshwater systems.

Information Available/categorization approach	AF
At least one short-term L(E)C$_{50}$ from one of the three trophic levels (algae, daphnia or fish)	1000
One long-term NOEC (either daphnia or fish)	100
Two long-term NOECs from two of these two trophic levels(combination of two: fish/daphnia/algae)	50
Three long-term NOECs from 3 trophic levels (normally algae, daphnia, and fish)	10

2.5.4. Calculations of predicted no effect Predicted environmental concentrations

In this section, in recognition of the model input data uncertainties (model input data were discussed in sections 2.3 and 2.4 as well as the results reported in section 2.5); this limitation was partly addressed herein by considering three release scenarios of TCS and TCC into the environment. The minimum (conservative) scenario aided to predict the likely lowest quantities of exposure to the environment linked to lower releases of TCS and TCC from PCPs. Conversely, maximum (worst-case) release scenario signified the optimal releases of both chemicals at elevated concentrations into the environment; thus with high likelihood to cause adverse effects to the aquatic organisms. The third case, the probable scenario represented an attempt to estimate the most realistic releases based on the available input model data from various sources (discussed in this chapter).

Therefore, using estimated TCS and TCC flow results in Section 2.5.2, PECs were determined for different environmental compartments in GP under minimum, probable, and maximum scenarios. Findings of TCS and TCC quantities released into the environment under different scenarios are presented in Table 2-11. Results indicated that the estimated quantities, and consequently, PECs were dependent on factors such as WWTPs removal efficiency of TCS and TCC, fraction of treated wastewater based on population with sanitation services in, dilution factor, and income per capita for different income groups as this influenced consumption per capita of the PCPs. The removal efficiencies of TCC and TCS, for example, are similar (Heidler et al., 2006; Lozano et al., 2013); however, in this study lower removal efficiencies for South Africa's WWTPs were used (Musee, 2011) in comparison to those of more developed countries where removal efficiency was about 97% for TCC and TCS (Heidler et al., 2006; Lozano et al., 2013). Based on the results in Table 2-11, quantities of TCC released into the environment were found to be 5 times higher than those of TCS. Subsequently, PECs in the freshwater and wastewater estimated and results are summarised in Table 2-12.

Table 2-10: Estimated quantities of TCC and TCS released into the environment based on model inputs under three different emissions: minimum, probable, and maximum scenarios.

Variable	TCC			TCS		
	MIN	PRO	MAX	MIN	PRO	MAX
Quantity released into Env.	1 675.54	1 763.73	1 851.91	315.63	332.25	348.86
f_{WWTP}: fraction of treated WW	0.93	0.91	0.89	0.93	0.91	0.89
f_R: fraction removed in WWTPs	0.83	0.68	0.58	0.84	0.67	0.59
Quantity released into WWTPs	1 558.25	1 604.99	1 648.20	293.54	302.01	310.48
Quantity in the sludge	1 290.99	1 097.00	957.05	245.70	201.57	182.63
Quantity in the effluent from WWTPs	267.26	507.99	691.15	47.84	100.45	127.86

Variable	TCC			TCS		
	MIN	PRO	MAX	MIN	PRO	MAX
Quantity released through run-off	117.29	158.74	203.71	22.09	30.23	38.37
Total quantity into freshwater	384.55	666.73	894.86	69.93	130.68	166.23

Abbreviations: Env.: environment, WW: wastewater, WWTPs: wastewater treatment plants. All fractions are dimensionless, and quantities are expressed in kga^{-1}

Using statistics of sludge generated from GP based on the flows and treated effluent data for 48 WWTPs; PECs in the terrestrial systems were determined. Among the input data were total daily flow of 2 552.80 ML/d (Table A3) where 350 kg dry solids are produced for every ML treated (Marx et al., 2004). Hence, the total sludge generated in GP was 326 082 t/a, and with about 80-97.4% estimated to be used for agricultural purposes (Musee, 2011). Estimated PECs in terrestrial systems under three application scenarios showed very low levels of TCS and TCC (through dilution via spreading of sludge over large area) with the maximum application worst-case concentrations being 3 μg/kg and 15 μg/kg, respectively (Table 2-13). Previous studies reported PEC values of 0.4-5.6 μg/kg in the Denmark soils when considered for agricultural applications – with application rates of 3000 kg/ha (Samsøe-Petersen et al., 2003).

Table 2-11: Estimated PECs, PNECs, and RQs for TCC and TCS in the freshwater and wastewater systems.

Environmental compartment	PEC, and dilution factor (DF)	TCC			TCS		
		MIN	PRO	MAX	MIN	PRO	MAX
Freshwater	DF: 10, (PEC, ng/ℓ)	39.3	70.7	99.8	4.7	14.6	34.8
	DF: 3, (PEC, ng/ℓ)	131.0	235.5	332.7	15.6	48.6	116
	DF: 1, (PEC, ng/ℓ)	393.1	706.6	998.2	46.8	145.9	348
	PNEC (ng/ℓ)	25	25	25	1.5	1.5	1.5
	RQ (DF =10)	1.6	2.8	4	3.1	9.7	23.2
	RQ (DF = 3)	5.2	9.4	13.3	10.4	32.4	77.3
	RQ (DF =1)	15.7	28.3	39.9	31.2	97.3	232.0
Wastewater (treated effluent)	$PEC_{influent}$ (ng/ℓ)	1 590	1 700	1 840	197	337	650
	$PEC_{effluent}$ (ng/ℓ)	270	540	770	30	110	270
	PNEC (ng/ℓ)	2500	2 500	2 500	21	21	21
	$RQ_{influent}$	0.6	0.7	0.7	9.4	16.1	31
	$RQ_{effluent}$	0.1	0.2	0.3	1.4	5.2	12.9

Table 2-12: Estimated PECs, PNECs, and RQ values for TCC and TCS in the terrestrial systems.

Variable	TCC			TCS		
	MIN	PRO	MAX	MIN	PRO	MAX
Sludge used in agriculture (%)	0.800	0.887	0.974	0.800	0.887	0.974
Total sludge used in farms (t/a)	2.61×10^5	2.9×10^5	3.18×10^5	2.61×10^5	2.9×10^5	3.18×10^5
TCC or TCS in sludge (kg)	1 290.99	1 083.11	944.94	245.70	201.57	182.63
Conc. in sludge (µg/kg)	4 950	3 740	2 980	940	700	570
PEC_{soil} (µg/kg)	15	11	9	3	2.4	2
$PNEC_{soil}$ (µg/kg)	40	40	40	20	20	20
RQ_{soil}	0.36	0.28	0.22	0.16	0.12	0.10

Using a similar approach as adopted in this study, previously several studies reported PECs of TCS in the soil systems. For example, an estimated PEC value of 68 *µ*g/kg (regarded as highly conservative) for TCS (with biosolids application rate of 8 300 kg/ha) (Government of Canada, 2016). Sánchez-Brunete et al. (2010) published measured TCS concentrations of 4.7 *µ*g/kg and 1.7 *µ*g/kg in agricultural soil sampled 1 day and 6 months, respectively, following biosolids application (application rate of 12 000 kg/ha). Hence, estimated PECs in terrestrial systems in this study are in the same order of magnitude with previous studies (Samsøe-Petersen et al., 2003; Government of Canada, 2016; Sánchez-Brunete et al., 2010), however, the differences of the results between the studies may be attributed to measured and predicted concentrations in addition to application rates used in different regions globally. This is an aspect that require attention as potential for risks of contaminants in the terrestrial are dependent on use of sludge sourced from WWTPs.

Overall, under the three likely emission scenarios considered herein, estimated PECs for the TCS and TCC were ≤ 1 000 ng/L in freshwater, in all three dilution factors (DFs) (1, 3 and 10), however, TCC concentrations were > 1 000 ng/L in the influent (Table 2-12). For GP, considerable part of the year is defined by low or no precipitation resulting to most river systems being seasonal. Therefore, very low dilution factors (DFs) are not abnormal but rather are exacerbated often by prolonged drought periods. Thus, lower DFs of 1 and 3 offered in this study were considered to have yielded more representative environmental conditions. For example, in certain months DFs < 1 are likely as result of high evaporation rates, and increased water use from the river systems for various purposes (Musee, 2011).

2.5.5. Comparison of MECs and PECs

The reported MECs of TCS and TCC in regions in GP were compared to PECs results derived in this study in the freshwater and wastewater systems. This was done for two reasons. First to check the reliability of the PECs derived from this study, and secondly, establish whether further refinement of the model was necessary. Results of estimated PECs for TCS in both influent and effluent (Table 2-12) were very low compared to MECs results of Amdany et al. (2014). The high MECs of TCS were based on specific local measured values at a given spot, as opposed to PECs estimated for whole GP. Nonetheless, such comparison is useful for already mentioned reasons.

The differences in MECs and PECs were attributed to complexities linked to model input data defined by both spatial and temporal features. However, considering PECs derived herein for GP, and similar results reported elsewhere for TCS, showed in certain instances this study's values were comparable values reported in other regions globally. For example, TCS results for GP (4.7-348 ng/L) (Table 2-12) and those of Zhao et al. (2013) in surface water (0.9-478 ng/L) were be in the same order of magnitude plausibly since China also experiences dry months (Tamura et al., 2013) as is the case in South Africa (defined by DFs of 1 and 3 in this model). Similar comparisons on MECs and PECs reported herein within the same range were observed for TCS in influent and effluent as reported by Lindström et al. (2002) for Switzerland.

2.6. Environmental risk assessment

Using PNECs and PECs results presented in sections 2.5.4 and 2.5.5, respectively, RQ calculations were performed to estimate TCS and TCC risks to the organisms in freshwater, wastewater, and soils. Herein, two approaches were followed to calculate RQs. The first approach entailed estimation of RQs using minimum toxicity value – i.e. for the most sensitive organism in a given compartment – and subsequently, PNEC value was calculated using an appropriate AF as prescribed by ECB (2003) (see rules in Table 2-10). Following this approach, and using chronic NOECs data for *D magna* (for TCC as 0.25 μg/L) and algae (for TCS as 0.015 μg/L) with AF of 10 (in accordance ECB (2003) categorization system, results obtained are shown in Table 2-12, risks were estimated. Although lower quantities of TCS compared to TCC were released into the environment (Tables 2-7, 2-11), however, as TCS is more toxic (Figure 2-1) compared to those of TCC (Figure 2-2); subsequently yielded higher RQs (Table 2-12). Results of higher RQs for TCS compared to TCC derived in this study had similarities to findings of Brausch and Rand (2011) (RQ_{TCS} = 19.17; RQ_{TCC} = 10.96) and Tamura et al. (2013) (RQ_{TCS} > 10; RQ_{TCC} ≈ 10 based on algae results) calculated using PNECs based on chronic toxicity, and MECs.

Using the reported MECs of TCC (Lehutso et al., 2017) and TCS (Amdany et al., 2014) in GP, a comparison of PECs and MECs was done as well as the RQs results derived based on each data type; and the findings are listed in Tables 2-15 (TCS) and 16 (TCC). Generally, PECs for both TCS (Table 2-15) and TCC (Table 2-16) showed good agreement with the minimum- to mid-values of MECs data; with an exception of TCC (Table 2-6) in freshwater where PECs were higher than MECs. The lower PECs values were either due to plausibility of the collected brand product data used in our model development did not account for all sources of TCC and TCS to the environments considered, and therefore, yielded lower predicted concentrations. Alternatively, given the MFA approach estimates average emitted concentration of a given contaminant into the environmental compartment as opposed to the localized concentrations obtained through local measurements reported as MECs values. Results of TCS in freshwater (based on PECs and MECs), influent and effluent yielded results of RQs > 1; hence indicating TCS poses elevated risks to the environment. The high RQs in freshwater derived using MECs indicate that despite WWTPs having had high removal efficiency for TCS of 67-86% (Amdany et al., 2014; Lehutso et al., 2017) in addition to dilution effect in the river system, TCS risks remained high especially to algae. In addition, in this study RQs for TCS based on PECs estimates were lower than those reported by Thomaidi et al. (2015) with RQ value of 4 914 calculated using MECs data; however, MECs based derived RQs values were in good agreement (587-5813) (Table 2-15).

Table 2-13: RQ values estimated using PECs and MECs of TCS in Gauteng Province, South Africa

	Freshwater		Influent		Effluent				
	MEC[1]	PEC	MEC[1]	PEC	MEC[1]	MEC[2]	PEC	MEC[1]	MEC[2]
Conc. (ng/l)	ND-1890	5-348	880-8720	197-650	2010-17600	78460-122700	30-270	990-13000	10700-22900
PNEC (ng/l)	2500	1.5	1.5	21	21	21	21	21	21
RQ	ND-0.8	3-232	587-5813	9-31	96-838	3736-5843	1-13	47-619	510-1091

[1]Luhutso et al., 2017; [2]Amdamy et al., 2014. *ND: not detected; MEC: measured environmental concentration; PEC: predicted environmental concentration.

Table 2-14: RQ values estimated using PECs and MECs of TCC in Gauteng Province, South Africa

	Freshwater		Influent		Effluent	
	PEC	MEC[1]	PEC	MEC[1]	PEC	MEC[1]
Conc. (ng/l)	39-998	ND-360	1590-1840	86-2840	270-770	ND-1890
PNEC (ng/l)	25	25	2500	2500	2500	2500
RQ	2-40	ND-4	0.6-0.7	0.03-1	0.1-0.3	ND-0.8

[1]Luhutso et al. (2017) *ND: not detected; MEC: measured environmental concentration; PEC: predicted environmental concentration.

Results also indicate that at low DFs, most flows are wastewater from WWTPs (DF ≤ 1), and subsequently, estimated risks in freshwater systems were significantly higher. However, at high DF of 40, TCC risks are only likely to occur under maximum release scenario for TCC (RQ_{TCCMax} = 1) whereas for TCS, this is plausible under probable (RQ_{TCSPro} = 2.43) and maximum (RQ_{TCSMax} = 5.8) release scenarios (data not in Tables 2-15 and 2-16). Nonetheless, there are potentially likely risks from the rest of release scenarios for TCS, and this was also observed in RQ results calculated using MECs (Table 2-15) where the later were higher and in range of 25-196 times more than PECs derived ones. However, MEC- and PEC-based calculated RQs values for TCC suggest low or no risk in the influent and effluent; but only in the freshwater (Table 2-16).

In terrestrial systems, TCS and TCC under all release scenarios had RQs < 1 (Table 2-13), and therefore, posed no risk to soil organisms based on estimated PECs (Table 2-11). However, caution is necessary since the highest mass of TCS and TCC were estimated to be in sludge (Table 2-11), this implies that as the use and release of these chemicals into the environment continues to increase, the PECs may likely exceed the PNECs, and could pose risk to the soil organisms. Moreover, given the full spectrum of metabolites of TCC and TCS formed in biosolids and during use phase in agricultural soils/terrestrial systems were not considered in this study raises the possibility that terrestrial systems may be adversely affected by these chemicals. This is compounded by lack of chronic toxicity for both TCC and TCS, and metabolites in the environmental systems especially in the terrestrial systems as the collected toxicity showed. This remain an open research question that merits further scientific research as the continued use of antibacterial products containing TCS and TCC.

The second approach entailed calculation of RQs for all organisms in different compartments using all acute and chronic toxicity collected during the tenure of in this study. For the purposes of achieving the objective of this study; only PECs-based results were considered in this approach. This is because MECs-based values were considered to be too few; thus are not representative of the entire GP region. In the second approach was informed by appreciation that; ecotoxicological data even for the same species, or on the same trophic level showed marked differences as evident in Figures. 2-1 and 2-2. The differences are due to many factors including intra-species differences in addition to life-history stage, non-uniformity of exposure media chemistry (abiotic factors), presence or absence of other environmental pollutants, testing protocols, consumption (feeding differences) of organisms dependent compartment of habit (water, sediments, etc.), biotransformation of the chemicals owing to abiotic and biotic factors. It is for this reason each toxicity data collected in the databases developed in this project was taken to represent a certain level of ecological risk into different environmental compartments.

Thus, from the second approach, the results derived for TCS and TCC, respectively, are pictorially summarised in Figures 2-3 and 2-4. Notably, results reported herein based on the second approach for RQs are under the following conditions: DF = 1, PEC for the probable release scenario, and AFs for acute and chronic toxicities as 1000 and 10, respectively, to indicate the most likely plausible worst scenario.

The results in Figures 2-3 and 2-4 generally suggest, on the basis of acute and chronic toxicities used for PNEC values estimations, that both TCC and TCS poses highly variant likely risks to organisms in different compartments with RQs > 1. Also, the results provide valuable insights that raise questions on the appropriateness of current approach (reported as first approach in this study) in selection of the most sensitive organism based on a single toxicity value as performed in the first approach. From a policy perspective, this is possibility of yielding undesirable scenarios where other organisms are compromised simply on the basis there reported toxicity were not the least in a given database. For example, choice of least toxicity assumes no impacts to organisms with what is regarded as higher toxicity – irrespective of the magnitude; however, results in Figures 2-3 and 2-4 show wide variability of adverse effects even of the same species, and at different life-history attested with numerous RQs > 1.

However, there is need for caution since as mentioned earlier, results presented in this study did not take into account potential risks associated with metabolites of TCS or TCC likely to be formed during transformation processes (e.g. adsorption into sediments, and phototransformation as well as chlorination, etc.) in freshwater and wastewater systems, or due to mixtures of TCS and TCC at various ratios, or together with their formed respective metabolites. For example, TCS can transform in the aquatic systems into methyl-triclosan – a chemical with similar properties to TCS but with very high bioaccumulation as well as biopersistence potential and aquatic toxicity, but poorly monitored in the environment. This aspect is crucially important, for example, a recent study by Villa and colleagues (Villa et al., 2014) observed that the IC_{50} toxicities bacterium *Vibrio fischeri* (values in parentheses) of individual TCC (0.91 mg/L), TCS (0.73 mg/L) and methyl-TCS (1.76 mg/L) were lower compared to their ternary mixture toxicity IC_{50} = 0.23 mg/L. Thus, in light of the predicted or measured concentrations (even if they are low) of TCC and TCS as well as the formation of their metabolites will result to their co-occurrence as mixtures; thus resulting to mixture toxic effects to organisms in aquatic systems such as bacteria but were not accounted in this study.

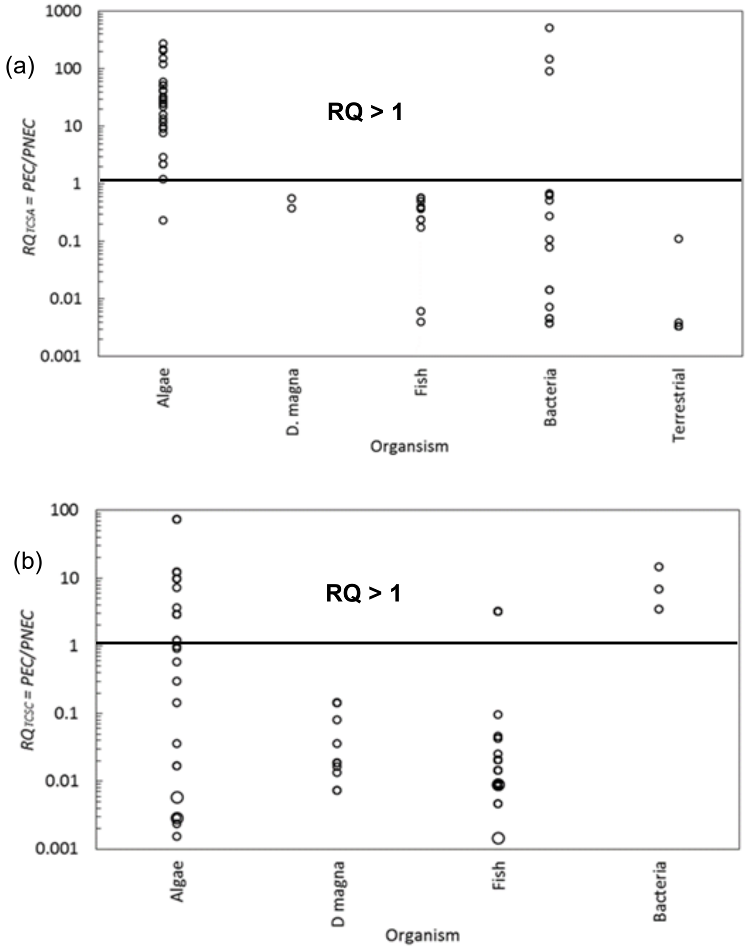

Figure 2-3: RQs calculated using (a) acute and (b) chronic toxicity of TCS to different organisms.

In addition, caution is essential in making comparison of RQ values derived using PECs- and MECs-based inputs as the former is for all plants whereas the latter is only for specific sampling locations. Overall, risk results presented herein, or reported elsewhere in previous investigation only provide partial the risks of TCS and TCC in the aquatic system, and hence this merits further research in order to bridge those data gaps.

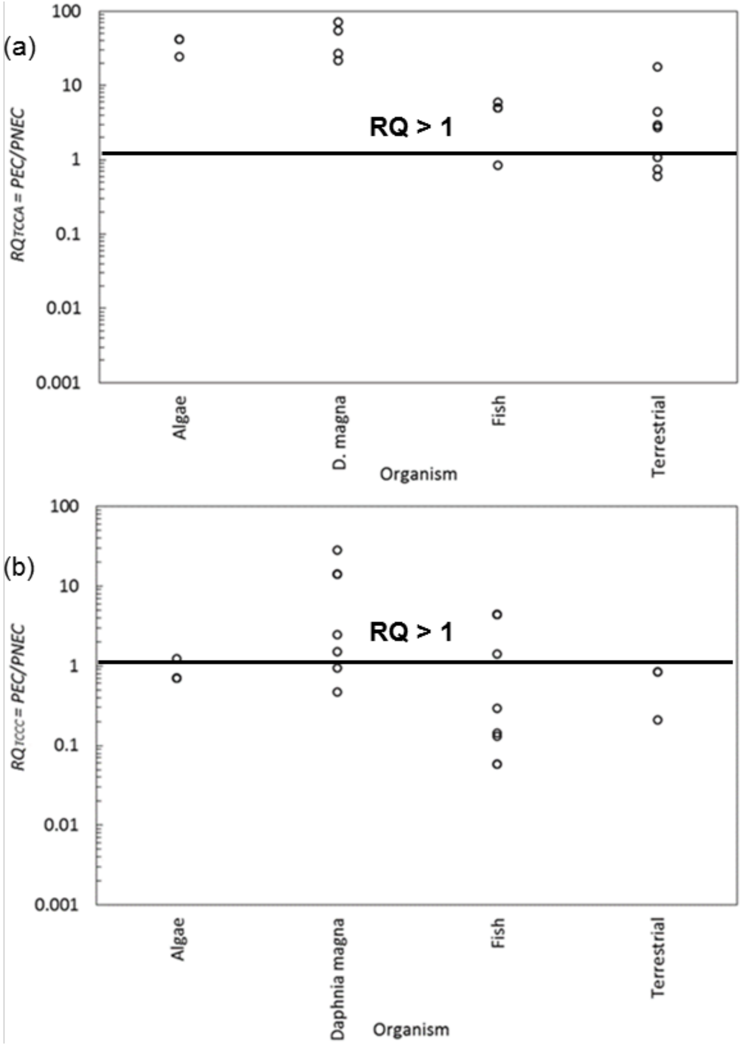

Figure 2-4: Estimated RQs using (a) acute and (b) chronic toxicity of TCC to different organisms.

Table 2-15: TCS and TCC measured concentrations in different regions in surface and WWTPs.

Location	Surface water (ng/l)	Sediment (ng/l)	Influent (ng/l)	Effluent (ng/l)	Removal efficiency (%)	Modified (%)	Sludge (%)	Reference
TCS								
China	0.9-478	46.2-1 329		162				Zhao et al., 2013
Lake Greifensee area	11-98	53-125		42-213	94	79	15	Singer, 2002
Tone Canal, Japan	11-31							Nishi et al., 2008
Ohio			3 800-16 600	200-2700	96			McAvoy et al., 2002
Johannesburg			78 460-127	10 700-22	81-6			Amdany et al., 2014
Gauteng Province	880-8 720		2 010-17 600	990-13 000	67			Lehutso et al., 2017
Durban			2 100-9 000	1 300-6 400	40			Madikizela et al.
Switzerland	1.4-74		500-1 300	70-650				Lindström et al. 2002
Ruhr, Germany	3-10		4 800-7 300	10-600	87-95			Bester, 2005
Dortmund			1 200	51	96	65	31	Bester, 2003
Mid-Atlantic			4 700	70	98	48	50	Heidler & Halden
China					28	19	32	Huang et al., 2014
United States					20-75			Ogunyoku & Young
Kansai region						30		Narumiya et al., 2013
TCC								
GP, South Africa	ND-360	86-2 840	LOD-1 890		97			Lehutso et al., 2017

*TCC: triclocarban, TCS: triclosan, GP: Gauteng province

2.7. CONCLUSIONS AND RECOMMENDATIONS

2.7.1. Concluding remarks

In this chapter, finings based on the bottom-up approach where the market data on PCPs was used to estimate the potential risks of TCS and TCC in different environmental compartments are presented. Owing to the versatile nature of the MFA approach, the likely risks of TCS and TCC in the aquatic systems were estimated, and the identified most affected compartments by these chemicals are freshwater and wastewater systems. Moreover, owing to lack of MECs data in South Africa impended mainly on the feasibility to ascertain the accuracy of the estimated PECs with respect to several proposed ranking criteria frameworks (Coetsier et al., 2009; Verlicchi et al., 2014). Despite this shortcoming, findings presented in this work have practical- and policy-related implications.

Given the complexity of environmental systems, and the spatial level targeted in this study, showed the estimated PECs were dependent on the quality and available data; and therefore, the accuracy of the findings are based on scenarios considered in this study and may change under different assumptions or in specific regions. Thus, based on the market penetration, purchasing power, dilution factors, among other variables considered in this study, RQs were > 1 in freshwater ranging from 2 to 232 for both TCS and CC (PECs values); whereas for TCS > 1 in influent and effluent wastewater, but < 1 for TCC. Hence, results show TCS can pose risks in wastewater and freshwater, whereas TCC poses risks to freshwater but none in wastewater. Using the available ecotoxicological data, both chemicals posed no risk (RQ << 1) to the terrestrial environments in GP, South Africa, although the ecotoxicological data in this compartment is scarce and defined by numerous data gaps.

2.7.2. Recommendations

In order to obtain better estimates on the flows of TCS and TCS, it is recommended that temporal and spatial MFA approaches are considered. This is key in effectively estimating the potential risks of chemicals in specific regions as shown in this study for triclo- and nanoscale-based chemicals. Moreover, it is essential to consider the development of dynamic material flow analysis (DMFA) model coupled with together spatial analysis which incorporates water flow, water bodies, population densities, income densities, etc. to refine the flows, and in turn, the potential of contaminants as those reported in this study.

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CHAPTER 3 : DEVELOPMENT OF DECISION SUPPORT SYSTEM TO ESTIMATE EXPOSURE POTENTIAL OF ENMS IN AQUATIC SYSTEMS USING FUZZY LOGIC THEORY

Ntsikelelo Yalezo, Ndeke Musee

3.1 INTRODUCTION

Nowadays, engineered nanomaterials (ENMs) are widely used in consumer products and industrial applications with the aim to improve products effectiveness and performance (Besinis et al., 2014, Vance et al., 2015). This is because of ENMs unique characteristics such as large surface area, huge band gap, antibacterial effects and high adsorption capacity per unit area (Reidy et al., 2013; Gottschalk et al., 2013; Vance et al., 2015: author) hence they find applications in automobiles, cosmetics, catalysis, coating and water treatment works (PEN, 2018; The Nanodatabase, 2018). Results from these inventories have highlighted diverse nanoproducts within the retail market, yet the potential risks associated with ENMs remain inadequately quantified (Art et al., 2015; Tolaymat et al., 2015; Coll et al., 2016; Musee, 2017).

To date, existing models estimate exposure of ENMs based on quantitative approach, e.g. mass flow analysis to predict environmental concentration (PEC). In addition, the risk assessment is based on the principle of deterministic ratio approach defined as (predicted environmental concentration PEC /predicted no effect concentration PNEC) (Mueller and Nowack, 2008; Gottschalk et al., 2009; Gottschalk et al., 2013; Dale et al., 2013, Keller et al., 2013; Scheringer et al., 2015; Dumont et al., 2015; Zhang et al., 2015; Musee, 2017). However, such approach do not consider the transformation behaviour aspect of engineered nanoparticles in estimating the risk (Dale et al., 2015; Nowack et al., 2017). Scientific evidence suggest otherwise as the exposure is dependent on both ENPs concentration, their intrinsic properties and abiotic factors. To address such limitation in the current knowledge, necessitates the development of universal conceptual framework which can link ENPs quantise and the behaviour mechanisms.

To date, for example there is increasing evidence of existence of ENMs in the aquatic systems as there are number of studies on their measured concentrations (Kaegi et al., 2008; Kaegi et al., 2010; Neal et al., 2011; Khosravi et al., 2012). Subsequently, the released ENPs interact with different species where evidence studies have their ability to induce adverse impacts to biological lifeforms (Reidy et al., 2013; Gkika et al., 2017; Mahaye et al., 2017; Hou et al., 2018). Owing to the diversity of fate and toxicity data as well as the large number of ENMs together with their derivatives that currently in use, render it unfeasible to undertake all the relevant experimental works required, due to cost, labour intensity, and urgency. Therefore, in this study, we adopted the use of a modelling approach to model the potential exposure of ENMs in the aquatic systems using artificial intelligence techniques – with specific reference to fuzzy set theory. Adoption of using fuzzy based models in the field of exposure assessment of ENPs in aquatic systems aid to fill knowledge gaps as well as extrapolate and predict missing data for decision-making based on the supposition from existing knowledge (Dale et al., 2015). Moreover, such a model aided to provide a unified framework to explain complex phenomena.in this case the fate and transformation of ENPs in the aquatic system.

To date, ENPs undergo transformation processes following their release into the aquatic systems, e.g. aggregation, dissociation, agglomeration, dissolution, adsorption, and deposition. These are widely investigated using the natural (Elzey and Grassian, 2010; Tso et al., 2010; Sillanpaa et al., 2011; Nur et al., 2014) and laboratory-based settings (Pettibone et al., 2008; Orma et al., 2014; Chekli et al., 2015; Vikesland et al., 2016). For example, deposition rate of ENMs has been quantified using the Stokes law – where heavier agglomerates were observed to settle faster than smaller particles – due to strong gravitational force pulling down large aggregates. The high gravitational settling (high deposition rate) results from rapid agglomeration, and subsequently, decreased the ENMs suspension in water column (Vikesland et al., 2016). As a result, the observed toxicity induced to biological species in the water column were likely to be minimal (Nur et al., 2014; Gkika et al., 2017; Hou et al., 2018) but higher effects to highly plausible the benthic organisms (Quika et al., 2010). In contrast, high dispersion in the water column due to adsorption and coatings of ENMs with negatively-charged natural organic matter was observed and evidenced by their mobility which was inversely proportional to aggregation rate(s) (Thio et al., 2011).

Most of such information and exposure data have been summarized in numerous reviews (Reidy et al., 2013; Wonga et al., 2013; Wagner et al., 2014; Dwivedi et al., 2015; Peng et al., 2017; Yu et al., 2018). However, with dramatic increase in data generated both in quantity and quality it is difficult for policy- and decision-makers to make decisions owing to current information being highly unstructured, uncertain and ambiguous. None of the existing conceptual network are organised for automation using fuzzy logic (FL). Previously, there have been attempts using artificial neural networks (ANNs) (Ondiaka, 2016) and Monte-Carlo simulation (M-CS) (Liu et al., 2010) to develop predictive models on the potential risk or effects of ENMs into the aquatic systems. However, use of ANNs and M-CS approaches to develop decision support system (DSS) are rather highly challenging especially in an emerging field due to lack of quantitative data, for example, as it is problematic to source data from graphs published in the scientific literature (Vafaeenezhad et al., 2013; Ondiaka, 2016). Hence, in this study an alternative approach – the fuzzy logic approach – which can address environmental problems where data and information are highly limited, or lacking is proposed as such applies in the case of ENPs in the environmental system.

3.2. SYSTEMATIC ARCHITECTURE FOR DECISION SUPPORT SYSTEM

Intelligent decision support systems (IDSS) framework can aid to organize and arrange the information into amenable format which non-experts can be able to make decisions (Jung and Burns, 1993; Musee et al., 2006; Musee et al., 2008). The strength of the preferred model approach relies on its capabilities to consider, two key factors such as, (i) the type of database and (ii) linearity or non-linearity of data (Dubois and Prade, 2003, García-Diéguez et al., 2015). To derive consistent decisions using database characterized by qualitative and quantitative format, statistical-driven methodologies are not usefully as they have drawbacks and such models rely on large numerical data sets. To develop a fuzzy model-based DDS to determine the likely fate of ENPs in the environment entailed three steps (i) knowledge elicitation. This entailed systematical outline and identification controlling factors on the exposure potential of ENMs in the aquatic systems. Development of a set of criteria to organize the solicited data into various categories amenable for automation. Next the data solicited from the literature was coded into a knowledge and rule bases required for decision making. The rules were used to test the functionality to estimate the potential risks of ENPs in the aquatic system. Figure 3-1; depicts the proposed developed system architecture of ENMs exposure assessment decision support system (EADSS).

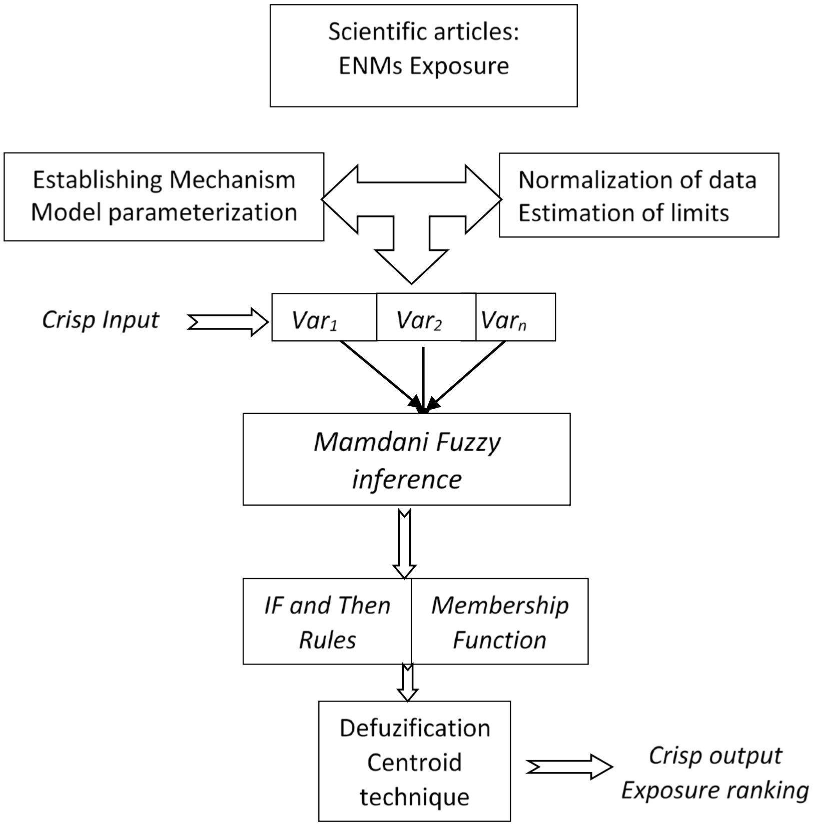

Figure 3-1: Systematic architecture for decision support system (DSS)

3.2.1. Knowledge acquisition

3.2.1.1. Data collection

Articles were accessed online from different scientific databases among them; Google scholar, ScienceDirect, American Chemical Society, SpringerLink. The following keywords such as fate, aggregation, adsorption, dissolution, surface transformation, deposition, dispersion of ENMs aided to search articles on the search engines in various data base. Pre-screening of the gathered scientific articles was undertaken in attempt reduce the sample size and improve the quality of the data collected in the process. The selected articles on exposure studies satisfied the following set criteria (i) reported data on metal / metal-oxide as ENPs of interest (ii) published between 2005-2018, (iii) synthesised water / natural water (e.g river, dams) were used as exposure medium and, (iv) provided information on one or more characteristics of ENMs and water chemistry .

To date, many scientific reports have been provided, regarding plausible estimated ENMs concentration in the aquatic system. However, the detected concentrations are based spot grabbed samples (kaegi et al., 2008; kaegi et al., 2010; Neal et al., 2011), or are defined on certain spatial boundaries (e.g. City or province) based on the models (Keller et al., 2013; Scheringer et al., 2015; Dumont et al., 2015; Zhang et al., 2015; Musee, 2017); therefore, are not representative of the entire ecosystem. Herein, bottom up approach for developing fuzzy models of ENPs was used. The study adopted to use variables such as product matrix (PM), user frequency (UF) and product concentration (PC) (Hansen et al., 2007; Teide et al., 2015; Zhang et al., 2015, Musee, 2017) quantification of ENMs. The qualitative and quantitative data retrieved from the sources was coded into spreadsheets and then was categorized for further analysis. The data included ENMs quantification properties (e.g. product matrix, user frequency), physicochemical properties (e.g. size, shapes/phase, crystal structure, zeta potential, coating agent, surface distribution, surface charge, etc.) and water chemistry properties (e.g. natural organic matter (NOM), ionic strength, and pH). Figure 3-2, shows the percentage distributions of the articles retrieved for different ENPs.

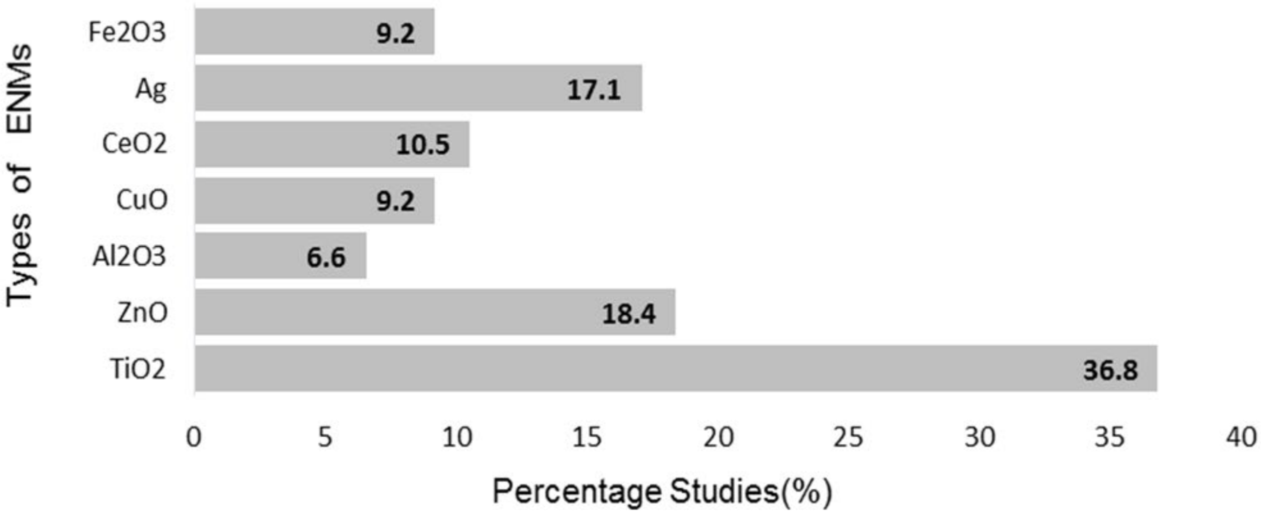

Figure 3-2: Distribution of articles per ENMs based on data sourced from literature (n = 76)

Following the intesive procedure discussed above, approximately (n = 76) articles met the set criteria. These were then retrieved, and further appraisal of the evidence was undertaken. The distribution of the articles per given ENPs is depicted in Figure 3-2: 36.8% represented articles on $nTiO_2$ and 9.21 for n-Fe_2O_3, correspondingly. Regarding the reviewed articles, 81.6% reported studies in synthesized water, and the rest (18.4%) were done in natural waters.

3.2.1.2. Model parameterization

Among the concerns as the exposure data increases in scientific literature relates to inability to adequately select the key controlling parameters for the exposure potential primarily due to many contradictions in the existing data (Chekli et al., 2015; Dwivedi et al., 2015). To date, there is lack of a framework that link the exposure potential of ENMs in the environment as a function of; physicochemical properties, water chemistry, and their quantities. A qualitative evidence-based procedure (Akobeng, 2005; Tolaymat et al., 2015) and multicriteria decision analysis (MCDA) are among the approaches for DSS used to solicit data (Yatsole et al., 2006, Rycroft et al., 2018). This is generally achieved through two stage processes: first, to establish the decision-problem of focus, and secondly, find adequate qualitative evidence through assessment of scientific database for modelling purposes. Herein, qualitative evidence-based procedure aided to analyse and prioritize both structured and unstructured exposure data of ENMs in the environmental systems. The selection of minimal input variables is critical for development fuzzy model, as the number of fuzzy rules are exponential function of the number of input variables. Hence, considering only significant variables reduces uncertainty and complexity, and this is difficulty in domain with non-linear data (Chiu, 1996).

The selection of inputs, intermediate and outputs was undertaken through problem solving principle of model parsimony (NRC, 2007). The parsimonious principle suggests (i) few parameters should be used in modelling and (ii) simplified explanation measures should be adopted over complex ones (Gigerenzer, 2010). The most reported properties in literature on ENPs are summarized in Figure 3-3 and Figure 3-4. In Figure 3-3; pH was found to be the most dominant water chemistry property reported at 100%, and temperature the least at 6.6%. In Figure 3-4; size was the most dominant physicochemical property reported at 97.7% and crystallinity the least at 19.7%. These trends were similar with the previous results reported elsewhere earlier (Chen el al., 2015; Tolaymat et al., 2017). Tolaymat et al., 2017, for example, reported 6% studies reported crystallinity and size was most reported property accounting for 61% of the articles considered.

In this study, we propose a novel and comprehensive aquatic environmental exposure modelling framework to assess exposure of metal- and metal-based ENMs in the aquatic systems. The conceptual framework is used to highlight the linkage between deposition and dispersion to both intermediate and input model parameters. The framework proposed herein adopted bottom-up approach where physicochemical properties (e.g. size, zeta potential, coating, etc.), and water chemistry (e.g. pH, natural organic matter (NOM), ionic strength, etc.), and ENMs quantification properties (e.g. product matrix, product concentration, and user frequency) relationships were mapped.

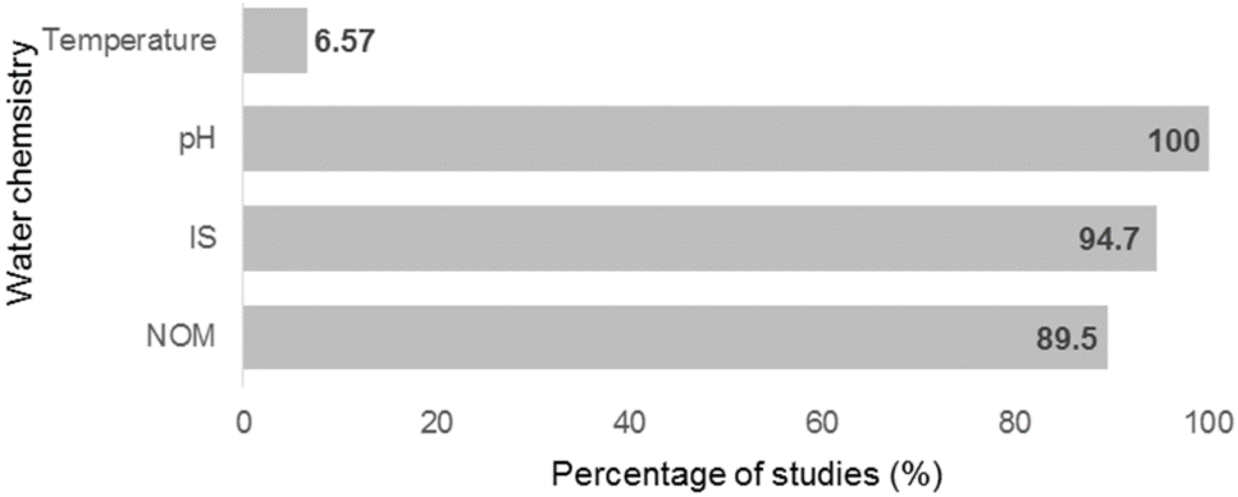

Figure 3-3: Distribution of articles reported on each aquatic environmental property

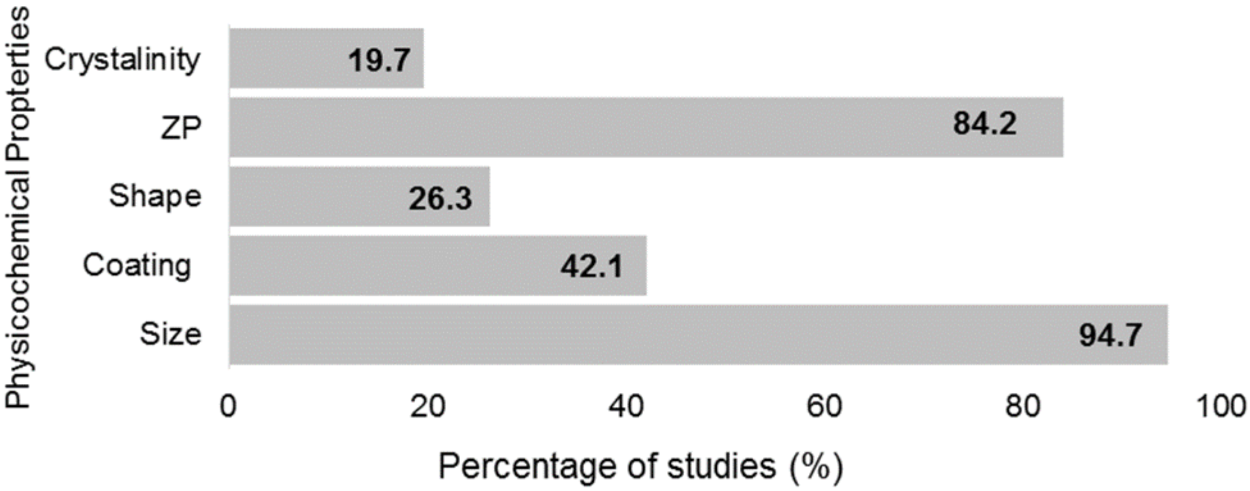

Figure 3-4: Distribution of articles on reporting each physicochemical property

Here we propose a novel and comprehensive aquatic environmental exposure modelling framework for assessing exposure of metal and metal based ENMs in aquatic systems. The conceptual framework is used to highlight the linkage of deposition and dispersion to intermediate and input parameters. The framework proposed bottom up approach derived from physicochemical properties such as size, zeta potential, coating. Conversely, the environmental factors such as pH, natural organic matter (NOM), ionic strength and ENMs quantification properties product matrix, product concentration, user frequency. Linked with transformation behaviour such as, e.g. aggregation, dissolution and adsorption to dispersion and deposition. The number of inputs identified it's not exhaustive in nature as there are other variables, however a herein the study seeks to use most reported properties as the bases.

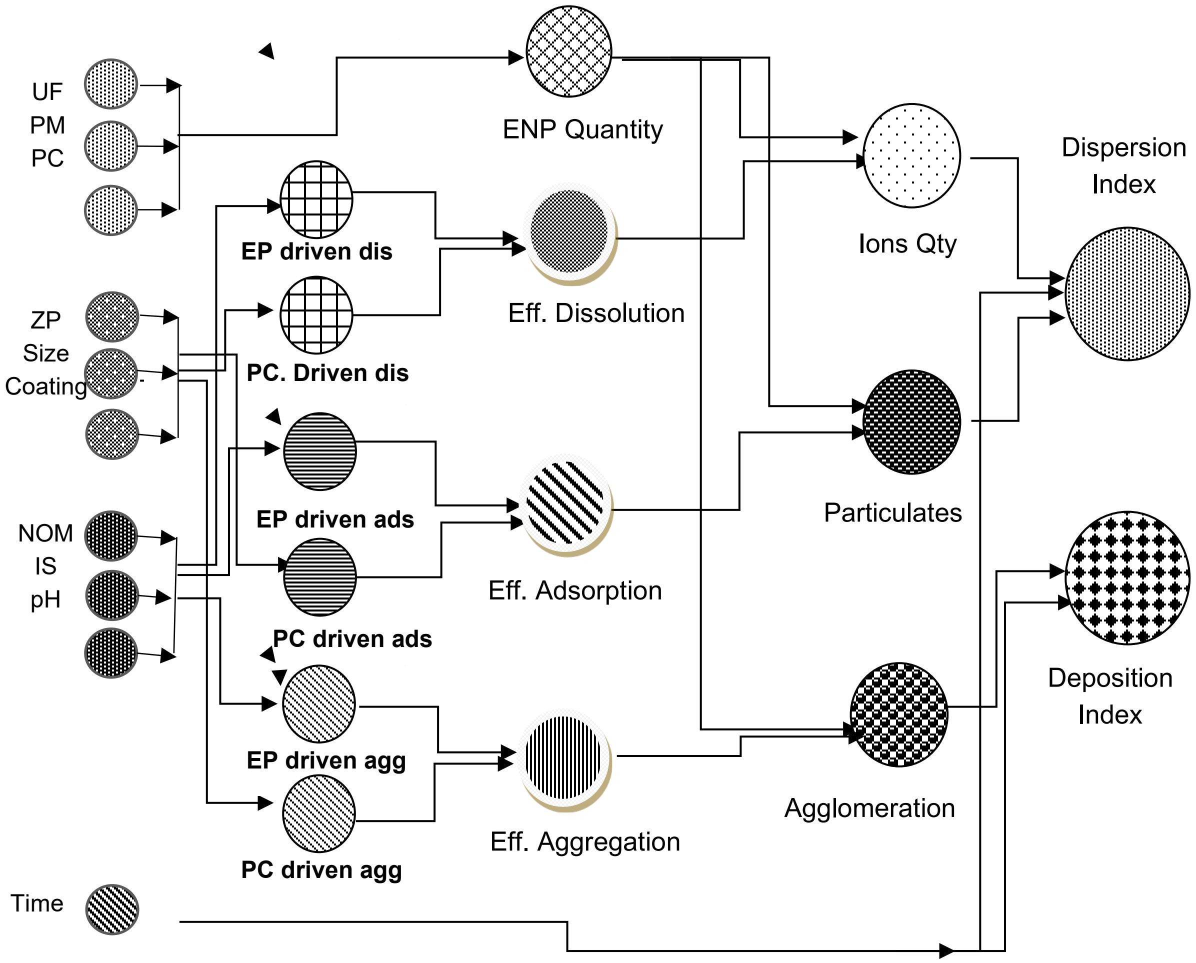

Figure 3-5: The structure of the inputs, intermediate, and output map for the exposure assessment of ENMs in the environment (NOM: natural organic matter; ZP: zeta potential; IS: ionic strength; PC *driven* agg: physicochemical properties-driven aggregation; PC *driven* ads: physicochemical properties-driven adsorption; PC *driven* dis: physicochemical properties-driven dissolution; EP *driven* agg: environmental-driven aggregation state; EP *driven* ads: environmental-driven adsorption state; EP *driven* dis: environmental-driven dissolution; Eff: effective; PM: Product Matrix; UF: User frequency; PC: Product concentration

3.2.2. Fuzzy set theory

Fuzzy set theory (Zedah, 1965) is a soft computing tool, and offers a rational and well-reasoned solution as a way of simulating human linguistic reasoning in complex problem domains (Shi et al., 1999; Barman and Choudhury, 2012). It is a non-monotonic reasoning mechanism and it generalises classic set to linguistic representation in n-dimensional space to enable modelling of information with ambiguity. Fuzzy sets are represented by a membership function μA(x) which is associated with each point in universe of discourse, in the interval [0,1] (Shi et al., 1999). Fuzzy logic comprises of fuzzy sets, which are a way of representing non-statistical uncertainty. Using mathematically definition, a fuzzy set A is defined as a set of ordered pairs described as shown Equation 3-1.

$$A=\{x,\mu\_A\ (x)I\ x\epsilon X\}, \qquad \mu\_A\ (x)x\epsilon\ [0,1] \tag{3-1}$$

where A is the fuzzy set x, described using linguistic variables and μA(x) is a value of a membership function in the interval [0,1].

FL allow multiple representations defined as nonlinear mapping of a vector inputs data set to a scalar output data in continuum set of membership function (Yeung and Tsang, 1997; Shi et al., 1999). This approach is representative of the nature of real-world problems. It is in this context that fuzzy logic offers mathematical formulations that can characterize uncertainty of data associated with parameters in a problem. FL in numerous studies have been shown to improve quality, reduce time in production and as powerful design and analysis technique for complex systems (Ressom et al., 2005; Musee et al., 2006; Vafaenezhad et al., 2013; Dieguez et al., 2015; Di addario et al., 2017). The fuzzy inference algorithm was generically developed through three phases namely; (i) fuzzification, (ii) knowledge-rule base and, (iii) inference and defuzzification system.

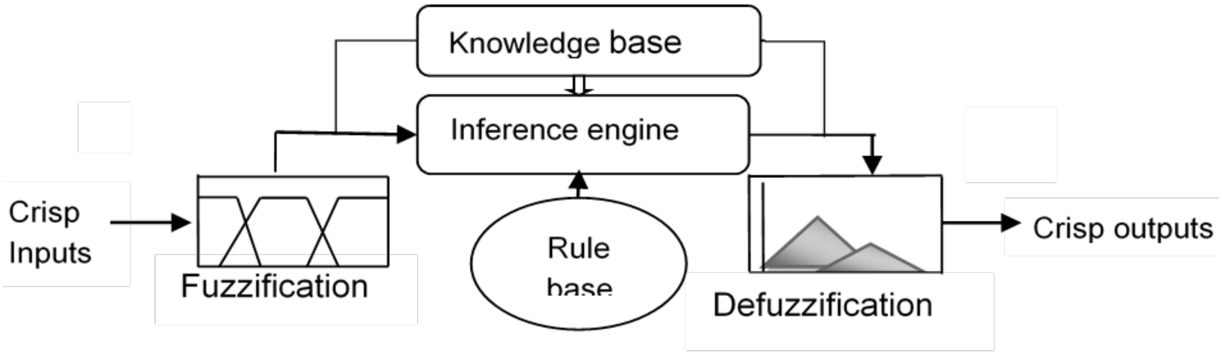

Figure 3-6: Systematic architecture for building fuzzy algorithm

3.2.2.1. Fuzzy membership function

Fuzzification of crisp inputs to linguistic variables was defined using a membership function (MF). MFs are widely used for fuzzy representation, and the widely used single MFs methods are; triangular, Gaussian, sigmoid, trapezoidal (Klir and Yuan, 1995; Mendel, 1995; Yeung and Tsang, 1997). In this work, the triangular and trapezoidal MF representation were used to code the non-linearity ENMs data into the model. (Musee, 2008; Erkan et al., 2016). Fuzzy inference system was built using Mamdani Fuzzy Logic Toolbox integrated in Matrix Laboratory (MATLAB). In this work, 10 primary inputs parameters (Figure 3-5) were fuzzified into fuzzy sets as listed in Table 3-1. The ranges in the universe of discourse for each input parameter were described based on minimum and maximum values relevant to the aquatic system. The primary inputs for trapezoidal and triangular-shaped MFs as a function of vector, x, were described using Equation 3-2 and 3-3, respectively.

$$\mu(x,a,b,c,d) = \begin{Bmatrix} 0, & x \le a \\ \frac{x-a}{b-a}, & a \le x \le b \\ 1, & b \le x \le c \\ \frac{d-x}{d-c}, & c \le x \le d \\ 0, & d \le x \end{Bmatrix} \tag{3-2}$$

where parameters *a* and *d* control the size of trapezoidal base whilst the parameters *b* and c define the "shoulders", and each of the input membership function for trapezoidal parameters are summarized in Table 3-1.

$$\mu(x,a,b,c) = \begin{Bmatrix} 0, & x \le a \\ \frac{x-a}{b-a}, & a \le x \le b \\ \frac{c-x}{c-b}, & b \le x \le c \\ 0, & c \le x \end{Bmatrix} \tag{3-3}$$

where the parameters *a* and *c* control the triangle base, and the parameter *b* locates its peak position, and each of the input membership value are defined in Table 3-1.

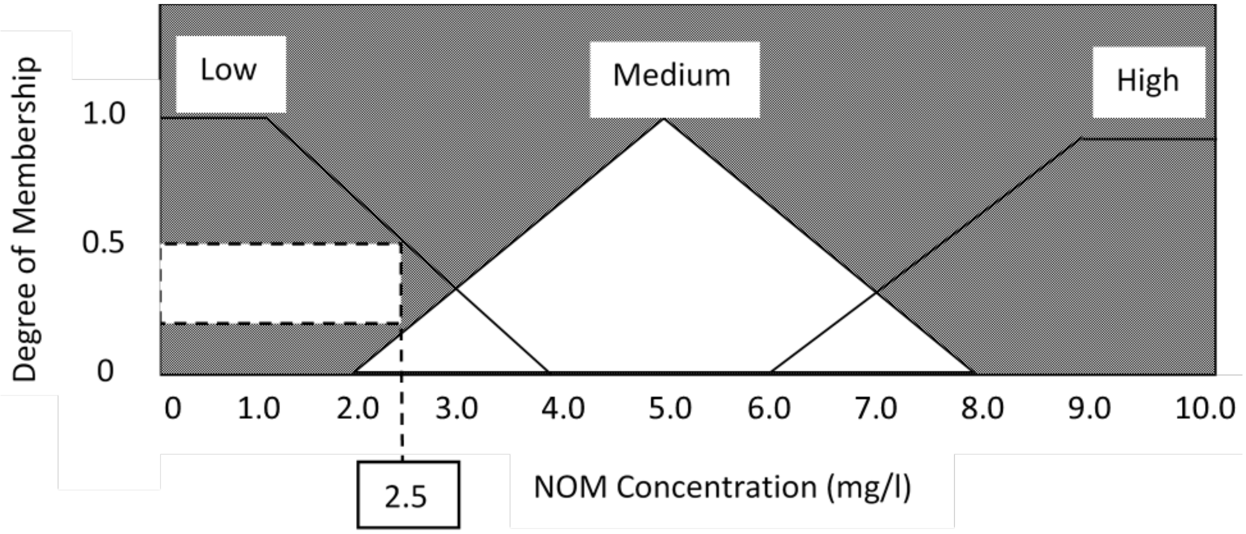

Figure 3-7: Distribution of triangular and trapezoidal membership function for NOM

Figure 3-7 depict the distribution of triangular and trapezoidal curves for fuzzification of NOM. The crisp input 2.5 in the universe of discourse [0,10] belonged to two fuzzy linguistic sets that are; "*Low*" and

"*Medium*". Using Equations 3-2 and 3-3, the crisp input of NOM= 2.5 generate two members function permitted by the combination of triangular and trapezoidal membership function and can be expressed as μ_1 (min, 2.5) = 0.2 and μ_2 (max, 2.5) = 0.5, representing low and medium fuzzy sets, respectively. Table 3-1 summarises fuzzification of other inputs using within the universe of discourse described.

Table 3-1: Parameterization of fuzzy inputs used in the development of ENMs exposure assessment decision support system (EADSS)

Inputs	Universe of Discourse	Units	Membership function and respective values										
			Low Trapezoidal				Medium Triangular			High Trapezoidal			
			a	b	c	d	a	b	c	a	b	c	d
NOM	[0 10]	mg/l	[0	0	1	4]	[2	5	8]	[6	9	10	10]
pH	[0 10]		[0	0	1.5	3.5]	[2	4	6]	[4.5	8	10	10]
IS	[0 1]	M	[0	0	0.1	0.4]	[0.2	0.5	0.8]	[0.6	0.9	1	1]
Coating	[0 1]		[0	0	0.2	0.4]		- - -		[0.3	0.6	1	1]
Size	[1 100]	nm	[1	1	10	35]	[20	50	80]	[60	90	100	100]
ZP	[0 40]	mV	[0	0	5	15]	[10	20	30]	[25	35	40	40]
PC	[0 25]	%	[0	0	20	40]	[20	50	80]	[60	80	100	100]
UF	[0 1]		[0	0	0.1	0.35]	[0.2	0.5	0.8]	[0.65	0.9	1	1]
PM	[0 1]		[0	0	0.1	0.35]	[0.2	0.5	0.8]	[0.65	0.9	1	1]
Time	[0-120]	hours	[0		20	45]	[30	60	90]	[70	100	120	120]

NOM: Natural Organic Matter; IS: Ionic strength; PM: Product matrix; UF: User frequency; PC: Product concentration; ZP: Zeta potential.

3.2.2.2. Linguistic Rules

Knowledge gathered was coded using fuzzy rules in the rule-based interface module using semantics *IF-THEN* in generic form in Equation 3-4. The *IF* component of the statement was used to capture the information and the *THEN* consequent component to give response. Theoretically, the maximum number of fuzzy rules can be computed as exponential function described as w^m, where m represents number of inputs of the system and w the number of the fuzzy sets (Yeung and Tsang, 1997; Shi et al., 1999; Zolghadri et al., 2007). Each rule is described in the product space $A \times M$.

$$R_g : \mathit{IF}\ x_1 \text{ is } A_{g1}\ \mathit{AND}\ x_2 \text{ is } A_{g2} \ldots \mathit{AND}\ x_k \text{ is } A_{gk} \tag{3-4}$$

THEN y_k is M_g with CF_g; g = 1, 2, . . . k

where, X=(x_1, x_2, x_3. . . . x_k) ∈ *A* represents antecedent, Y= (y_1, y_2, y_3. . . y_k) ∈ *M*1; M_g M2 . . . M_k is the consequent class of the rule, A_{gk} is the fuzzy set associated to x_k, CF_g is the rule weight of rule R_g, and k is the number of fuzzy antecedents in the rule-base. The use of rule weights on the coded *IF-Then* rules in the knowledge base was to improve the accuracy of model outputs in the fuzzy inference system (FIS).

From a theoretical view point, the 10 crisp inputs can yield a maximum of *IF-Then* rules computed as 3^{10} x 2^1 =118 098. However, due to errors and time consuming and tedious process such high number of rules, an hierarchical approach to link inputs to intermediate outputs and then the final model outputs was done in a similar version as done by Musee et al. (2006) on classification of hazardous composite waste. Thus the input parameters were categorised into three layers to reduce the complexity as shown in the conceptual framework summarized in Figure 3-5. In this approach, water chemistry, inherent physicochemical, and ENMs quantifications attributes yielded a total of 27 rules under each rule-base since had a set of 3^3 matrix. In the design of *if-then* rules water chemistry factors were assigned higher weights as the controlling factors, and any rules that were in conflict with what is practically feasible, such rules were regarded as retardant rules, and hence, were removed from the rule base.

3.2.2.3. Fuzzy Inference and defuzzification

A fuzzy rule composition technique namely 'MAX-MIN' was used to combine two or more MF (Zadeh, 1975; Mandani, 1999). A defuzzification technique namely a center of gravity method (COG) defined mathematically in Equation 3-5 was used for defuzzification of the crisp out. In this approach a weighted average of the membership function curve is computed as a distinct arithmetical crisp output value (Yager and Zadeh, 1992; Mendel, 1995; Mandani, 1999, Musee et al., 2008).

$$z^* = \frac{\sum_{j=1}^{n} \mu_z(\omega_j)\acute{\omega}_j}{\sum_{j=1}^{n} \mu_z(\omega_j)} \qquad (3\text{-}5)$$

Where *z** is the deffuzified output, *r* is the number of the rules, w_j is the output value in the *j* subset and *μ* is the MF value of w_j.

3.3. SYSTEM TESTING AND EVALUATION

To demonstrate the functionality of the developed EADSS, simulations were carried using case studies summarised in Table 3-2 and 3-3. The FIS system required the user to provide 10 inputs to initiate the evaluation process. Data collated on physicochemical, water chemistry, and quantification parameters were used to test the functionality of model under diverse set of scenarios as depicted by the case studies. Data on the fate and behaviour of nZnO and $nTiO_2$ were used as inputs to illustrate the functionality of proposed fuzzy model developed in this study. For completeness, hypothetical scenarios were developed to test the functionality of the model with respect to how the output(s) correlated to a set of inputs. This entailed, first, having both qualitative and quantitative attributes being transformed to numerical values to render them compatible for use in the fuzzy algorithm. And secondly, Input variables were fuzzified and assigned linguistic values or membership function based on the universe of discourse. Tables 3-4 and 3-5 lists the aggregated fuzzy system results obtained using the set of inputs values listed on Tables 3-2 and 3-3, respectively. The procedure followed was based on the designed hierarchical approach in Figure 3-5. The pH and zeta potential in Tables 3-2 and 3-3, were computed using Equations 3-6 and 3-7, respectively.

$$pH = \mathrm{Abs}(pHm - pH_{PZC}) \tag{3-6}$$

$$ZP = \mathrm{Abs}(ZP_{ENP}) \tag{3-7}$$

where pHm denotes the pH of the medium, pH_{PZC} represents the point of zero charge of the ENPs, and the ZP_{ENP} is the actual surface charge of ENPs, and can be negative or positive.

The aggregated fuzzy values of EPA (0.925), EPD (0.075) and EPAd (0.300) shown in (Table 3-4, Column 2-4, Case 1 of nZnO) were assigned linguistic rankings of *very high*, *very low* and *low*, respectively, computed using water chemistry values in Table 3-2 (Column 2-5, Case 1 of nZnO). These findings suggest very high aggregation of nZnO. Similar observations have previously been reported in experimental studies (Bain et al., 2011; Odzak et al., 2014; Majedi et al., 2014) at pH close to the point of zero charge ~9.1, low natural organic matter concentration, and under high ionic strength conditions. High concentration of NOM at10.00 mg/l as shown in Table 3-2 (Column 2, Case 2 of nZnO), resulted to EPA of 0.925, linguistically ranked as *very high* indicating the stabilizing ability of the natural organic matter as the negatives charged material. Results of further high adsorption capacity in relation to $nTiO_2$ and NOM are 0.921, 0.803 and 0.668 in Table 3-5 (Column 4, Case 3, 7 and 8). These findings suggest likely linear relationship between NOM concentration and adsorption of $nTiO_2$; where high adsorption were attributed with high electro negative surface of the NOM.

Table 3-2: Set of model input parameters based on nZnO

Case#	NOM (mg/l)	pH_{Medium}	Δ pH	IS (mM)	Coating	Size(nm)	ZP_{ENP}(mV)	ZP (mV)	PM	UF	PC	Time (h)
C_{Zn1}	1.000^{a}	8,100	0.650a	1.000^{a}	0.120	20.00^{a}	+22.00	22.00	0.800	0.650^{J}	15.50^{J}	0.250^{a}
C_{Zn2}	10.00^{a}	7,450	1.300	0.100^{a}	0.210	20.00^{a}	-28.00	28.00	0.700	0.700^{k}	0.001^{k}	1.000^{a}
C_{Zn3}	2.000^{b}	7,900	0.950	0.001^{b}	0.140	70.00^{b}	$+13.00^{b}$	13.00	0.600	0.200^{l}	3.000^{l}	24.00
C_{Zn4}	0.000	8,000	0.750	1.000^{c}	0.700	20.00^{c}	+15.00	1500	0.850	0.640^{k}	3.000^{k}	24.00d
C_{Zn5}	2.000^{d}	6,400	2.350	0.732	0.003	52.00^{d}	-23.00^{d}	23.00	0.837	0.880^{l}	10.00^{m}	24.00
C_{Zn6}	3.000^{e}	10,00	1.050	0.572^{d}	0.001	4.000^{e}	-25.00^{e}	25.00	0.600	0.550^{l}	5.000^{L}	90.00
C_{Zn7}	0.900^{h}	9.800	0.850	0.080^{e}	0.520	$15.,00^{e}$	-25.00^{e}	25.00	0.860	0.200	3.000	3.000
C_{Zn8}	4.650^{f}	7,000	1.950	0.080^{e}	0.120	20.00	$+26.00^{f}$	26.00	0.600	0.690	3.000^{k}	5.000
C_{Zn9}	0.500^{l}	6,100	2.650	0.029^{g}	0.184	4.500^{g}	+23.00	23.00	0.850	0.180	0.372	76.00a
C_{Zn10}	3.100^{h}	6,400	2.350	0.300^{h}	0.600^{h}	15.00^{h}	$+30.00^{l}$	30.00	0.837	0.550	0.001^{n}	12.00

NOM: Natural Organic Matter; IS: Ionic strength; PM: Product matrix; UF: User frequency; PC: Product concentration; a=Zhou and keller, 2010; b=Zhang et al., 2008; d:Majedi et al., 2014; e:Bian et al., 2011; f: fang et al., 2017; g: Odzak et al., 2014; h: Odzak et al., 2017; I: Omar et al., 2014; J: Boxel et al., 2007; k: Tiede et al., 2015; l: Lorets, 2006; m ; Zhang et al., 2015, n: Weir et al., 2012.

Table 3-3: Set of model input parameters based on $nTiO_2$

Case#	NOM (mg/l)	pH_{Medium}	ΔpH	IS (mM)	Coating	Size(nm)	ZP_{ENP}(mV)	ZP	PM	UF	PC	Time(h)
C_{Ti1}	0.400[a]	6,500	0.250	1.000[a]	0.114	24.30[a]	+6.060	6.060	0.862	0.700[l]	0.001[j]	15.00
C_{Ti2}	0.100[a]	7,000	0.700	0.120[c]	0.760	5.000[c]	+32.00	32.00	0.820	0.310	5.000[f]	24.00
C_{Ti3}	8.340[d]	5,000	1.500	0.050	0.849	30.00[e]	-20.00[d]	20.00	0.720	0.830[h]	13.00[g]	10.00
C_{Ti4}	3.000	9,400	2.700	0.850	0.000	17.70[b]	+18.00[d]	40.00	0.700	0.280	0.001[j]	1.000
C_{Ti5}	0.350	8,000	2.050	1.000[a]	0.020	7.000	+23.00[e]	23.00	0.720	0.120[i]	0.500[f]	36.00
C_{Ti6}	0.400[a]	8,000	2.750	0.200	1.000	15.00[d]	+25.00	25.00	0.500	0.360	4.000[m]	12.00
C_{Ti7}	7.320	7,450	1.000	0.005[c]	0.320	36.20	-10.00	10.00	0.620	0.200	0.740[g]	3.000
C_{Ti8}	8.340	6,400	2.300	0.630[d]	0.001	26.20	-16.10[a]	16.10	0.650	0.100[i]	0.001[j]	72.00
C_{Ti9}	2.000[c]	4,600	1.200	0.124[c]	0.120	56.00	+15.00	15.00	0.670	0.320	3.000	76.00
C_{Ti10}	10.00[b]	7,900	1.700	0.497	0.450	4.000	-22.00	22.00	0.500	0.340	0.790	48.00

NOM: Natural Organic Matter; IS: Ionic strength; PM: Product matrix; UF: User frequency; PC: Product concentration; a: Tso *et* al., 2010; b: Chowdhury et al., 2012; c: Domingos et al., 2009; d: Loosli et al., 2013; e: Thio et al., 2011; f: Boxel et al. 2007; g: Tiede et al., 2015; h: Lorets, 2006; I: Zhang et al., 2015; J: Weir et al., 2012; k: Biesterbos et al., 2013; l: Hall et al., 2007; m: Muller et al., 2007.

The results of EPA (0.925) and EPA (0.291) in Table 3-4 (Column 2, Case 1 and 9 for nZnO) and results of EPA (0.912) and EPA (0.500) in (Table 3-5, Column 2, Case 5 and 6 for TiO_2) showed the effect of the pH which in the model was computed as difference between media pH and the point of zero charge. The results showed negative linear relationship between absolute pH and aggregation of the ENPs for nZnO and $nTiO_2$.This can be attributed to the factor that pH has influence on the electric double layer (EDL) where if the pH is away from point zero charge, the ENPs were stable, and vice versa (Domingos et al., 2009; Thio et al., 2011). The aggregated fuzzy values tabulated in Tables 3-4 and 3-5 (Columns 5-7) are the results of the physicochemical-driven transformation of the ENPs. These results were computed using the zeta potential, size and coating fuzzy values. A coating value of 0.700 assigned linguistically ranking of *high* in Table 3-2 (Column 5, Case 6 of nZnO), yielded a *very low* physicochemical-driven aggregation (PCA), dissolution (PCD) and adsorption (PCAd) with fuzzy output values of 0.224, 0.2342 and 0.2845, respectively. This is attributed to the stability of coated ENPs through addition of capping agents, for example, electrostatic stabilized (citrate coated) or sterically stabilized (polyvinylpyrrolidone coated) as these have been previously reported to reduce aggregation, dissociation, and adsorption (Badaway et al., 2010; Ellies et al., 2016). The extent of reduction in aggregation, adsorption and dissolution depends on the structure of the capping agent as influenced by the chain length and functionality of the capping agent (Peng et al., 2017).

The ENPs size has effect on their aggregation, dissociation, and the ability to be adsorbed by organic materials (Nel et al., 2006; Bian et al., 2011). The results in Tables 3-4 (Column 5-7, Case 6 of nZnO) and in Table 3-5 (Column 5-7, Case 5 of $nTiO_2$) depict the influence of size in increasing the physicochemical-driven aggregation, dissociation and adsorption. The aggregated fuzzy values in Tables 3-4 and 3-5 (Column 8-10) was computed using inputs in Columns 2-7 as inputs in respective tables. The results of effective aggregation in Table 3-4 (Column 8, Case 4 nZnO) had fuzzy output of 0.267 determined as *very low* with EPA (0.500) and PCA (0.281) as inputs in Table 3-4 (Column 2 and 5) as model inputs. The input variables for determining effective transformation were designed such that water chemistry inputs were assigned high weights during the coding of fuzzy rules as they exert greater influence in determining the transformation behaviour. Tables 3-4 and 3-5 (Column 11) depict aggregated fuzzy values for the ENP concentrations ranking. The aggregated fuzzy value NPQ (0.738) had linguistic ranking of *high,* and was computed using the PM of 0.800 (ranked as *high*), user frequency index of 0.650 (ranked as *moderate*), and 15.5% (ranked as *moderate*). These were computed using the inputs in Table 3-2 and 3-3 (Column 8-10), namely; product matrix, frequency use of the product, and the weight percentage with a given product. To determine the deposition and dispersion ranking for nZnO and $nTiO_2$, fuzzy values in Column (2-14) were used as inputs. Figure 3-8 summarises exposure ranking index for dispersion and deposition of nZnO and $nTiO_2$ in relation to the primary inputs listed in Table 3-2 and 3-3, respectively.

Table 3-4: Results based on ZnO inputs in Table 3-2

Case (1)	EPA (2)	EPD (3)	EPAd (4)	PCA (5)	PCD (6)	PCAd (7)	EA (8)	ED (9)	EAd (10)	NPQ (11)	AI (12)	IQI (13)	PI (14)	DepI (15)	Deposition ranking (16)	DisI (17)	Dispersion ranking (18)
CZn_1	0.925	0.075	0.300	0.750	0.918	0.750	0.921	0.268	0.267	0.738	0.931	0.321	0.470	0.767	VH (0.89)	0.300	L (1)
CZn_2	0.300	0.075	0.925	0.808	0.910	0.808	0.268	0.268	0.914	0.367	0.268	0.268	0.595	0.179	VL (0.71)	0.320	L (0.80)
CZn_3	0.700	0.300	0.300	0.776	0.531	0.776	0.732	0.267	0.267	0.250	0.423	0.217	0.217	0.340	L (0.60)	0.150	VL (1)
CZn_4	0.500	0.075	0.300	0.281	0.281	0.281	0.267	0.077	0.077	0.500	0.400	0.217	0.216	0.300	L (1)	0.150	VL (1)
CZn_5	0.391	0.601	0.267	0.621	0.750	0.750	0.421	0.735	0.267	0.718	0.398	0.702	0.321	0.300	L (1)	0.600	L (1)
CZn_6	0.700	0.300	0.500	0.750	0.914	0.750	0.632	0.267	0.402	0.250	0.476	0.217	0.312	0.452	M (0.98)	0.110	VL (0.60)
CZn_7	0.700	0.300	0.300	0.281	0.281	0.281	0.500	0.077	0.077	0.500	0.567	0.217	0.217	0.492	M (0.58)	0.17	VL (0.80)
CZn_8	0.500	0.300	0.700	0.342	0.342	0.534	0.268	0.081	0.730	0.349	0.219	0.080	0.587	0.124	VL (0.74)	0.499	M (0.51)
CZn_9	0.291	0.624	0.210	0.478	0.702	0.750	0.321	0.733	0.275	0.500	0.288	0.601	0.124	0.214	VL (0.36), L (0.14)	0.502	M (0.48), H (0.02)
CZn_{10}	0.309	0.448	0.508	0.281	0.281	0.281	0.017	0.301	0.332	0.500	0.178	0.400	0.442	0.212	VL (0.38), L (0.12)	0.390	L (0.10), M (0.40)

AEI: attachment efficiency index; DepI: deposition index; IDisI: ionic dispersion index; IDisI: particulate dispersion index; EA: effective aggregation; ED: effective dissolution; EA: effective adsorption; EPA: environmental driven aggregation; EPD: environmental driven dissolution; EPAd: environmental driven adsorption; PCA: physicochemical driven aggregation; PCD: physicochemical driven dissolution; PCAd: physicochemical driven adsorption; NPQ: nano-particle quantity; PI; particulate index; IQI, ions index;, AI: agglomeration index; M: medium; L: low; VL: very low; VH: very high; H: high; N: none

Table 3-5: Results based on $nTiO_2$ inputs in Table 3-3

Case (1)	EPA (2)	EPD (3)	EPAd (4)	PCA (5)	PCD (6)	PCAd (7)	EA (8)	ED (9)	EAd (10)	NPQ (11)	AI (12)	IQI (13)	PI (14)	DepI (15)	Deposition ranking (16)	DisI (17	Dispersion ranking (18)
C_{Ti1}	0.912	0.075	0.300	0.915	0.750	0.519	0.925	0.258	0.267	0.500	0.682	0.321	0.300	0.525	M (0.25), H (0.25)	0.300	VL (0.85)
C_{Ti2}	0.700	0.300	0.300	0.379	0.750	0.379	0.731	0.077	0.267	0.500	0.567	0.217	0.217	0.474	M (0.76)	0.320	VL (1)
C_{Ti3}	0.330	0.080	0.921	0.279	0.279	0.750	0.077	0.077	0.700	0.722	0.231	0.124	0.672	0.220	VL (0.30), L (0.20)	0.150	M (0.65)
C_{Ti4}	0.331	0.231	0.769	0.919	0.750	0.518	0.331	0.268	0.721	0.377	0.217	0.291	0.621	0.192	VL (0.58)	0.150	M (0.72)
C_{Ti5}	0.797	0.096	0.296	0.923	0.750	0.517	0.887	0.301	0.288	0.399	0.571	0.332	0.332	0.421	M (0.71)	0.600	VL (0.44), L (0.06)
C_{Ti6}	0.500	0.300	0.300	0.632	0.793	0.537	0.585	0.267	0.077	0.250	0.403	0.217	0.067	0.325	L (0.75)	0.110	VL 0.70
C_{Ti7}	0.369	0.197	0.803	0.519	0.222	0.909	0.339	0.265	0.734	0.250	0.258	0.217	0.521	0.170	VL (0.80)	0.17	M (0.71)
C_{Ti8}	0.300	0.162	0.668	0.280	0.280	0.750	0.267	0.271	0.660	0.250	0.217	0.217	0.492	0.110	VL (0.60)	0.499	L (0.66)
C_{Ti9}	0.700	0.300	0.300	0.264	0.705	0.359	0.832	0.267	0.267	0.308	0.642	0.218	0.281	0.487	M (0.63)	0.502	VL (0.9)
C_{Ti10}	0.300	0.077	0.700	0.314	0.520	0.750	0.112	0.077	0.500	0.250	0.067	0.067	0.380	0.071	N (0.58), VL (0.21)	0.390	VL (0.7)

AEI: attachment efficiency index; DepI: deposition index; IDisI: ionic dispersion index; IDisI: particulate dispersion index; EA: effective aggregation; ED: effective dissolution; EA: effective adsorption; EPA: environmental driven aggregation; EPD: environmental driven dissolution; EPAd: environmental driven adsorption; PCA: physicochemical driven aggregation; PCD: physicochemical driven dissolution; PCAd: physicochemical driven adsorption; NPQ: nano-particle quantity; PI; particulate index; IQI, ions index;, AI: agglomeration index; M: medium; L: low; VL: very low; VH: very high; H: high; N: none

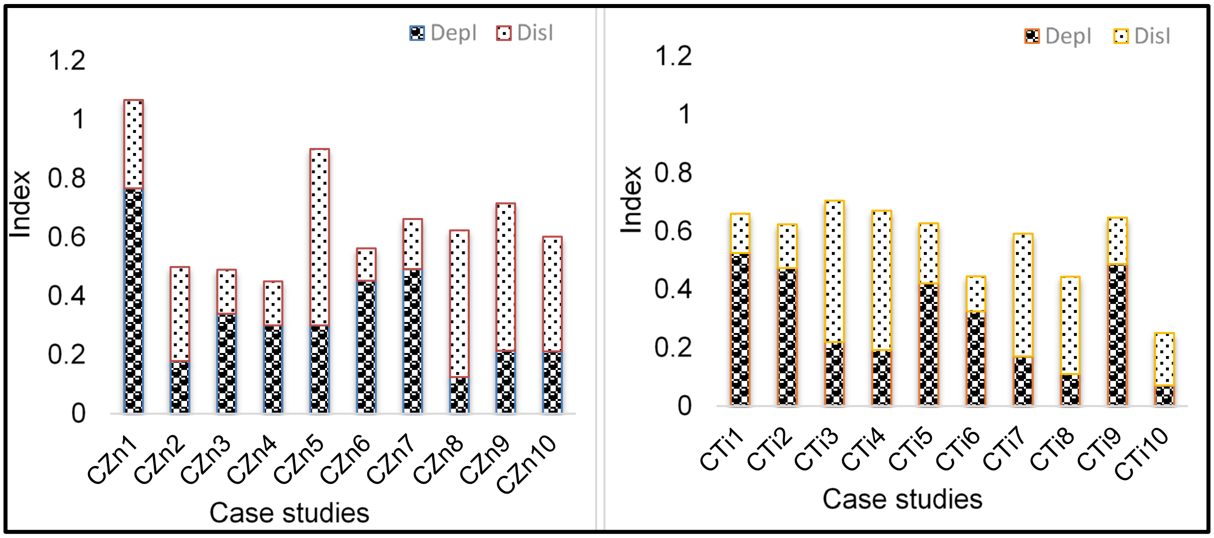

Figure 3-8: Dispersion and deposition ranking for (a) nZnO, (b) $nTiO_2$.

In this work, a case of cosmetics was used as the basis of the ENPs concentrations used in this model for nZnO and $nTiO_2$. Figure 3-8a, case 1 of nZnO show defuzzied crisp value for deposition of 0.767 linguistically ranked as *very high* and dispersion ranking of 0.30 linguistically ranked as *low.* These results can be attributed to low NOM (1.00 mg/l) concentration, low absolute pH (0.650), and low effective coating (0.120) values used for nZnO as depicted in case study 1. Figure 3-8b, cases 3, 4, 7 and 8 for $nTiO_2$, show high dispersion ranking when compared to deposition. This is attributed to high NOM concentration in respective cases in the range of 3 to 8.340 mg/l. The results indicate that dispersion of $nTiO_2$was likely attributable to the presence of the NOM and coating as $nTiO_2$ have been previously reported to have limited or no dissolution (Tso et al., 2010; Chowdhury et al., 2012; Loosli et al., 2013)

Under acidic conditions of pH < 6 the nZnO have been previous reported to exhibit high dissociation due to positive charge that builds on the surface of nZnO with positive ZP potential (Majedi et al., 2014) as the pH is far from point of zero charge from nZnO which ranges between 8 to 9.5 (Troester et al., 2016). In Figure 3-8a, cases 1, 3, 4, 6, 7, for nZnO the actual pH values were 8.1, 7.9, 8.0, 10.0, and 9.8, respectively, hence deposition was found to be high when compared to dispersion. Moreover, influence of ENPs concentration is highlighted in the results in Table 3-4 (Columns 15 and 17, Cases 3, 6 and 8). In these case studies deposition crisp outputs were 0.340, 0.452, and 0.214 linguistically ranked as *low*, *medium* and *very low*, respectively. These results were computed by aggregating fuzzy inputs in Table 3-4 (Columns 11 and 8). The results of 0.423 were ranked *low* to *medium* in Table 3-4 (Column 12, Case 3 for nZnO) computed using the inputs that are *0.250 ranked* as low and 0.732 ranked *high* and in Column 8 and 11, respectively. This is attributed to likely high rate of collisions in due to high ENPs concentrations. The fuzzy algorithm was able to show it was possible to examine the exposure of ENPs, with considering the influence of the attribute for each properties of hypothesized case studies. Hence, the DSS reported herein is a tool that can aid decision makers to formulate policy instruments and/framework to support risk assessment of ENPs in the environmental systems.

3.4. CONCLUDING REMARKS AND RECOMMENDATIONS

In this chapter, a fuzzy based decision system was proposed and developed to aid estimation of exposure potential of ENPs following their release into the environment. The fuzzy based system results derived in this study were an attempt to mimic closely human experts' reasoning approach in domains characterized by high degrees of data ambiguity particularly where data has both qualitative and quantitative attributes such as the exposure potential of ENMs. The developed fuzzy logic systems is deemed to be robust, transparent, and suitable as a computational tool to aid rapid first-level screening tool to estimate the exposure potential of ENPs in the aquatic systems. Moreover, the modular development approach (without the need to re-configure the entire model) used in this work render the model to be flexible and transparent where new data can be easily incorporated in the knowledge-rule base, and can allow additional input parameters be added as new data becomes more accessible. . Given the costly and time consuming nature of experiments on fate of ENPs in the environment, the DSS can provide early warning system of likely adverse implications of ENPs, and in turn, can provide science-evidence to trigger the development of proactive measures as means to protect the aquatic lifeforms.

Due to the uniqueness of different classes of ENPs and their variant behaviour in the environment, the system herein is unlikely to take into account potential exposure of the ENPs not considered in this study. Thus, it is recommended that data for other types of ENPs be tested, and attest the robustness of the proposed fuzzy-based system. It is recommended that as more data in the field of exposure of ENPs in the environment and particularly based on commercial products; the tool described herein can be refined further to ascertain its degree of reliability, validity, and dependability.

Moreover, given the fuzzy logic limitation is that, the strength and breath of this approach rely on coding of expert information, and thus, likely to be subjective to human error. Due to static nature of fuzzy system reported herein, it is recommended that a dynamic DSS be developed, such as neurofuzzy model that can aid better understanding of ENPs exposure potential in the aquatic systems, for example, identify key influencing parameters for environmental processes, e.g. dissolution, adsorption, aggregation, and dispersion of ENPs.

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CHAPTER 4 : DEVELOPMENT OF NANOPRODUCTS INVENTORY IN SOUTH AFRICA AND ESTIMATION OF NANOMATERIALS FLOWS INTO THE ENVIRONMENT

Ernst H. Bekker, Ndeke Musee

4.1. INTRODUCTION

Over the past two decades the applications of engineered nanomaterials (ENMs) in consumer products and industry have increased dramatically in diverse economic sectors. These applications of ENMs are intended to improve the performance of consumer products and provide a competitive advantage in certain manufacturing sectors (Hansen et al., 2016; Tiede et al., 2015; Zhang et al., 2015; Musee, 2011; The Nanodabase, 2016). Regulations regarding the use of nano-based products have, however, not advanced at the same pace for most of the world, especially in developing countries, as the potential risk these products could pose is still not fully understood. To date, several developed countries have, however, recognised the potential risks of ENMs to the environment and human health. To establish the extent of the likely impacts, national databases and/or nanoproduct inventories have been developed. For example, various European countries like Belgium, Denmark, France, Germany (Hansen et al., 2016), and United Kingdom (Tiede et al., 2015) as well as for the Asian continent such as Singapore (Zhang et al., 2015) have developed national databases for nanoproducts (products containing ENMs) especially widely used by consumers.

In the last few decades, rapid economic growth in developing as well as countries with economies in transition, have also joined the global economy, resulting in a steady rise of nanoproducts in such countries. This raises the question, to what extent are ENMs present in such countries in consumer products, and more specifically in the African economies? For example, South Africa’s Total Gross Domestic Product (GDP) is in the top two of a total of fifty-five countries in Africa (IMF, 2017), and also to optimise the benefits of nanotechnology as envisaged in the National Nanotechnology Strategy (NNS, 2005).

Yet, for risk assessment of ENMs to be undertaken systematically to support sustainable use and applications, their sources needs to be quantified in the region of focus. Thus, to quantify the potential risks of a given contaminant in the environmental systems, e.g. ENMs both detection and modelling approaches can be employed. However, data on the concentrations of ENMs are scarce including in South Africa, and therefore, mass balance and material flow analysis (MFA) models to estimate the predicted environmental concentrations (PECs) will be applied. This is because although risk quantification based on measured environmental concentrations (MECs) is generally regarded as more accurate; but such data are costly, require some prior knowledge of the region in question, and generally based on few spot grab measured points; which are not representative to the entire region of consideration.

MFA can be executed using two approaches namely: the top-down or bottom-up where the later approach was used in this work. Until now, the top-down approach has been favoured over the bottom-up in estimating the potential risks of ENMs in the environmental system (Muller and Nowack, 2008; Musee, 2011; Domercq et al., 2018; Hellweg and Canals, 2014; Dumont et al., 2015; Tiede et al., 2015), and only handful studies have adopted bottom-approach (Musee, 2017; Caballero-Guzman and Nowack, 2018). It is for this reason herein, that a nanoproduct inventory was developed in order to apply MFA bottom-up model where the input data is specific to the region of focus.

More specifically in the case of South Africa, two previous studies have applied top-down approaches to quantify ENMs potential risks in the environmental systems (Musee, 2010; 2017) where the input data were sourced from international sources for ENMs production data, or inventory of consumer nanoproducts of another country. For example, Musee (2010) reported environmental risk of cosmetics in Johannesburg, South Africa and the potential risk of ENMs was shown to be particularly high in regions with high population densities and high income per capita, such as cities. In another study, Musee (2017) developed a model aimed to screen and prioritize consumer nanoproduct risks. This study used input data for various nanoproducts derived from consumer products database for United Kingdom (Tiede et al., 2015) and adapted to South Africa context. In both studies, to estimate masses of ENMs likely to be released into the South Africa environmental systems, a set of correction factors were used. However, as the data used to estimate various correction factors had certain degrees of uncertainty, it required the models to be refined further to enhance them to be more reflective of the South African context.

To date, however, there is a lack of a category-based nanoproduct inventory or a bottom-up MFA that takes into account South African market data for various categories of nanoproducts. This, as a result, inhibits to predict the environmental risks of ENMs in South Africa as done elsewhere due to lack of such inventory. Therefore, in this chapter, a database developed for nanoproducts and their categories in South Africa is reported. Moreover, we herein demonstrate how the data in the database to present estimation of ENMs into the environment using a bottom-up MFA approach specific to the South African retail market for various nanoproducts with Gauteng Province as the case study.

4.2. METHODOLOGY

4.2.1. Inventory of nanoproducts in South African market

Google, Google Scholar as well as the University of Pretoria library website were used as search engines for various nano databases and nano inventories using keywords such as: nano database, nano inventory, nano products or nanoproducts, and products containing nano. The searches objective were to establish existing nanodatabases and journal articles (inventories) as a starting point for market penetration of nanoproducts globally, followed by specific reference to the South African retail market. The list of products found in the scientific and technical literature in nanodatabases and nano-inventories were then cross checked against products present in the South African retail market by visiting major retail outlets as well as online retail websites of most dominant retail groups in South Africa. In this study, online shopping was excluded due to low retail market share as it only contributes approximately 1% of total retail sales in 2016 and defined by very slow growth (World Wide Worx, 2018).

At the start, there was no specific focus of particular ENMs. This was to fully understand what is present in the South African retail market without any bias to ENMs being most produced worldwide. The search started with all different types of ENMs reported in the databases and inventories. As more data was collected from the South African retail market, the ENMs present in products found in commerce, it became more apparent which ENMs would be of interest.

An inventory for each product type was then established based on data collected from the retail shops where, the presence or lack thereof, ENMs based on information supplied as ingredients in a given sub-product. In this work, sub product(s) refer to products found within a product brand, for a specific kind of product. For example, brand 1 may have 30 sub products or variants of toothpaste while brand 2 may have 5 sub products and so on. This approach also aided to exclude “dead products” from the inventory to avoid over estimation of risks with non-existing products in the market. Dead products herein refer to products that may appear online in the retail stores but were no longer in the market based on same principle adopted in Musee (2018).

As a basis to categorise product data collected, a set of criteria was applied to identify and select products or applications to be included in the inventory. Thus, the products were grouped in three categories; namely: nano-based, indeterminate or unknown, with respect to the incorporation of ENMs in products. Details of the conditions a given product had to satisfy for classification in a given category were as follows:

(i) Product contains ENMs (nano-based) – evidence of ENMs in the product was ascertained in a given brand product based on data from based on information in patent documents, published literature, or indicated list of ingredients.

(ii) Indeterminate – the information from the patent documents or published literature was found inadequate to ascertain whether same parent material in the brands in question were of nano- or bulk-form.

(iii) Unknown – the brand had no evidence in the ingredient information, patent documents, nor in the published literature to ascertain the nature of its composition.

Nano- and indeterminate-based products were also categorised according to the matrix within which the ENMs were embedded. The matrix-classification of nanoproducts have been discussed elsewhere (Hansen et al., 2007; Musee, 2011b, Tiede et al., 2015), and therefore, will not repeated here. The classifications are summarised as either ENMs may be:

(i) Suspended in liquid (SL) product matrix (e.g. toothpaste, car polish, sunscreen, etc.), and considered to have a high release potential and expected to reach the environment;

(ii) Surface bound (SB) product matrix (e.g. refrigerator, shaver, washing machine, etc.), and considered to have a likely to unlikely release potential; thus may or may not reach the environment; or

(iii) Suspended in solid (SS) product matrix (e.g. CPU, RAM, batteries, etc.), and deemed unlikely to be released and not expected to reach the environment.

4.2.2. Estimations of ENMs quantities

In this study, the bottom-up MFA approach was employed to predict the likely ENMs quantities released into the environment in a specific region where Gauteng Province (GP) was chosen as the study area. The selection and setting of the problem boundary based within the GP as region of choice has been outlined elsewhere (Musee, 2017; 2018), and will not be repeated here. Thus, following the use of nanoproducts by consumers, ENMs are eventually released into the environmental systems. The likely released quantities of ENMs from a specific nanoproduct based on bottom-up approach was found to be dependent on: market share, ENMs concentration, and daily usage for a given nanoproduct within a given income bracket. Hence, these parameters were used to estimate the quantities of ENMs released from all products considered with the exception of mobile phone, personal computer (PC) & laptops and televisions using the following expressions (modified from Musee, 2017):

$$M_{ENM\,i,j,k} = Pop_k * MS_{j,k(s,b,v)} * DQ_{ENM\,i,j,k} * C_{ENM\,i,j} * C_{F\,k} \tag{4-1}$$

$$MS_{j,k(s,b,v)} = \sum S_k * \sum B_j * \mu V_j \tag{4-2}$$

Where $M_{ENMi,j,k}$ is the quantity of ENMs *i* (*i* = $nTiO_2$, nAg, etc.) from nanoproduct *j* (*j* = cosmetics, textiles, etc.) from household income group *k* (very low, low, medium, high) annually (kg), Pop_k is the population under the income group *k*, $C_{ENMi,j}$ is the concentration of ENMs *i* in nanoproduct *j* (expressed as percentage) $DQ_{ENMi,j}$ the daily usage per person of a nanoproduct *j* containing ENM_i (g/p/d), and $MS_{j,k(s,b,v)}$ represents market penetration of nanoproduct *j* in South Africa in terms of shops *s*, brands *b* in shops, and probability *v* of purchasing a variety of a product within a specific shop within a specific brand found in household income group *k*, and $C_{F\,k}$ is a correction factor calculated by multiplying the population in group k by the range of disposable income and normalising.

$\sum S_k$ was calculated by summing shop market share obtained from the annual media and products survey for 2015 (AMPS, 2015) for GP. The term $\sum B_j$ was the summation of brand share and calculated by summing the total number of products within a specific brand and divided by the total number of products in a given shop after which the different brand shares were then summed for all shops found in that income group. μV_j denote the probability for a person buying a specific sub-product in a brand *b* and was calculated by dividing by the total number of sub-products in a brand and then taking the mean of the shops in that income group. The model discussed and developed herein was hinged on several assumptions, and these are articulated in the results and discussion section. As an example, the mobile phone and personal computer ENM quantities were calculated as follows:

$$M_{ENM\,i,j,k} = Nett_{discard-GP} * MS_j * C_{ENM\,i,j} * C_{F\,k} \tag{4-3}$$

$$MS_j = V_j \div \sum V_j \tag{4-4}$$

Where $Nett_{discard-GP}$ is the total number of products disposed-off in a given year in GP. MS_j as the market share of ENMs in consideration and was calculated by dividing the variety of products containing the ENMs *i* by summing variety of brands in a given shop.

To calculate the most likely mass, probability density function (PDF) analysis was incorporated using Markov Chain Monte Carlo (MCMC) methods in R (Martin et al., 2011). The Markov Chain (Figure 4-1) was weighted according to accepted standards as previously applied by Sun et al. (2014). The weighting is based on the principle of degree of belief, for example, if there are three values but only one is closest to the trusted number then it will be given a 50% degree of belief followed by the second most trusted value with 30% and the least trusted, 20%, which is then arranged into a matrix and the calculation iterated 10,000 times or until the matrix stabilised. In the case of this study, there were only two values available, a min and a max, thus a third value was generated by taking the geometric mean of the min and max which served as the middle value and was considered to be second most trusted. The stabilised matrix coefficients were then multiplied by their respective mass values and the most likely value obtained.

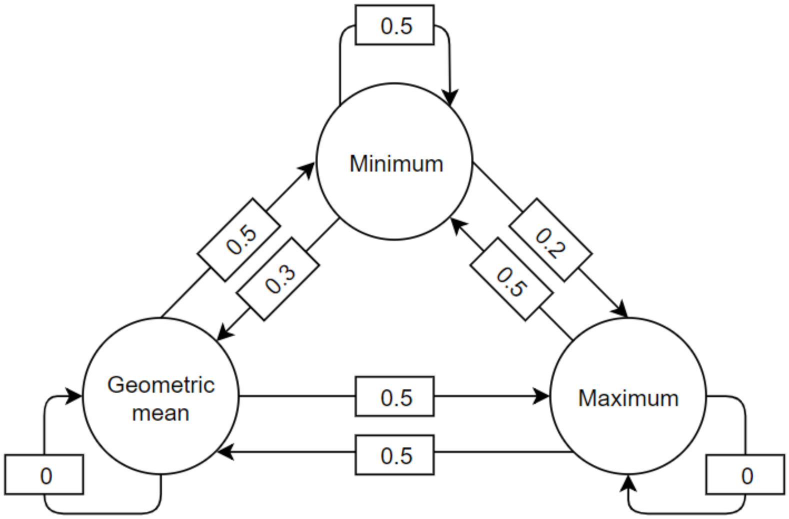

Figure 4-1: An illustration of Markov Chain method used to calculate most likely release of ENMs into the environment.

Products considered to contribute to most likely produced ENMs mass in the South African retail market were calculated using both confirmed nano and indeterminate nano product masses. The reason for including the quantities released from indeterminate products owing to their plausible released of ENMs. The degree of belief principle was, however, also applied by adding a weight to the respective product masses as discussed previously and the result distributed accordingly.

4.2.3. Material flow analysis to different compartments

An MFA was then applied to the most likely ENM masses. Journal articles and reports were used to establish the most likely routes for each ENM as well as the income group of the product. According to a report by Statistics SA (2015), 13% of Gauteng residents had no access to sanitation connected to the public sewerage system and thus that mass is assumed to end up as run-off for the very low income group. Recycling was also considered as a potential pathway for electronic waste as 11% of all electronic waste is recycled in South Africa according to a report by the Department of Environmental Affairs (DEA, 2012). It is thus assumed that 11% of all electronic are recycled. Gauteng is also regarded as the central hub for the collection, consolidation, pre-processing and processing of electronic waste with 55% of material handled in 2015 (Green Cape, 2018).

4.2.4. Data acquisition

This section summarizes the approach followed in obtaining data for various parameters specific to this study. Population and income data (supplementary Table S4-15 and Table S4-16, respectively) were obtained from Statistics South Africa (Statistics SA, 2017). Due to high income disparities per capita, income in South Africa motivated for usage of products for different categories was used in this work as determined by Musee (2018). The retail shop information such as shop market share were sourced from annual specific shop-retail group reports as well as annual media and products survey (AMPS, 2015). Annual shop group reports were also used to further confirm shop market share based on number of shops, annual turnover, and types of products in commerce at given time.

4.2.5. Ingredients

In terms of ascertaining the product presence and ingredients in a given product category, visits were made to various retail groups as identified in the shop group reports and AMPS (AMPS, 2015) data. Targeted on-line searches using Google, Google Scholar as well as the University of Pretoria library website were used based on a set of key words for products and ENMs. The search words included: ‘nano’, + general product (e.g. conditioner, personal computer, laptop, toothpaste, shampoo, shaving cream, etc.), brand names associated with these products, ENM found to be prevalent in product as per nanodatabases and articles (e.g. titanium dioxide, silver oxide, silver, etc.) An example of such a search was ‘brand toothpaste nano titanium dioxide ingredients’, ‘brand toothpaste titanium dioxide ingredients’ and ‘brand toothpaste ingredients' from which variants of that brand could be further investigated. Similar phrases were used for all products identified in the South African retail market.

4.2.6. Concentrations of ENMs

The concentrations of ENMs in different product categories were acquired either as reported in scientific and technical literature, and/or patents specific to the product using key single words and compound phrases such as: nano, toothpaste titanium dioxide nano, silver nano bar soap, etc. Synonyms of product types were also considered as general names of the products under consideration. An example of such a synonym term is “dentifrice”, a synonym for toothpaste. Product parent companies, for example, the owners of a specific brand, were also used during the search of patent information as these are patents that are specific to their products and products in question for the study. The patent information was searched using Google Patent search engine. Patent information was obtained using key words with a combination, e.g. ‘dentifrice, titanium dioxide, product parent company”.

Where the concentration information of ENMs in articles were not available, values were calculated, e.g. for devices such as integrated circuits (ICs) or ‘computer chips’ specifically in cases like central processing units (CPU) or random access memory (RAM), and rechargeable lithium ion (Li-ion) batteries. The concentration for ICs containing SiO_2, for example, were estimated based on technical data sourced from published literature and manufacturers (Flynn and Luk, 2011; Intel Newsroom, 2009; OMRON Corp., 2008). In the case of Carbon nano tubes (CNT), which are used extensively in Li-ion batteries for mobile phones and notebook computers, concentrations were also calculated.

4.2.7. Usage per capita

Usage data per capita (supplementary Table S4-17) was derived from the technical Guidance Technical Document on risk assessment (TGD) (ECB, 2003), and USEPA (2011). To take into account huge disparities in South Africa on usage per income group for the PCPs the values estimated by Musee (2018) were used, and only exception was car polish usage derived in this study as discussed in Tiede et al. (2015).

4.2.8. Quantification of material flows

Quantification of material flows of given products were also determined using secondly data sources. To illustrate this aspect, we present the electronics, appliances and accessories (EAA) products category as a case study to clarify this aspects. Import and export data sourced from the South African Revenue Service (SARS) were used to calculate number of mobile phones, personal computers and laptop sales as reported by e-Waste Association of South Africa (Finlay and Liechti, 2008). Parameters considered entailed, for instance, second hand sales, storage of devices, average mass of product, average lifespan of product, population growth and presence surveys (e.g. how many households had a mobile phone in 2007). The

Finlay and Liechti (2008) report projected electronic waste (e-waste) of selected electronic devices up to 2012. This data, together with the number of households owning a particular product by province in reports published by Statistics South Africa (Statistics SA, 2001; 2011; 2012; 2013; 2015; 2016; 2017) was used to calculate electronic waste quantities in Gauteng from 2000 to 2020.

4.3. RESULTS AND DISCUSSION

4.3.1. Inventory of ENMs found in South Africa

Inventories and databases of nanoproducts used to acquire possible products of interest are summarised in the supplementary information Table S4-1 and S4-2, (PEN, 2018; The Nanodatabase, 2018; BUND, 2018, etc.). A summary of different nanoproducts grouped according to ENMs and categories are summarised in supplementary Tables S4-3 to S4-8.

4.3.2. Inventory and data analysis

From the data collected, six major nanoproduct categories were identified likely exist in the South African retail market. These were automobile (AUTO); electronics, appliances and accessories (EAA); food and beverages (FB); fitness and textiles (FT); medical (MED), and personal care products (PCP). In total, 2002 products (Figure 4-2) were identified consisting of 31 different types of products. PCP, for example, accounted for 54% of all products in the inventory consisting of 9 product types; whereas EAA accounted for 26% under 13 different types, and AUTO contributing 9.6% of total products in the inventory. The FB, FT and MED categories made up the remaining 9.9% of total products but none had confirmed ENMs. ENMs were confirmed to be present in 8.8% of products in the AUTO category, which were 3 tyre products from a single brand, whilst car polish had 14 confirmed products from two brands but car shampoo had none.

The EAA category had most products with ENMs, with over 65% of all products in this category confirmed to contain ENMs comprising of cameras, personal computers, laptops, mobile phones, printer ink, printer toner, rechargeable batteries, televisions, and washing machines. ENMs could not be confirmed in electric shavers, flat irons, hair dryers, and refrigerators. Only 21% of products found in the PCP category contained ENMs. For example, ENMs were confirmed in bar soaps, conditioners, deodorants, lotions, shampoos, shaving creams, shower gels, sunscreens, and toothpastes. Detailed tabulated results for all products are summarised in supplementary Tables S4-9 to S4-14.

The FB category had 2 products types, namely: the beer and noodles. However, no clear evidence was accessible to confirm the presence or absence of ENMs in these products in the South African market. Zhang et al. (2015) suggested likely incorporation of nano-TiO2 in instant noodles as reported by Weir et al. (2012) which reported that approximately 39% of food-grade TiO2 particles were less than 100 nm in at least one dimension and that they readily dispersed into water as fairly stable colloids. While for beer cans, a thin layer of few nanometres of SiO2 is incorporated on the surface of the aluminium can to protect the metal surface from oxidizing and reduce reflectivity (Kasaai, 2015). Both these claims, however, were not specific to a brand and the concentration data was unavailable.

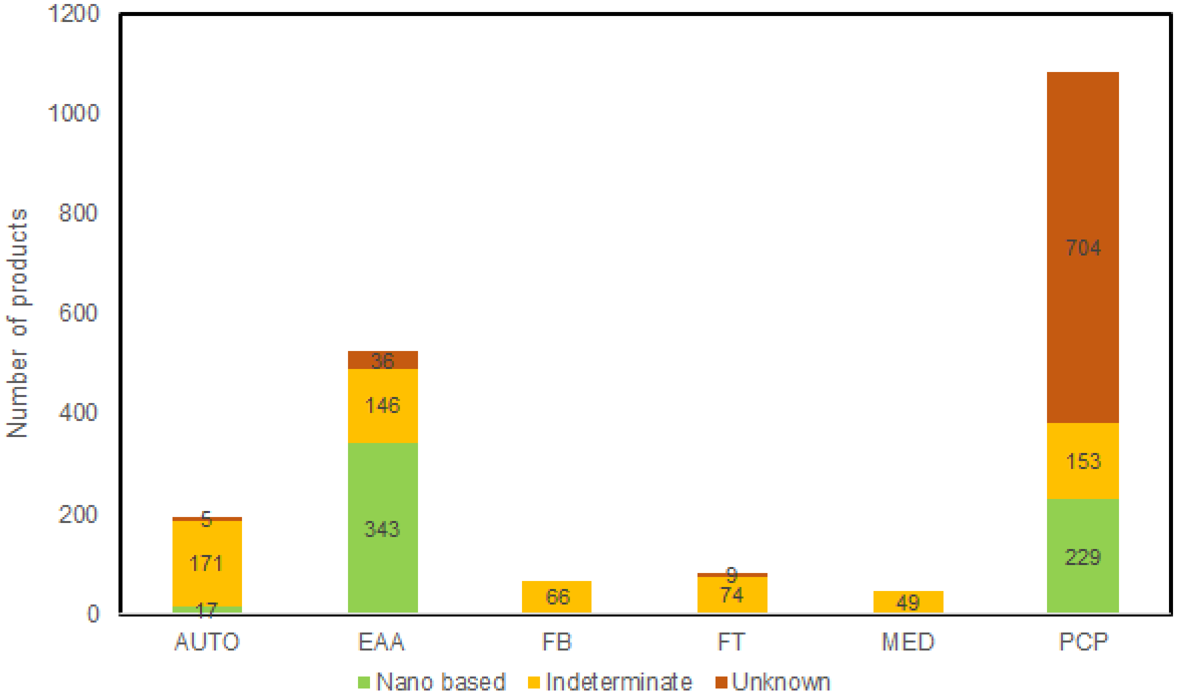

Figure 4-2: Summary of products in the South African retail market based on their ENM status.

The other two remaining categories FT and MED had minor contribution to overall products as well as not having any confirmed ENMs. FT consisted of sports rackets, sport shirts and sport socks of which all sports rackets except one of unknown ENM status. The MED category consisted of one band aids, all of which were deemed of indeterminate status.

4.3.3. Inventory of nanoproducts

The composition of the inventory of products for each ENM in Figure 4-3 for each category of products as either nano-based or indeterminate. It is, however, important to note that each ENMs were counted based on presence in a product including the complex mixtures of ENMs found in most products. Nonetheless, in this work on mixture analysis of product composition, was not done. The data should thus be interpreted as percentage presence of a specific ENM, for example in AUTO, 100% of nano-based products are SiO_2 based but approximately a third of SiO_2 based-products were classified under indeterminate category, bearing in mind that SiO_2 also were a constituent in a mixture with carbon black (CB) and CNT in certain products. This approach is useful in assessing which ENMs are widely used in different product categories based on occurrence frequency in this inventory.

Of the total nano-based products, SiO_2 were in 34.0% of all products with 70.1% and 25.8% being in the EAA and PCP categories, respectively, and the remainder in the AUTO category. TiO_2 and CNT accounted for 18.9% and 18.3% of all products, respectively, and 100% of CNT were identified in EAA.

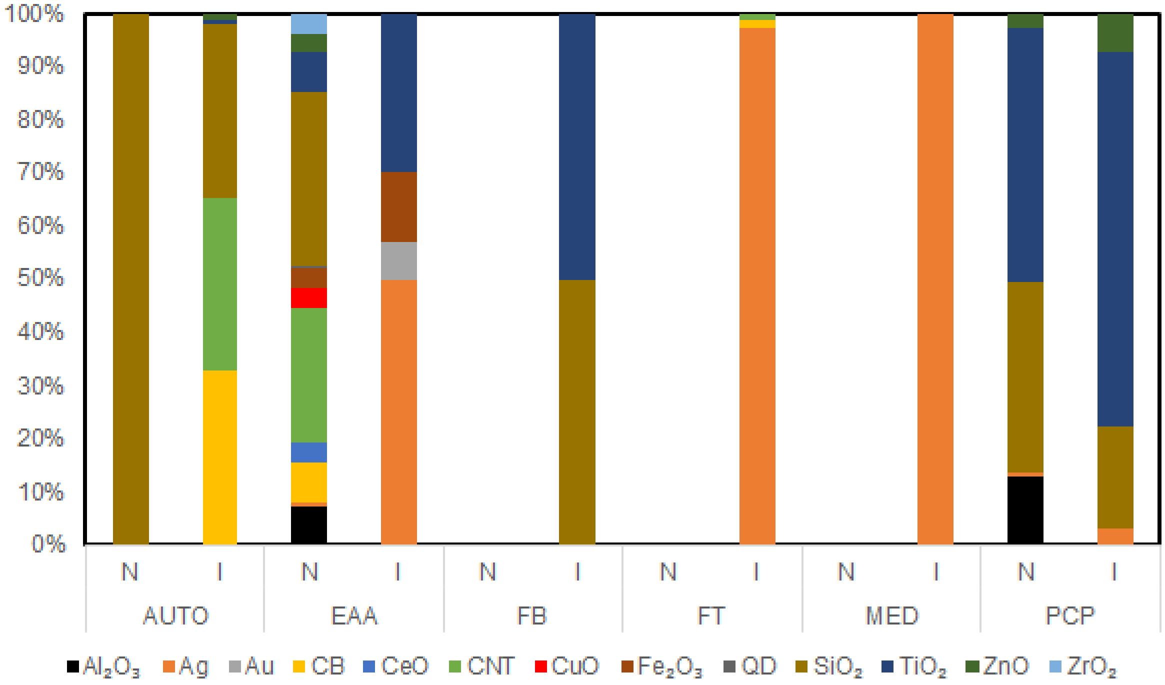

Figure 4-3: Summary of ENM composition for nano-based and indeterminate products.

The PCP accounted for 71.7% of products containing TiO_2, and the remainder were in the EAA category. Results revealed that Al_2O_3 and CB were 8.5% and 5.3%, respectively, of the products analysed in this system with 62.5% of Al_2O_3 found in EAA, and 37.5% by PCP. Hundred percent CB was only found in the EAA products category. The remaining eight of the 13 ENMs combined only contribution 15.0%, and individually under 3.4%, with no Au found in any product considered in this study.

Conversely, for indeterminate products, Ag constituted the highest number of products (23.2%) followed by TiO_2 and SiO_2, respectively, at 22.2% and 20.2%, where these three ENMs accounted for about two-thirds of products under this category. CB and CNT both contributed 13.7% whereas Fe_2O_3, ZnO and Au 3.3%, 1.9% and 1.8%, respectively, were considered to be incorporated into various products. From the analysis, the remaining ENMs could not be assigned to specific products under the intermediate category.

4.3.4. Nano-based and indeterminate products matrixes

A summary of product matrix composition results for different categories are illustrated in Figure 4-4 both for nano-based and indeterminate products. Each product in the inventory was categorised according to matrix holding ENMs under a given product category. The classification used by other researchers (Hansen et al., 2007; Musee, 2011b; Tiede et al., 2015) was used in this work as basis to determine the likely release potential of ENMs from a given product (see section 4.2.1), and the results in Figure 4-4. These results were valuable as they aided to prioritise which category of product(s) likely to pose the greatest concern to different natural and technical environmental systems.

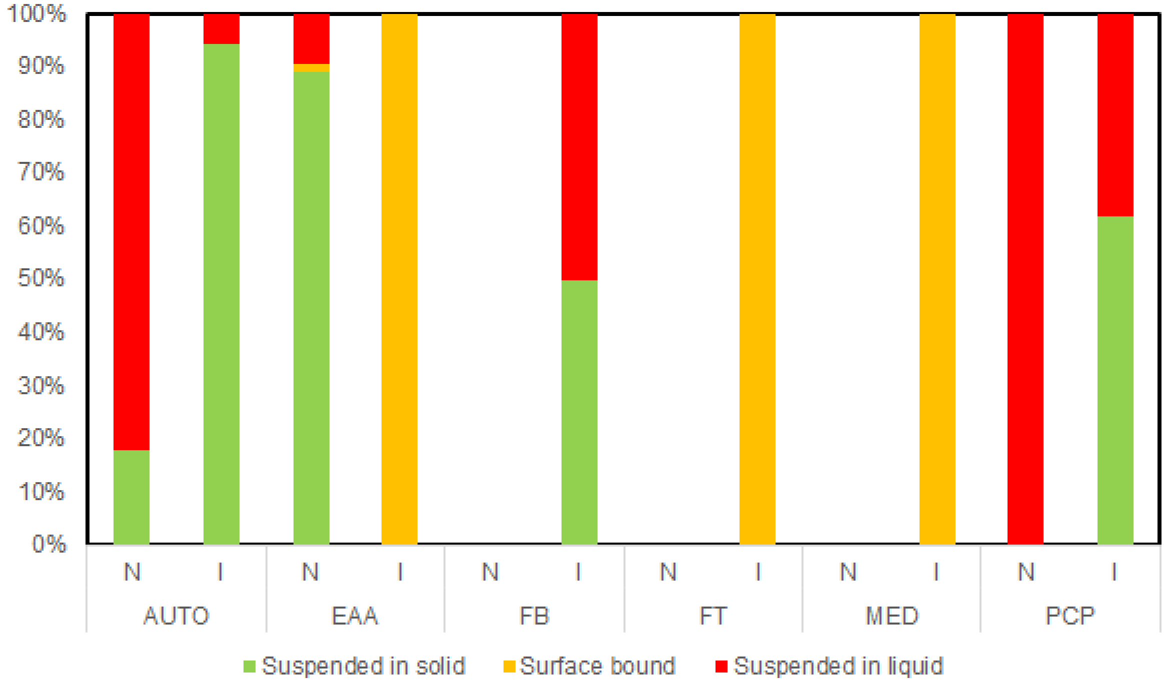

Figure 4-4: Percentage distribution of matrix composition for nano-based and indeterminate products.

Results indicated that ENMs in the PCP category had the greatest release potential, and therefore, most likely to account the largest quantities of ENMs in the environment, followed by AUTO category. Moreover, this is also attested by the large number of nano-based products confirmed ENM status under PCP category when compared to other categories, e.g. AUTO. The EAA category products results also indicated some concern as was found to be a major product contributor. The indeterminate products in PCP also pose some concern and will require further attention in future investigations. Usage rates of products were also greatest for PCP category which additionally gave further evidence of their likely higher contribution of ENMs into the environmental systems.

AUTO products considered likely to release ENMs into the water sources were car-polish and shampoo, while in the EAA category only washing machines had likely release of reasonable quantities of ENMs. It should be noted that although certain PCP products (e.g. bar soap) had ENMs in SS, overall still posed high release potential due to release during the use phase. Thus, all PCPs were considered to exhibit high release potential, and in turn, account for large quantities of ENMs into different environmental compartments.

4.3.5. Quantification of ENMs in Gauteng Province

Based on results in Figure 4-2, PCPs constituted the second highest number of nanoproducts in the inventory and most probable likelihood to release into the environment during the use and disposal phases based on ENM matrix (Figure 4-4). Similarly, the EAA category had the highest number of confirmed nanoproducts, but only with limited product types likely to release appreciable ENMs quantities into the environment. Such deductions can qualitatively provide valuable insights on the potential risks of ENMs, but are non-conclusive. Therefore, using the MFA approach, quantities of ENMs flows from various products into various environmental compartments were determined in this study.

ENMs quantities from various products were estimated; save in cases where required data were inadequate. For the AUTO, EAA and PCP categories each had confirmed ENMs at least in a single product type. Thus in the AUTO category, quantities from car polish were estimated, whilst in EAA category, estimates were done for the mobile phones, personal computers, laptops, televisions and washing machines. For the PCP category, quantities of ENMs were calculated for products, including: bar soaps, conditioners, deodorants, lotions, shampoos, shower gels, sunscreens and toothpastes.

4.3.5.1. ENMs flows in Gauteng Province

Following the approach outlined in Section 4.2.2, quantities of ENMs from various products within a given income group were determined. Herein, three scenarios are considered, namely: the minimum, medium, and maximum. Minimum (min) scenario denotes calculated quantities based on confirmed nano-based products. Maximum (max) scenario represents estimated quantities based on the nano-based and intermediate products identified, and coded in the inventory discussed in section 4.2.1. The medium (med) quantities is envisaged to represent the most probable release of ENMs into the environmental system, and was calculated as a geometric mean of the minimum and maximum quantities.

Using the three estimated quantities, the most likely ENMs quantity (medium) were calculated by means of PDF using the MCMC method. From this method, a stable matrix was acquired, and then used as the probability fraction of a certain mass (e.g. probability of min, probability of med, etc.) which represents the most probable mass – herein regarded as likely quantity. The matrix stabilised with the coefficient values for 0.500, 0.267 and 0.233 for min, med, and max, respectively. Next, the coefficients were then multiplied by the respective masses to calculate the most likely mass of ENMs. A summary of the results for the min, likely, and max quantities for different ENMs are presented in Figure 4-5 as per different income group contribution.

Results indicate that the *very low* income group had insignificant contribution to overall ENMs likely to be released into the environment on average of about 1% on average with highest being 4.4% for CNT. This is unsurprising although the group accounts for 46% of GP, and on average shop market share of 21%. This is most likely owing to low usage and limited variety of nanoproducts in the shops mostly serving this population segment. The high CNT contribution is likely due to high mobile phone usage since penetration is ranked at 98.3% of households in GP by 2015 (Statistics SA, 2015). The *low* income group represents 36% of total GP, had average shop market share of 22%, and contributed about 5% on average with CNT accounting for 17% but only 0.7% for the Ag.

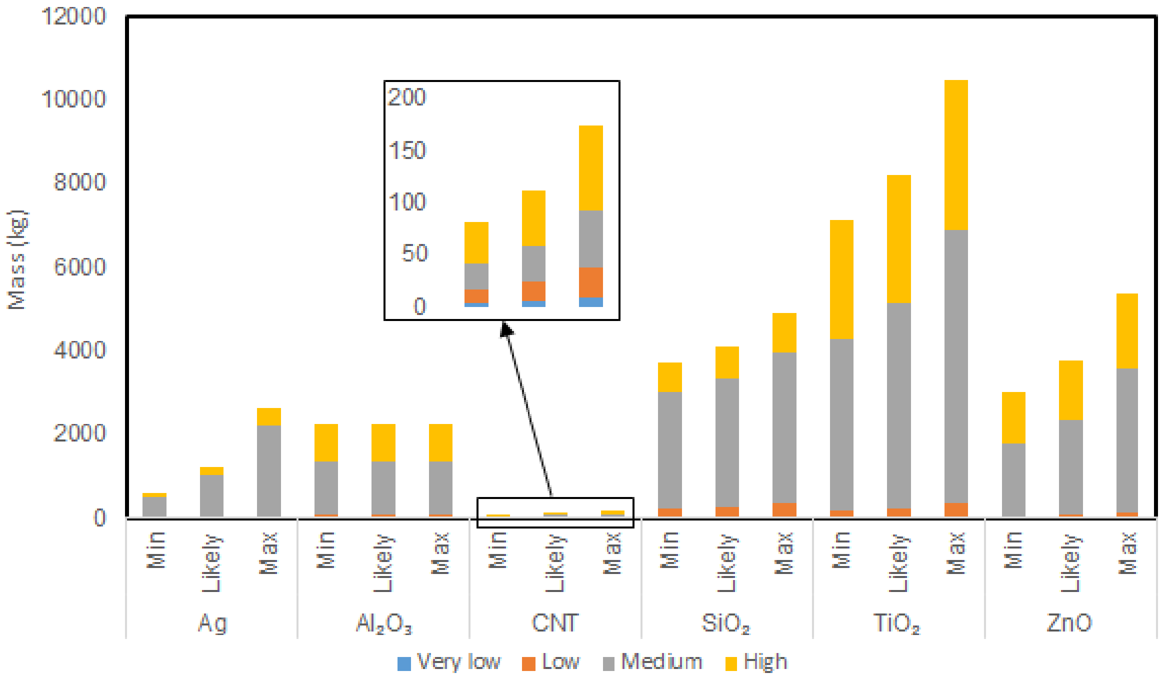

Figure 4-5: Estimated ENMs quantities per different income groups: very low, low, medium and high income groups under three scenarios: min, med, and max.

The main contributor of ENMs into the environment were the *medium* income group as it accounted on average 61.5% of all ENMs considered in this study. More specifically, estimated quantities of ENMs linked to usage of nanoproducts were: 82.8% of Ag, 74.8% of SiO_2, 61.9% of ZnO, 60.6% of TiO_2 and 57.7% of Al_2O_3. The *medium* income group represents 15% of GP population; however, accounted for 44% on average shop market share. Notably, the usage of nanoproducts in this income group was higher compared to that of *high* income group. The highest quantities of CNTs were generated by the *high* income group accounting for 47.6%, and the *medium* income group accounted for 31.0%. The high CNT generation is likely due to the high purchasing power of this income group. On average the *high* income group accounted for about 32.5% of all estimated quantities of ENMs, but only constitutes about 3% of GP's population. However, this income group had the lowest shop market share of 8% but also had the highest usage in comparison to other income groups. The next aspect entailed elucidating the source of ENMs owing to the apparent significance in future decision making, for example, with respect to regulating nanoproducts. Thus, a breakdown of ENMs sources, and the results are given in Figure 4-6, and the attendant most likely masses generated from different nanoproducts.

Most Ag (93%) was released from the washing machines, a product primarily highly used by the *medium* and *high* income groups. The remaining Ag estimated in this study (about 81kg) were from the bar soaps, deodorants, and shower gels. It should be noted that Ag was confirmed only in washing machines (Samsung Electronics Co. Ltd., 2003; Daewoo Electronics Co. Ltd., 2003) and deodorants (Beiersdorf AG, 2012). Nonetheless, there is likelihood that bar soaps (Adawi et al., 2018) and shower gels (Zhuo Hongsi, 2008) may contain nano Ag since in the packing ingredients information indicate the inclusion of silver in these products although the form (bulk or nano) is unspecified (Martirosyan and Schneider, 2014). Toothpastes and sunscreens products contained Al_2O_3 with only single toothpaste brand (total brands were thirteen) with ENMs concentration of 3% (Glaxo Group Ltd, 2010). Sunscreens had Al_2O_3 with concentration of about 3% (Beiersdorf AG, 2013), and only found in three of eight brands. Overall, the sunscreens contribute about 81% of Al_2O_3, and the toothpastes the rest.

CNTs are used extensively in Li-ion batteries in mobile phones and notebook computers. The batteries have about 1 wt % CNTs loading in $LiCoO_2$ cathodes and graphite anodes, and blended with active materials and a polymer binder (de Volder et al., 2013). Theoretically, the capacity of $LiCoO_2$-based batteries is about 274 mAh/g $LiCoO_2$ (Deng, 2015; Dai et al., 2012). As such, the CNTs concentration was calculated for the rechargeable Li-ion batteries used in mobile phones and laptops based on their power rating in mAh. Despite personal computers and laptop products contained greater CNT amounts per product; however, accounted only about 30% of total mass whereas the mobile phones contributed 70% of total CNTs largely to wide use of the later in all income groups and lower price in comparison to the former.

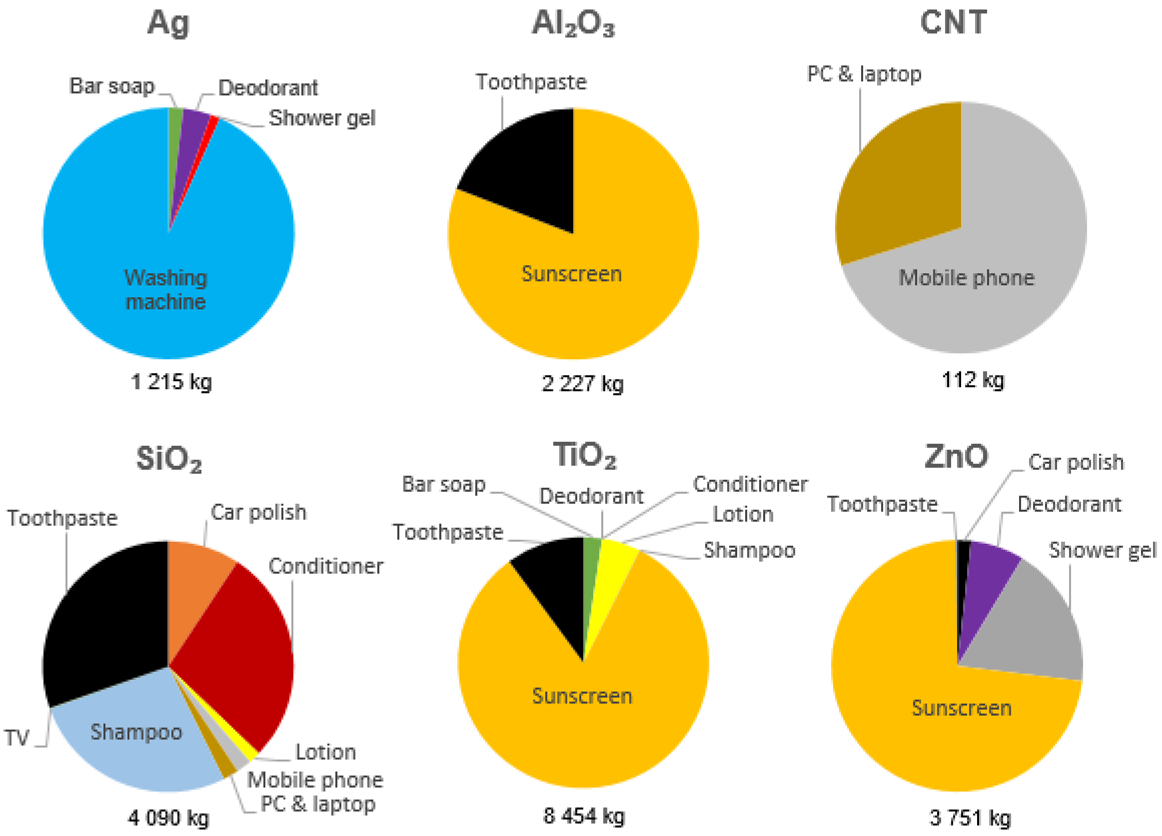

Figure 4-6: Products considered to contribute to most likely produced ENM mass in the South African retail market.

Three main sources of SiO_2 were toothpaste (being the highest contributor of *ca* 30% of SiO_2) followed by conditioner (28%), and shampoo (27%) in that order; and the remaining 15% were from car polish (9%) with rest from lotions (~2%), mobile phones (~2%), PC & laptop (~2%), and televisions (0.08%). Only a single brand of toothpaste contained SiO_2 (Colgate-Palmolive Co., 1984) with 3% concentration silica aerogel (Syloid 244®), and particle size <1 µm (Jesionowski et al., 2004) with the remaining twelve products grouped under indeterminate category with concentrations of 1% aerogel (Lever Brothers Company, 1974). Conditioner had six of thirty-one brands with confirmed nano SiO_2 with concentrations ranging from 0.4% to 0.5% (L'Oreal SA, 1995a; Unilever, 1992). Moreover, shampoo had SiO_2 confirmed in three of twenty-five brands with a concentration of 1.5% (Unilever, 1990); and the estimated quantities from these products were high due to their high usage within different income groups.

Car polish usage was considerably low (0.3 g/c/d) compared to PCPs – and mostly used by the *medium* and *high* income groups plausibly due to high ENMs concentration used of between 5% and 15% thus making it highly priced. In the car polish product type, ten of eighteen products contained $nSiO_2$ (3M Innovative Properties Co., 2010). For the lotions, only a single product (total of 207 products in the inventory) contained nano SiO_2 with a concentration of 5% (L'Oreal SA, 1995b), and accounted about 1.54% of total SiO_2 emissions into the environment. The concentration for ICs containing SiO_2 (Intel Newsroom, 2009) was made based on technical data acquired from articles and manufacturers. According to Flynn and Luk (2011) the average size for a computers IC (referred to in industry as a die) is 10-15 mm square with a height of 160 µm (OMRON Corp., 2008). Smart televisions contributed only 0.08% of the total mass while mobile phones were at 1.98% and PC & laptops were at 2.01%, all of which were due to their low usage and concentrations.

The highest estimated quantities of TiO_2 were from sunscreens (79.75%), only three out of eight brands had TiO_2 at concentration of 10% (Beiersdorf AG, 2015), followed by toothpaste (9.72%), bar soap (5.69%), and lotion at 4.81% in that order; with the remaining products (deodorant, conditioner and shampoo) with negligible contribution constituting about 0.03% (2.54 kg) of total estimated quantities. Toothpaste had the highest number of products containing nano accounting for about 55% (TiO_2 with 56 of 101) with concentration per article ranging from 0.74% to 0.83% for three of thirteen brands (Weir et al., 2012). For the Bar soaps product type, twenty-one of one hundred and eighty-four products were nano-based or rather three of twenty-six brands, all with 0.4% TiO_2 concentration (Unilever, 2002; Unilever, 2005). Deodorants, conditioners, and shampoos had the lowest concentrations of TiO_2 ranging from 0.00006% to 0.001% in 11.2% of total products in this category (fifty-two out of four hundred sixty-six) (Weir et al., 2012).

Based on study results, sunscreens were the highest source of ZnO (72.92%), and then shower gel (18.27%) with the remaining 8.81% (about 330 kg) coming from deodorant, car polish and toothpaste. A 5% concentration of ZnO was confirmed in a single brand of sunscreens (Beiersdorf AG, 2007) and 1% in a single brand (six of fifty-one products) of shower gel (Colgate-Palmolive Co., 2003). Deodorant had a single brand with confirmed ZnO (two of two hundred and forty-five products) with a concentration of 7% (Colgate-Palmolive Co., 2000). Toothpastes and car polish had no ZnO; although there is likelihood some of products may have nano forms. The total contribution (1.92%) of these two products were very low due to the low degree of belief associated with the medium and maximum masses, with resulted in toothpaste contributing 6.02 kg and car polish 65.90 kg.

4.3.5.2. Distribution of ENMs in different environmental compartments

From the results summarised in Section 4.3.2.1, the next step entailed the estimating distribution of ENMs into different environmental compartments. Among the parameters considered to estimate the ENMs flows entailed product matrix (e.SL, SS or SB), usage patterns (e.g. washed down the drain, disposed of in refuse bin, etc.), extent of sanitary services in GP to households (e.g. household connected to sewerage line, refuse collection, etc.), and recycling pattern for the region – with specific reference to household income groups. Estimated total quantities for each type of ENMs released into different environmental compartments are presented in Table 4-1. A similar approach as discussed in Musee (2017) was followed with specific reference to simultaneous release of ENMs into the environment from various nanoproduct categories. Thus, the results reported herein are a summation of quantities of the same ENMs released from different nanoproducts; and the mass distribution (as percentage) are depicted in Figure 4-7. Overall, the released quantities were strongly dependent on the nature of product, use as well as disposal method. For example, SS bound ENMs were likely to end-up in the landfills whereas from the liquid-based products were released into the environment via the wastewater and run-off.

Both the PCPs and washing machines released ENMs into the environment mainly via the wastewater pathway. On model input(s), several assumptions were made. First, it was assumed that wastewater were generated by entire populations in the *high*, *medium*, and *low* income groups, and 72% in the *very low* income group since 13% of the GP population had no access to sanitation by 2015 (Statistics SA, 2015). It should also be noted that washing machines containing ENMs were considered as high cost product, and therefore, was not used in *very low* and *low* income households, and this was partially attested by low sales of these products. For the very low income group, 28.37% of the generated ENMs were assumed to be released via run-off (direct release to the environment due to lack of sanitation and waste management services). Car polish accounted significant release of ENMs via run-off pathway into the environment. Quantities of ENMs to the landfills were considered to about 5% as used in Musee (2017) from the sunscreens generated from different income groups. Other products considered to end up in landfills were mobile phones, personal computers and laptops, and televisions with the exception of 11% of these electronics being recycled for low, medium and high income groups as per a report by the Department of Environmental Affairs (DEA, 2012). CNT had the least quantities released into the environment while Ag has the greatest potential flow as illustrated in Figure 4-7. TiO_2 and Al_2O_3 have a similar flow profiles.

Table 4-1: Anticipated distribution of low, likely and max scenarios of produced ENM mass (kg).

		Environmental compartment				
ENM	Scenario	Wastewater	Landfills	Run-off	Recycled	Total
Ag	Low	589				589
	Likely	1 214		1		1 215
	Max	2 624		6		2 630
Al_2O_3	Low	2 137	90			2 227
	Likely	2 137	90			2 227
	Max	2 137	90			2 227
CNT	Low		73		7	80
	Likely		102		10	112
	Max		159		15	174
SiO_2	Low	3 215	93	384	8	3 701
	Likely	3 531	160	385	14	4 090

	Max	4 173	298	387	27	4 886
TiO_2	Low	7 075	300	7		7 382
	Likely	8 110	336	8		8 454
	Max	10 070	401	12		10 483
ZnO	Low	2 897	106			3 002
	Likely	3 520	138	94		3 752
	Max	4 746	200	403		5 350

4.3.5.3. Nanoproduct case study: toothpaste

In this section, using the case of toothpaste as a case study, it will be illustrated how the information in the inventory (Section 4.2) was employed to estimate the total flows of ENMs into the environment. Here, toothpaste will be used for illustrative purposes as it accounted for product category with highest confirmed ENMs in GPs market. Using the approach outlined in Section 4.2; quantities of ENMs from various products within a given income group were determined. For toothpaste, 13 brands which comprised of 101 sub-products were identified in the South African market, with only three brands containing ENMs (Brand 2, Brand 4, and Brand 12). Of the 101 sub-products, the presence or lack thereof was distributed as follows: 60% (nano-based), 39% (indeterminate), and 1% unknown (Table 4-2). Moreover, Brand 4 contributed *ca.* 33% of for the entire market size.

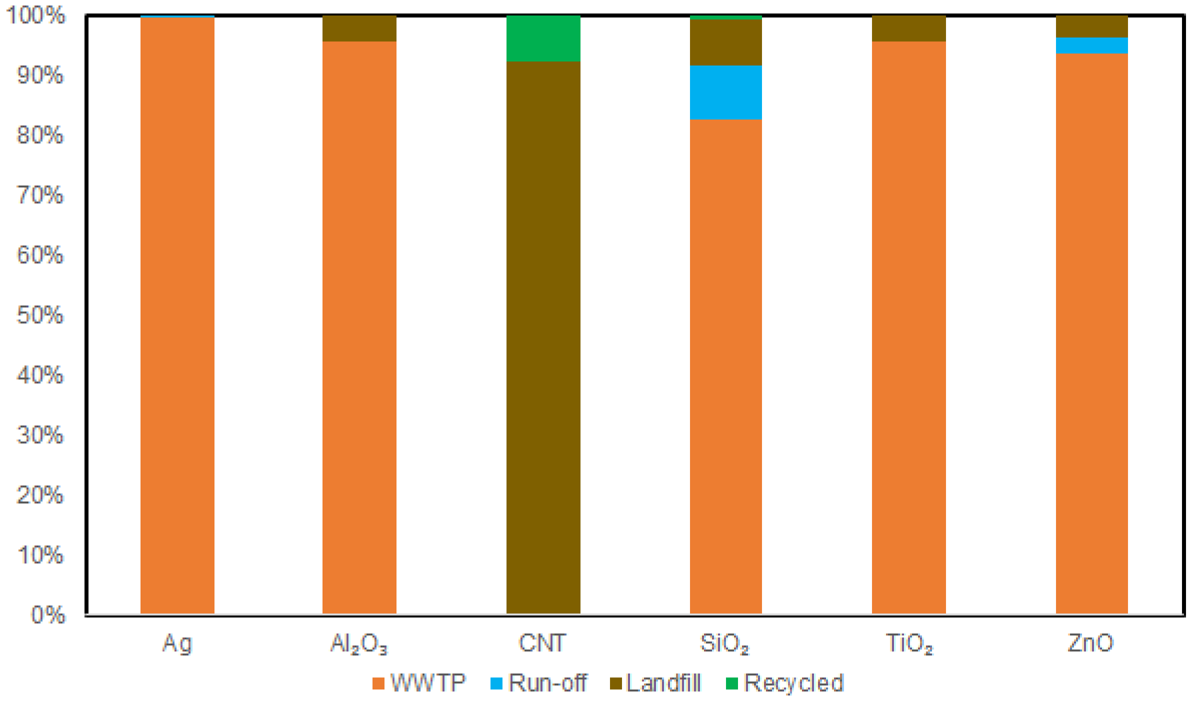

Figure 4-7: Percentage distribution of most likely produced ENMs.

Table 4-2: Distribution of the toothpaste in the South African Retail Market (2017 data).

Toothpaste Brand	Sub-brands	Nano-based	Indeterminate	Unknown
Brand 4	34	33		1
Brand 2	18	18		
Brand 12	10	10		
Brand 7	7		7	
Brand 6	8		8	
Brand 5	4		4	
Brand 10	4		4	
Brand 11	4		4	
Brand 13	4		4	
Brand 1	3		3	
Brand 3	2		2	
Brand 9	2		2	
Brand 8	1		1	
Sub-Total	**101**	**61**	**39**	**1**

Next, to account for the distribution of toothpaste in the market based on income population group, each brand and its sub-groups (products) was linked to the retail shops. Results of this exercise yielded the results summarised in Figures 4-8 and 4-9. For the very low and low income population groups, the dominant brands were 2 and 4 (and mostly were served by three shops (shops 1 to 3) (Figure 4-8); and the extent of the dominance was shop dependent; with shop 3 (under low income) with 43 variant products, and brand 7 being least with only one product (Figure 4-8b). Similar trends on distribution of key brands (brands 2 and 4) dominant in the medium and high income groups as well (Figure 4-9). Except for shop 9 in the high income group (Figure 4-9b), all other shops had high variability of toothpaste products exceeding 40 in shops 4 to 8 (Figure 4-9a and b).

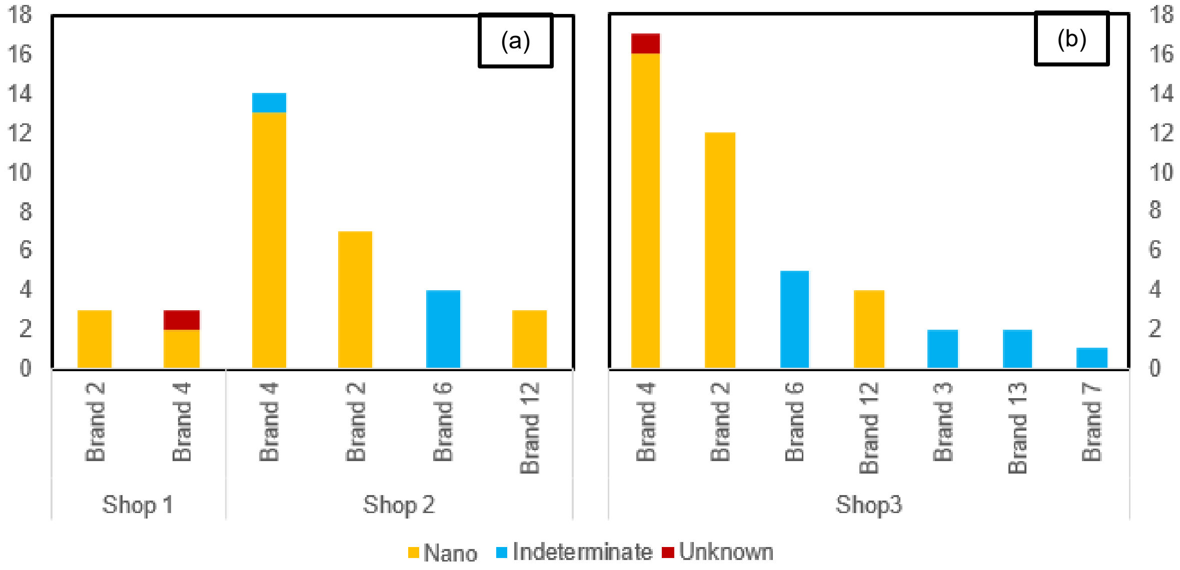

Figure 4-8: Distribution of toothpaste products in (a) very low and, (b) low population income group retail market segment with ENMs and, indeterminate and unknown status.

Results in Table 4-3 lists quantities of ENMs likely to be released into the environment from toothpastes in GP based on type, and income group. Brand 4 had three types of ENMs (SiO_2, TiO_2 and ZnO) whereas the rest had a combination of two of the three only (Al_2O_3, SiO_2 and TiO_2). In addition, Brand 4, accounts for the highest released of SiO_2 and TiO_2. Overall, the released ENMs into the environment for Al_2O_3, SiO_2, TiO_2 and ZnO were 425 kg, 1 241 kg, 824 kg and 6 kg, respectively. With respect to ENMs flows based on income group variant, the low and medium income groups accounted for most of the ENMs emissions (irrespective of type), and the very low income group negligible, for example, where this group's contribution did not exceed 1.5%.

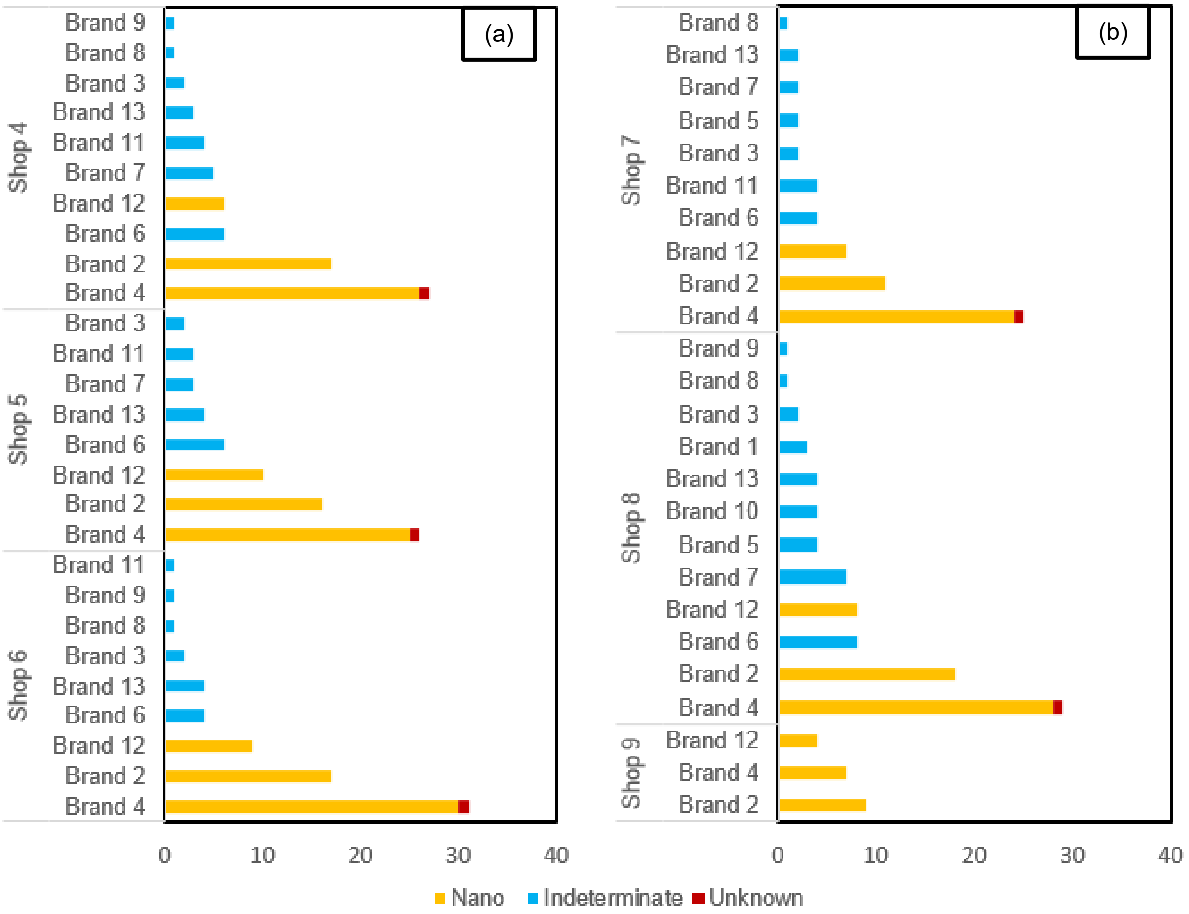

Figure 4-9: Distribution of toothpaste products in (a) medium and, (b) high population income group retail market segment with ENMs and, indeterminate and unknown status.

Table 4-3: Estimated quantities of ENMs of from the toothpaste brands in GP, South Africa, and their distribution across different income groups (2015).

	Brands				Income groups				
ENMs	BR 2	BR 4	BR 12	Total	VL	L	M	H	Total
Al_2O_3	425			425		40	305	80	425
SiO_2		1241		1241	16	100	925	200	1241
TiO_2	318	348	158	824	13	66	600	145	824
ZnO		6		6			4	2	6

BR: brand, IG: income group.

These results confirm the soundness of the assumption applied in earlier models reported by Musee (2011; 2017); where the contribution of *very low* income groups were not considered. This is partly because nanoproducts are generally higher priced compared to brands with no ENMs, and for this, reason most population cannot afford such products. Secondly, usage per capita of PCPs is least in this group (Table 4-3 from Musee, 2018). Since the Gauteng Province has approximately 87% sanitation services available to the population, a large portion of the ENMs are likely to be released into wastewater and landfills based on a formalism developed and reported by Musee (2017). To, for example, ascertain how results developed in this model, this study's results were compared with results Musee (2017) (Figure 4-10).

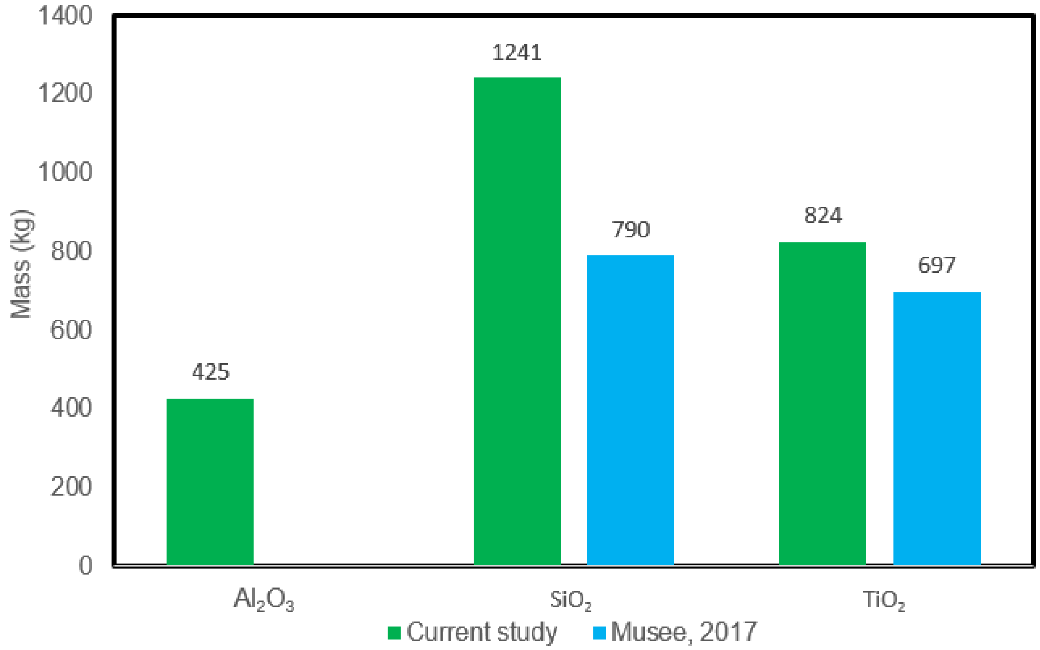

Figure 4-10: Comparison of estimated releases of ENMs in current study (bottom-up approach) and findings of Musee (2017) (top-down approach).

Two notable aspects are apparent. First, the results of the bottom-up approach (this study) and top-up approach (Musee, 2017), yielded comparable results but with differences of 36.3% and 15.5% for SiO_2 and TiO_2, respectively. Secondly, to improve the quality of risks estimation for a given pollutant, it is essential to use market data for products that are sources in a given country, or region. For example, such approach will allow to take into account differences in economies by scale and character; therefore, ENMs in a given brand (e.g. toothpaste) may differ from one database (country specific) to another. For example, no Al_2O_3 ENMs in the toothpaste brand in the in the database for UK (Tiede et al., 2015), and this explain why in Musee (2017) study; in the risk hazard ranking model, no Al_2O_3. Yet, calcium peroxide ENMs from toothpaste were estimated for GP (Musee, 2017) but no single brand had such ENMs based on the database reported herein. Hence, the database reported herein aids South Africa to consider ENMs of priority and eliminate the possibility of considering risks based on non-existing contaminants of concern based on other country's inventory.

A similar approach as described for the toothpaste can be applied to all other products to estimate quantities likely to be released into the environment. To improve the estimation of the low and high emissions of ENMs into the environment, the quantities based on confirmed nano-based products will be used as the minimum value. In addition, the sum of the likely mass from nano-based and indeterminate products will yield the estimated maximum released quantities into the environment.

4.3.5.4. Top-down and bottom-up mass production compared for selected products

The results of the top-down model (Musee, 2017) and bottom-up model (Figure 4-11) yielded comparable outcomes, with the exception of Al_2O_3 which was found to be present using the bottom-up approach as the inventory used by Musee (2017) is United Kingdom based and contain different ENM. Only these products were comparable as studies are based on independent inventories.

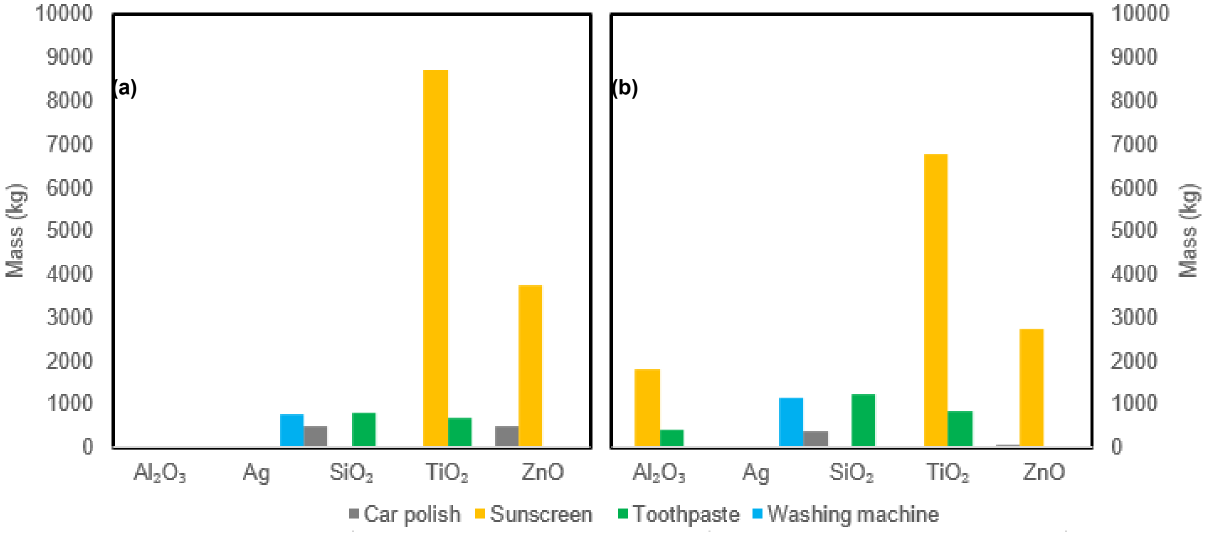

Figure 4-11: (a) Top-down approach (Musee, 2017) vs. (b) likely mass of bottom-up approach.

The results of the bottom-up approach (this study) and top-down approach (Musee, 2017), had several observable dissimilarities such that the absolute average difference in ENM mass between the study products were 45.7% (32.2% when excluding Al_2O_3) yet there is only a 1.95% (or 300 kg) difference in overall mass of ENMs specific to these products.

4.4. CONCLUDING REMARKS

The database reported herein provides an updated and detailed account of the nanoproducts available in the South Africa market. It further indicates that the ENMs are present in various product categories as well as their occurrence and release potential based on usage, market penetration, and the nature product matrix. The studies conducted in major stores that account for *ca* 92% of the retail market in GP provides insights on the distribution of nanoproducts. Moreover, the database currently contains a total of 2002 products distributed as follows: AUTO, EAA, FT, FB, MED and PCP under nano-based, intermediate, and unknown categories, respectively. Most nanoproducts were found in the EAAs category followed by PCPs. Based on estimated ENMs generated, the largest quantities of ENMs were in descending order (based on most likely mass) as TiO_2 > SiO_2 > ZnO > Al_2O_3 > Ag > CNT likely to be discarded into the GP environment.

Currently, there are only handful studies globally that have reported measured environmental concentrations (MECs) of ENMs in different environmental compartments. Therefore, the database can serve a valuable source of information required to estimate the potential risks of ENMs, and in turn, identified ENMs of concern to the South African environment. Secondly, the data can aid to determine the level of human exposure of ENMs in South Africa; and likely implications to human health, an aspect yet remain to be quantified in this country. This is of great significance since most products are in PCPs category; and therefore, likely to not only through dermal but digestion as well via drinking water.

One great concern in light of these findings is that a large portion of nanoproducts (37.6%), are of unknown status. This, together with largely unknown effects of ENMs to the human health and the environment implies that both short- and long-term implications of these emerging contaminants with nanoscale dimensions remain unquantified. As a result, this limits government's ability to develop mechanisms and tools to effectively govern nanoproducts from cradle to grave. One way to address potential risks of nanoproducts is through the development of policy and legislative frameworks that, for example, require manufacturers to declare if their products include ENMs, and safety-related data. However, such mechanisms may lead to overestimation or underestimation of nanoproducts as recently Hansen et al. (2016) highlighted as to how manufacturers may report by either exaggerating "nano" claims, or fail to report as has been found in the food industry. Thus, it is in this context, results in this report offer valuable insights on the nature and extent of quantities of ENMs likely to reach the environment; and their probability to induce deleterious effects to organisms.

4.5. RECOMMENDATIONS AND FUTURE WORK

The model results are not a replacement for the analytical approach for the quantification of ENMs in actual environmental matrixes using analytical techniques. Therefore, it is recommended that future studies to include detection and quantification of ENMs in actual environmental matrixes (e.g. freshwater, groundwater, wastewater, to mention a few) where available techniques permit. The proposed approach will allow the validation of the model and improving its degree of accuracy in future predictions. Further analysis of the results and uncertainties with respect to population, daily usage, concentrations per product(s) as well as other model parameters will need further refining. This approach will seek to improve the parameterization of the model inputs, which is a key step in improving the usefulness of the proposed model.

CHAPTER 5 : SINGLE AND BINARY TOXICITY OF ENMS TO BACTERIA IN RIVER WATER SYSTEM

Samuel K. Leareng, Eunice Ubomba-Jaswa, Ndeke Musee

5.1. INTRODUCTION

The transition of engineered nanoparticles (ENPs) from development to commercialisation has driven their growing use in consumer products and applications (Suresh et al., 2013; Gottschalk et al., 2013; Hedge et al., 2016). Furthermore, applications and products are predicted to utilise ENPs due to their favourable properties, increasing their production to millions of tonnes by 2020 with investment values estimated at three trillion dollars (Roco et al., 2011). These estimated production volumes of ENMs and increased usage have resulted in increasing release into environmental systems.

Among the metal oxide ENPs, zinc oxide nanoparticles (nZnO) are among of the most widely used (Thwala et al., 2013; Hedge et al., 2016; Baek et al., 2017), for example, in cosmetics, rubber, chemical fiber, electronics and other industries due to their large surface area (Hou et al., 2018) including consumer products already in the market in South Africa (Musee, 2017). Due to their magnetic properties, Iron oxide nanoparticles (including nFe_2O_3) have found use in applications such as biomedical imaging and environmental remediation (Sarahan et al., 2014; Demir et al., 2015; Taze et al., 2016; Hu et al., 2017), and are among the least studied nanoparticles with respect to their potential implications in the environmental systems (Wang et al., 2016; Dinali et al., 2017).

ENMs are released into the environment through their life cycle including; (i) during production of raw nanomaterial and manufacturing of nano-enabled products; (ii) during use; and (iii) after disposal of NP-containing products (waste handling and waste disposal points) (Musee et al., 2011). The released ENMs may enter the environment either directly from use and release or indirectly via technical systems such as effluents of WWTPs, application of biosolids to soil, or leachates from landfills (Musee et al., 2011; Bundschuh et al., 2018). Quantities of released ENMs to date have largely been estimated using modelling approaches reported as predicted environmental concentrations (PECs) due to lack of suitable analytical techniques. Estimations of current environmental concentrations from prediction models, for instance, in aquatic systems ranged from 10 ug/L to 400 ug/L of nZnO concentrations in the environment (Gottschalk et al., 2009, 2011). Moreover, PECs for IONPs in European surface waters has been estimated at up to 36 ng/L (Wang et al., 2016). With recent advances in technology, analytically determined quantities of ENMs are gaining traction to support modelling efforts (Bitragunta et al., 2017).

Consequently, over the last 15 years considerable efforts have been exerted to understand the ecotoxicological effects of single ENPs on biological lifeforms at different position of the taxa in the food chain such as bacteria (Heinlaan et al., 2008; Baek et al., 2011; Hou et al., 2018), algae (Aruoja et al., 2015), plants (Thwala et al., 2013; Thwala et al., 2016), invertebrates (Heinlaan et al., 2008; Azevedo et al., 2016; Lu et al., 2017) and vertebrates (Zhu et al., 2012; Kteeba et al., 2017; Hou et al., 2018). nZnO has been shown to induce toxicity in bacteria by nanoparticle contact with cells, Zn^{2+} ion released from the particulate and oxidative stress (Kumar et al., 2011, Li et al., 2011, Li et al., 2013, Kim et al., 2016; Rago et al., 2018). nFe_2O_3 toxicity arises from oxidative stress and ENM-contact (Auffan et al., 2008; Arakha et al., 2015; Ranmadugala et al., 2017). The toxicity of ENMs is dependent on their physicochemical properties, which are affected by water chemistry or exposure media (Thwala et al., 2013; Zhu et al., 2014; Hedge et al., 2016; Yi et al., 2017). Although the influence of environmental factors such as pH, ionic strength, light, and natural organic matter (NOM) (Li et al., 2013; Dasari et al., 2013) is well studied, there is still scarcity of information on the toxicity of ENMs in the real aquatic environment. A number of emerging studies have explored the fate and behaviour of ENMs in freshwater systems (Odzak et al., 2017, Peng et al., 2017, Conway et al., 2017), however, these studies have lacked the toxicity aspect, and thus, have not explored the implications of the fate of studies ENMs to the eventual toxicity of organisms in the same media.

Furthermore, in the aquatic environment, released ENPs will co-exist together and along with other pollutants. Due to the reactivity of ENPs, their transformation and reactivity will alter their physicochemical properties, which will also alter their toxicological profiles (Tong et al., 2015; Wilke et al., 2016). Hence, the toxicity findings of single ENPs would be likely limited to predict the effects of mixtures as has been shown for counterpart bulk chemicals (Heys et al., 2016). ENPs will mostly likely interact with other contaminants in the environment, including organic contaminants such as triclosan (TCS). Triclosan is widely used antimicrobial agent in household consumer goods such as personal care products and medical products; and as a result, is highly detected in the aquatic environment (Brausch and Rand, 2011; Zhang et al., 2016), and modelled environmental concentrations exceed safety threshold (Musee, 2018). In the aquatic systems, TCS ecotoxicological data show it can induce antibiotics resistance in microbial communities (Drury et al., 2013; Oggioni et al., 2013), lead to shifts and altercations in microbial communities (Stasinakis et al., 2008; Carey and McNamara, 2015), and induce high toxicity on aquatic microorganisms compared to other disinfectants (Brausch and Rand, 2011). Toxicity of TCS to microorganisms occurs through the inhibition of fatty acid synthesis (Heath et al., 1999; Ricart et al., 2010; Grandgirard et al., 2015). However, to date, there is little information on the chemical interactions and toxicity of ENPs and TCS on microorganisms.

To protect the environment from adverse effects of pollutants, understanding the toxicity of ENPs on bacteria is of significance as they play a vital role in the ecosystem such as nutrient recycling, decomposition of organic materials, and a primary source of food in both aquatic and terrestrial systems (Holden et al., 2014; Qiu et al., 2016). Therefore, in the environment, taxa at different positions of food chain will be exposed to mixtures of different stressors, or contaminants either concurrently, sequentially or both (Løkke et al*.,* 2013). The mixtures are made up of different classes of contaminants, e.g. ENPs and antimicrobials. Further, each contaminant class have different toxicological profiles, which may change as they interact with organisms and other classes of contaminants as well as are transformed due to water chemistry-based factors like pH, ionic strength, etc. For instance, toxicity of nZnO is known to be primarily driven by the release of ions which are driven by water chemistry (Aruoja et al., 2009; Kumar et al., 2011; Li et al., 2013). whereas minimal toxicity reported due to aggregation and reduced ROS production for iron oxide nanoparticles (including nFe_2O_3) (Auffan et al., 2008; Arakha et al., 2015; Ranmadugala et al., 2017).

The effects of ENPs on bacteria as primary targets to ENM exposure has likely adverse impacts (Ma et al., 2013; Luche et al., 2016). *Bacillus Subtilis*, an environmentally ubiquitous organism, has been widely used as a model organism in many ENPs toxicity studies, and has been shown to be more susceptible to ENMs compared to organisms such as *Escherichia coli, Aeromonas hydrophila and Pseudomonas aeruginosa* (Ma et al., 2013; Ivask et al., 2013; Binh et al., 2014; Arakha et al., 2015; Hseuh et al.. 2015; Ye et al., 2017). Furthermore, there is necessity to establish toxicity of ENPs at sub-lethal concentrations (concentrations below 1 mg/L) in water, which is still lacking as most studies have been done at concentrations magnitudes higher than environmentally predicted (Sheng and Liu, 2017).

In this study, the effects of the nZnO and nFe_2O_3 on B *subtilis* both as individual and mixtures, were assessed in river water. For the single ENMs, cell viability, oxidative stress from ROS and ATP production were assessed as toxicity endpoints. Furthermore, cell viability was measured to aid in predictive models used to calculate expected toxicity of mixtures on *B. subtilis* under the varying water chemistry parameters.

5.2. METHODOLOGY

5.2.1. Materials and reagents

nZnO (< 100 nm, 20% dispersion in H_2O, CAS 1314-13-2), nFe_2O_3 (γ- Fe_2O_3, < 50 nm, nano powder, CAS 1309-37-1), 2′,7′-Dichlorofluorescin diacetate (DCF-DA), Dimethyl sulfoxide (DMSO) and Acetone were purchased from Sigma-Aldrich, South Africa. According to the manufacturer, the particle sizes were < 100 nm and < 50 nm for nZnO and nFe_2O_3, respectively. All chemicals were analytical grade reagents and used as received without further purification. The *Bacillus subtilis* (ATCC 11774) strain was purchased from Anatech, (Johannesburg, South Africa).

5.2.2. Freshwater sampling

Freshwater samples were collected from two river systems, the Elands river (ER) (25°32'58.4"S 28°33'53.4"E), Gauteng Province, South Africa), and Bloubank river (BR) (26°01'20.3"S 27°26'31.6"E), North West Province, South Africa) in May 2018. Notably, these water samples were used as experimental exposure matrixes to represent complex environmental surface freshwater systems. The collected river water was filtered using Whatman No. 1 filter paper (pore size: 11 µm) followed by filtration through 0.2 µm pore sized membrane filters to remove microorganisms and larger particles. All the water samples were stored at 4°C until analysis. Physicochemical properties of the river water were analysed, and results listed in Table 5-1.

Table 5-1: Physicochemical parameters of freshwater samples from Bloubank and Elands River

Parameter	Unit	Bloubank River water	Elands River water
pH		7.9	8.1
DOC[a]	mg C L^{-1}	8.25	5.51
Electrical conductivity	ms/m 25°C	39.8	19.6
COD[b]	mg/L	21.3	6.67
Alkalinity	mg/L	217	75.6
NH_4	mg/L	3.4	4.27
NO_3	mg/L	0.2	0.33
Cl^-	mg/L	12.9	17.1
SO_4	mg/L	6.77	9.03
PO_4	mg/L	1.23	0.57
Fe^{3+}	mg/L	<0.004	<0.004
Zn^{2+}	mg/L	0.01	0.008
Ca^{2+}	mg/L	36	14
Mg^{2+}	mg/L	31	9.82
Na^+	mg/L	22.4	15.6
K^+	mg/L	3.13	4.24
Ionic strength	mM	4.94	2.45

[a]Dissolved organic matter; [b] Chemical oxygen demand; [c] Ionic strength

5.2.3. Characterisation of ENMs

X-ray diffraction (XRD) was used to determine the crystalline properties of the nZnO and nFe_2O_3. Since nZnO were dispensed in de-ionised water upon purchase, the sample was dried in the oven for 2 h at 100 ºC to remove all solvents before XRD analysis were performed. The sample was then left to cool in the oven overnight. The oven was air-sealed to prevent any contamination of the nZnO. The individual nanoparticle powder patterns were analysed at room temperature (20˚C) using a Bruker D2 Phaser powder X-ray diffractometer (Bruker n.d.; Diffraction & Detector, 2009). A CuK-α radiation of λ = 1.54 Å was generated at 30 kV and 10 mA. The diffraction patterns were programmed at 7 s step intervals, and increased by a size of 0.1°. The powder pattern data was collected in reflection geometry at 2θ ranging from 5-45˚. The XRD spectra was analysed using the Diffrac. Eva. Suite software where quantitatively determined the material present in the experimental pattern, and in turn, determine the percentage match between the reported XRD pattern and the calculated XRD pattern.

The ENPs size and morphology were characterised by transmission electron microscope (TEM) (JEM, 2010F, JEOL Ltd., Japan); with ENPs diameter measured using imageJ software (National Institutes of Health, USA) based on particle size analysis from several micrographs. Phase composition was determined using Bruker D8 Advance powder X-ray diffractometer (XPRD) with monochromatized Cu Kα radiation of wavelength (λ) 1.54 Å. The hydrodynamic diameter (HDD) and zeta potential (ζ-potential) for ENPs suspensions in river water were characterised using dynamic light scattering (DLS) on a Zetasizer Nano-ZS instrument (Malvern Instruments, UK). Concentrations of 20 mg/L ZnO and 5 mg/L nFe_2O_3 were used for hydrodynamic size and ζ-potential measurements.

5.2.4. Dissolution of ENMs in freshwater

Ddissolved metal concentrations of nZnO and nFe_2O_3 were determined in suspensions of ENPs under similar experimental conditions (bacterial culture and exposure preparation), without the bacteria. ENM solutions were filtered through 3 kDa molecular weight cut-off centrifugal filters (Merck Millipore, Darmstadt, Germany) by centrifuging for 30 min at 4000 xg (Eppendorf 5810 R, Eppendorf, Germany) and the dissolved fraction acidified with concentrated HNO3.Iinductively coupled plasma mass spectrometer (ICP-MS) (ICPE-9820, Shimadzu, Japan) was used for analysis of the dissolved ion concentrations.

5.2.5. Bacterial culture preparation

Bacterial strain *B. subtilis* was plated on sterilized lysogeny broth (LB) agar plates and maintained at 4°C until ready for use. For viability studies, a single colony was inoculated in Lysogeny broth (LB broth) and incubated overnight at 30°C, wish shaking at 150 rpm, until they reached approximately 0.5 at OD_{600nm}. Following centrifugation at 7 500 g for 5 min, cells were subsequently washed twice using filtered river water. Following the washing step, bacteria were re-suspended in the filtered river water to an optical density of 0.3 at 600 nm corresponding to 10^8 cells/mL as measured by plate counting.

5.2.6. Single ENM exposures

ENP stock solutions of 100 mg/L were prepared in ultrapure water (18 MΩ cm resistivity, Elga PureLab Option System, United Kingdom) and sonicated for 20 min in an ultrasonic bath prior to exposure experiments. TCS stock solution was prepared in analytical grade acetone (> 99%) to a concentration of 1 g/L. All exposure experiments in both river water samples were conducted in 250 mL flasks, with different nominal concentrations of ENMs (nZnO; 0.01, 0.1 and 1 mg/L, nFe_2O_3; 0.01, 0.1, 1 and 10 mg/L; concentration gradients for dose response-curves) and cells at 0.3 of OD_{600nm} (10^8 cells/mL) and river water to a final volume of 25 mL. All exposure studies were done at room temperature (20-23°C) on a rotating shaker at 100 rpm for 2 h under visible light (338 lux).

5.2.7. Cell viability

Both exposed and unexposed (control) bacteria exposure samples were serially diluted in 0.85% NaCl and plated on LB agar to determine cell viability using the modified drop count method. Nine drops (20 µL per drop) were transferred onto solid LB agar medium. The plates were incubated at 37°C overnight. The viability percentage of the bacteria in the ENPs suspensions were calculated by dividing colony forming units (CFU/mL) to that of the control (bacterial preparations without ENPs). The viability experiments were done in three replicates, and data expressed as the mean with corresponding standard deviation (SD).

5.2.8. Membrane integrity

The cell membrane integrity of both the exposure and control samples was analysed using L13512 LIVE/DEAD BacLight viability kit (Invitrogen, Eugene, OR, USA). The kit contains two stains, namely: propidium iodide (PI) and SYTO9, which are used to differentiate between cells that were intact (live organisms – stained in green) and damaged cells (dead organisms – stained in red), respectively. Hundred-µL aliquots of ENPs exposed and non-exposed (control) samples were placed in 96-well microplates (Greiner Bio-One, Austria), combined and mixed thoroughly with 100 µL of SYT09/PI mixture (10/60 µM), followed by incubation in the dark (covered by aluminium foil) for 15 minutes at room temperature (20-23°C). Fluorescence intensity was measured using a Flouroscan Ascent FL microplate reader (ThermoFisher, USA). The excitation/emission maxima for the dyes were 485/538 nm for SYTO9 and 485/635 for PI, respectively. A calibration curve was obtained using cells with known percentages of intact cells. For each test, three replicates of each treatment were included per plate, and two plates were used to ensure reproducibility.

5.2.9. ROS assay

DCF-DA which is oxidized to the fluorescent 2′, 7′-Dichlorofluorescin (DCF) after reacting with ROS was used as an indicator of ROS. Hundred and fifty µL aliquots of the exposed and non-exposed samples incubated with DCF-DA (100 µM final concentration) in 96-well microplates for 30 min at 37°C under dark conditions (covered using aluminium foil). A Flouroscan Ascent FL microplate reader (ThermoFisher, USA) was used for measuring DCF fluorescence intensity at an excitation and emission of 485 and 538 nm, respectively. ROS production was expressed as percentage fluorescence of the control over the exposed samples. For each test, three replicates of each sample were added per plate, and two plates were used to ensure reproducibility.

5.2.10. Bacterial ATP levels

Bacterial ATP response to ENMs exposure was measured using the BacTiter-Glo Microbial Cell Viability assay (Promega, Germany). In 96-well microplate (Greiner Bio-One, Austria), Hundred-µL of ENPs aliquots of the exposure samples and controls and 100 µL of the BacTiter-Glo reagent were combined, mixed thoroughly and incubated for 5 min at room temperature (20-23°C) under dark conditions (covered with aluminium foil). The luminescence signal was measured using the Flouroskan Ascent FL microplate reader, and the results were expressed as relative percentage of ATP of exposed bacteria to the control. Three replicates were included in each treatment per plate, and two independent microplates were used to ensure reproducibility of the results.

5.2.11. Microscopic observations of bacterial cells

TEM was used to observe the direct contact between the NPs and the bacterial cells. A drop of bacteria exposed to the NPs and the NPs-free control was air-dried onto a copper grid and was then imaged by the TEM. To observe the internalization and localization of the NPs in the cells and the changes in cellular structure as affected by the NPs, both NPs exposed and non-exposed bacteria were fixed in 2.5% glutaraldehyde, dehydrated in graded concentrations of ethanol (50%, 70%, 80%, 90%, 95% and 100%) for 15 min at each step and transferred to absolute ethanol for 20 min. The samples were immersed in 1:1 and subsequent 1:3 mixtures of acetone and epoxy resin for 1 h and 4 h, respectively, and then let to polymerize for 36 h. Ultrathin sections were cut, stained with uranyl acetate and lead citrate and finally observed with TEM (Huang et al., 2015).

5.2.12. Mixture studies exposure

5.2.12.1. Data Analysis

Dose-response relationships were described by non-linear regression analysis using a four-parameter Hill model in the Dr-Fit software (Di Veroli et al., 2015). The software also allows and automatically generates dose-response models of multiphasic features using the generalised Hill model given by the expression:

$$E(C) = \left(1 + \frac{EC_{max\ 1} - 1}{1 + \left(\frac{EC\,501}{C1}\right)^{H1}}\right)\left(1 + \frac{EC_{max\ 2} - 1}{1 + \left(\frac{EC\,502}{C2}\right)^{H2}}\right) \qquad (1)$$

where effect *E* is obtained at a given concentration *C*, E_{max} is the maximum effect, *H* is the hill component.

The derived dose-response curves were then used to calculate the EC_x (x being the percentage mortality observed at effect concentration) values of the constituent toxicants. These EC_x individual values were then used to estimate the toxicity of the mixtures. The individual component's concentrations were first expressed as fractions of the total concentration. Next, the calculated fractions were used in the concentration addition (CA) model as described in Equation 2 (Villa et al., 2014). This was used to calculate various EC_{mix} valves. This equation is used to predict the concentration:

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i}\right)^{-1} \qquad (2)$$

where ECx_{mix} denotes the produced total concentration of the mixture that results in a x % of the effects. EC_{xi} gives the i^{th} component's concentration resulting in the same *x* % effected as the mixture, and p_i is the fraction of a single component *i* present in an *n*-mixture. An alternative model, the independent action (also regarded as response addition) was applied to the individual components to predict the mixture effects; based on the expression:

$$E(C_{mix}) = 1 - \prod_{i=1}^{n}(1 - E(C_i)) \quad (3)$$

where $E\ (C_i)$ is the effect that the i^{th} mixture component would provoke if applied singly at an exposure concentration C_i, and $E(C_{mix})$ is the overall effect caused by the mixture (Bliss, 1939; Faust et al., 2003; Villa et al., 2014; Wang et al., 2016).

Equitoxic mixtures of the chemicals were then prepared based on their individual toxicities and evaluated to determine their joint effects. Herein, the joint effects are described by the sum of the toxic unit indexes as follows (Wang et al., 2014; Wang et al., 2016):

$$TUx = \left(\sum_{i=1}^{n} \frac{C_i}{ECx_i}\right) \quad (4)$$

where C_i is the concentration of the i^{th} component in an n-compound mixture and EC_{xi} denotes the concentration of substance i that provokes an effect equal to x% when applied individually. According to Broderius et al. (1995), a simple addition is characterized by $1.2 > TU > 0.8$, whereas $TU > 1.2$ represents antagonism, and $TU < 0.8$ indicates synergism.

5.2.13. Statistical analysis

Data herein are expressed as mean with corresponding standard deviation (SD). Two-way analysis of variance (ANOVA) was used to evaluate statistical differences followed by *post hoc* Tukey's multiple comparisons tests. Differences between samples were considered statistically significant when at $p < 0.05$. All analyses were done with GraphPad Prism V7.04 (GraphPad Prism software Inc., San Diego, CA, USA).

5.3. RESULTS AND DISCUSSION

5.3.1. Freshwater results

The average particle sizes of nZnO and nFe_2O_3 were 22.5± 5.1 nm and 40.5± 24.9 nm, respectively, from TEM observations and measurements. nZnO were predominantly irregularly shaped, and nFe_2O_3 had tetragonal and cubic structures as shown in Figs. 5-1a and b, respectively. XRD results revealed the crystalline phase of nZnO as zincite whereas that of nFe_2O_3 was predominantly maghemite (γ-Fe_2O_3) (Figs. 5-1c and d). The ζ-potential for both ENPs was negative in all river water samples (Table 5-2) within a narrow range from -12.3 to -15.1 mV.

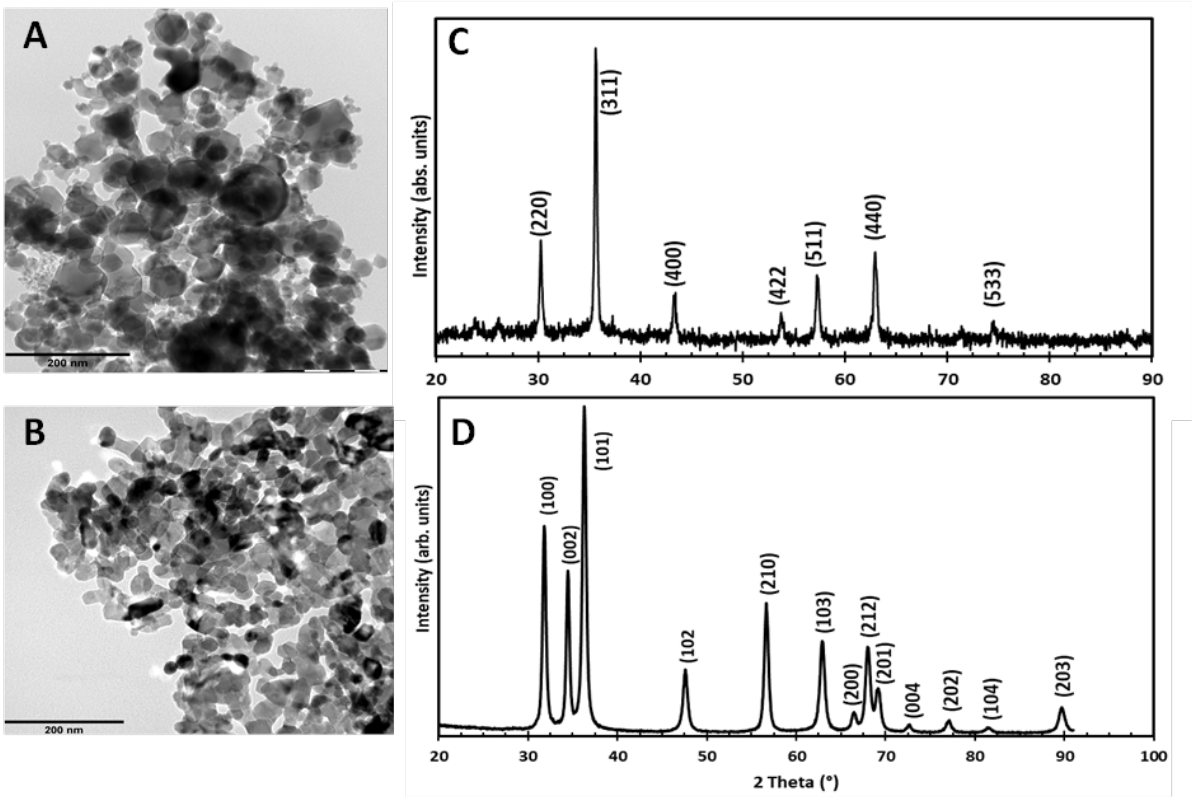

Figure 5-1: TEM micrographs of (a) nFe$_2$O$_3$ and (b)nZnO. XRD patterns of (c) nFe$_2$O$_3$ (d) nZnO

Rapid agglomeration was observed post-sonication in both river water samples for nZnO and nFe$_2$O$_3$, as shown by high hydrodynamic diameter (HDD) from the ENM sizes (Table 5-2). nZnO sizes varied in river water samples, with significant average size of 1069 ± 186.9 nm in BR water compared to 512 ± 22.3 and in ER water. Similar results were observed for nFe$_2$O$_3$ where aggregate sizes increased over time in both river water systems. Notably, BR had higher HHD of 1626.5 ± 193.9 nm compared to 1098.69 ± 286.5 nm in ER water (Table 5-2) after 2 h. The increased aggregation over time in both river water samples was associated with notably low ζ-potential of between -12.3 ± 0.6 and -15.1 ±1.3 mV. The surface charge is considered to play a significant role in the stability of ENPs, and determines their interaction with biological systems (Demier et al., 2015; Lowry et al., 2016). In this study, the zeta potential was considered to be too low for charge stabilisation and ENP dispersion (Philippe and Schaumann et al., 2014; Lowry et al., 2016), and may therefore account for the high aggregation.

Table 5-2: Hydrodynamic diameter and zeta potential of ENMs in river water samples

	NZNO (20 MG/L)		NFE$_2$O$_3$ (5 MG/L)	
PARAMETER	Bloubank river	Elands river	Bloubank river	Elands river
Z^A (MV)	-13.41 ± 0.44	-15.07 ± 0.63	-12.25 ± 1.25	-15.12 ± 1.25
D_H^B (NM), 0 HRS	1069.08 ± 186.90	512.27 ± 22.28	1056 ± 120.30	957.64 ± 188.01
D_H (NM), 2 HRS	1372 ± 257.28	557.48 ± 28.51	1626.5 ± 193.98	1098.69 ± 286.52

Z^a: Zeta potential, D_H: Hydrodynamic diameter

The release of ions by both ENPs was measured in both river water samples, and the results are shown inn Fig. 5-2. At nominal exposure concentration of 100 µg/L, 14.33 µg/L of Zn^{2+} ions were measured in ER, and < 2 µg/L in BR water. At higher nominal exposure concentration of 1000 µg/L, higher dissolution of nZnO was observed in ER and BR water at values of 366 and 183 µg/L respectively. Therefore, the released Zn ions were concentration dependent, as observed in earlier studies (Li et al., 2010; Ma et al., 2014). In contrast, nFe$_2$O$_3$ was found to exhibit very little to no dissolution as Fe ions were not detected or were below analytical detection limit, in agreement with literature (Wang et al., 2016).

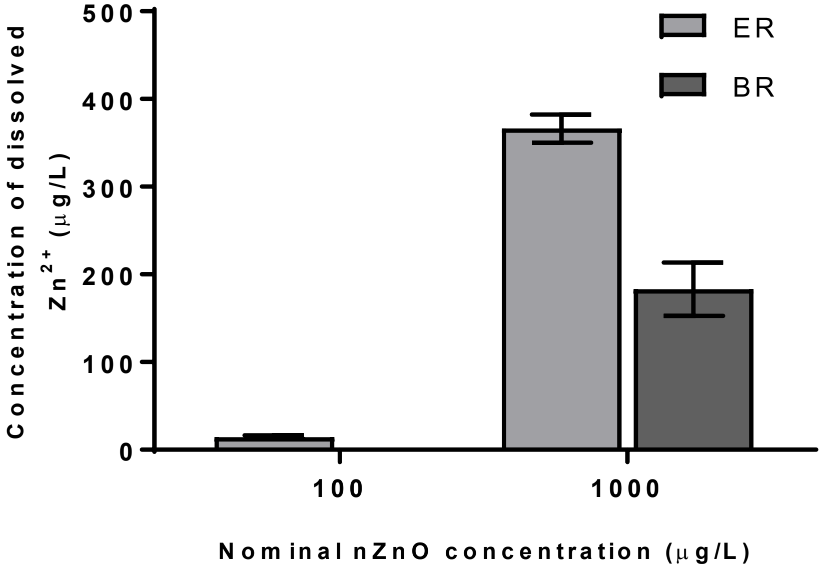

Figure 5-2: Dissolved zinc concentrations in the river water samples following 2h incubation under visible light. Errors bars denote standard deviation (*n* = 3). Concentration of 100 µg L^{-1} ZnO in BR below detection limit (10 µg L^{-1}, not shown). Nominal nZnO exposure concentrations used were 100 and 1000 µg L^{-1}.

In this study, high nZnO aggregation occurred in water with high ionic strength and NOM, which in turn showed reduced dissolution. NOM and ionic strength have been found to be critical to both the stability and dissolution of ENMs (Peng et al., 2015; Odzak et al., 2017). The presence of elevated cations (e.g. Ca^{2+}, Na^{+}, etc.) and anions (e.g. SO_4^{2-}, PO_4^{2-}), present BR water was correlated with the increased aggregation, which was comparably lower in ER water. Odzak et al. (2017) showed that enhanced aggregation was more significant in river and lake water with higher ionic strength (Between 3.4 and 6.4 mM) exhibited enhanced aggregation and dissolution of nZnO compared to water with lower ionic strength. These findings are comparable to findings from this study where the differences in ionic strengths of 2.45 and 4.95 mM in ER and BR, respectively, contributed the differences in aggregation and dissolution. Herein, differences in ionic strength and NOM may plausibly account for higher aggregation in BRW compared to ERW (Table 5-1). In this instance, increases in NOM and ENPs interactions led to higher aggregation resulting to a reduction in dissolution.

5.3.2. Cell viability

Cell viability results following interaction of nZnO and nFe_2O_3 with *B. subtilis* in ERW and BRW are shown in Figs. 5-3a and b. The obtained results showed that nZnO significantly induced reduction in viability at concentrations of 100 and 1000 µg/L in ER water, whereas there no observed reduction was observed at 10 µg/L in ER (Fig. 5-3 a). The toxic effects of nZnO on bacteria have been reported in other studies (Li et al., 2013; Ma et al., 2014; Kumar et al., 2011), and were linked to released Zn^{2+} concentration, ROS production and surface contact. In this study we also evaluated the water chemistry differences on the behaviour and resulting toxicity. It was observed that the low aggregation in ER water and increased dissolution were the likely contributing factor to the differences in the toxicity of the ENM in the two water systems. Previous studies showed that release of Zn^{2+} ions from nZnO was primarily responsible for inducing toxicity in bacteria (Li et al., 2013; Dasari et al., 2015; Wang et al., 2016). In this study, we observed markedly significant reduction in viability in *B. subtilis* in ERW at 1000 µg/L ($p \leq 0.001$) of nZnO which had high concentrations of Zn^{2+} ions, at 336 183 µg/L (Fig. 5-2). In contrast, there was no observed reduction in viability all concentrations in BR water. Although there were dissolved Zn ions at 183 µg/L, these did not induce any effects on the cells. These observed effects are consistent with findings from Li et al. (2013) who observed mitigated effects from Zn^{2+} on bacteria. In our study, there were elevated concentrations of Ca^{2+} and Mg^{2+} in BR water compared to ER, which would likely compete for binding sites on the bacteria with released Zn^{2+} ions.

Following *B. subtilis* exposure to nFe_2O_3, no significant effects were observed, and similar results were observed, irrespective of exposure concentration in both river waters, relative to the control. The nFe_2O_3 results are in agreement with the literature where no toxic effects were observed on microorganisms at concentrations < 70 mg/L. The low cytotoxic effects of nFe_2O_3 observed in this study was attributed to instability leading to high aggregation, and low solubility, and hence, very low or none release of ions (Wang et al., 2016).

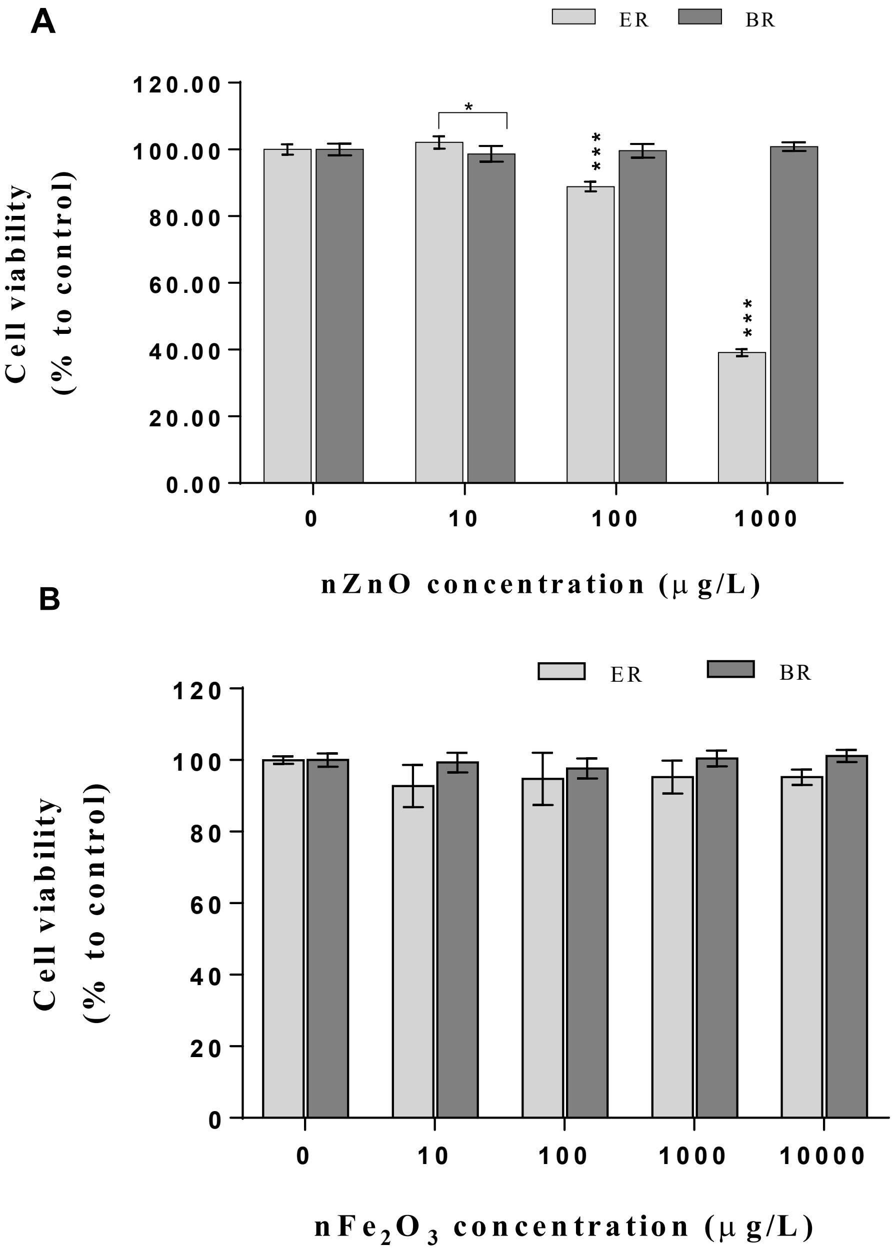

Figure 5-3: Effects of (a) nZnO and (b) nFe$_2$O$_3$ on *B. subtilis* viability in river water. Data represents the average ± SD (n=3). Asterisks (*) represent significance levels from Tukey's *post hoc* tests in two-way ANOVA (*p < 0.05, **p ≤ 0.01, ***p ≤ 0.001).

5.3.3. Membrane integrity

Fig. 5-4 shows the effects of nZnO and nFe$_2$O$_3$ on cell membrane integrity in both water samples. For nZnO, A concentration-dependent reduction in cell membrane integrity of *B. subtilis* was observed in both water systems. The greatest effect was observed at all concentrations in ER water compared to BR water, where the maximum reduction of 53.8% observed at 1 000 µg/L (highest concentration) compared to 25.5% in BRW (Fig. 5-4 a). The observed differences on cell membrane integrity were attributed to two factors: (i) site specific water chemistry conditions (Table 5-1) wherein a marked reduction was observed in ER water, and (ii) the type of ENPs with nZnO inducing a higher disruption (Fig. 5-4). The results herein indicate that nZnO induced significantly higher effects on cell membrane integrity disruption compared to nFe_2O_3. The observed high nFe_2O_3 aggregation mitigated toxicity where sedimentation could be seen at the bottom of exposure flasks. For nZnO, aggregation and dissolution played a significant role in the observed toxicity. It was noted that the maximum cell membrane integrity significantly decreased ($p \leq 0.01$) in BRW (1000 µg/L), and the results were similar to those of 100 µg/L in ERW. Previous studies have shown that intracellular Zn^{2+} can cause cell membrane disruption by lipid peroxidation (Anders et al., 2017).

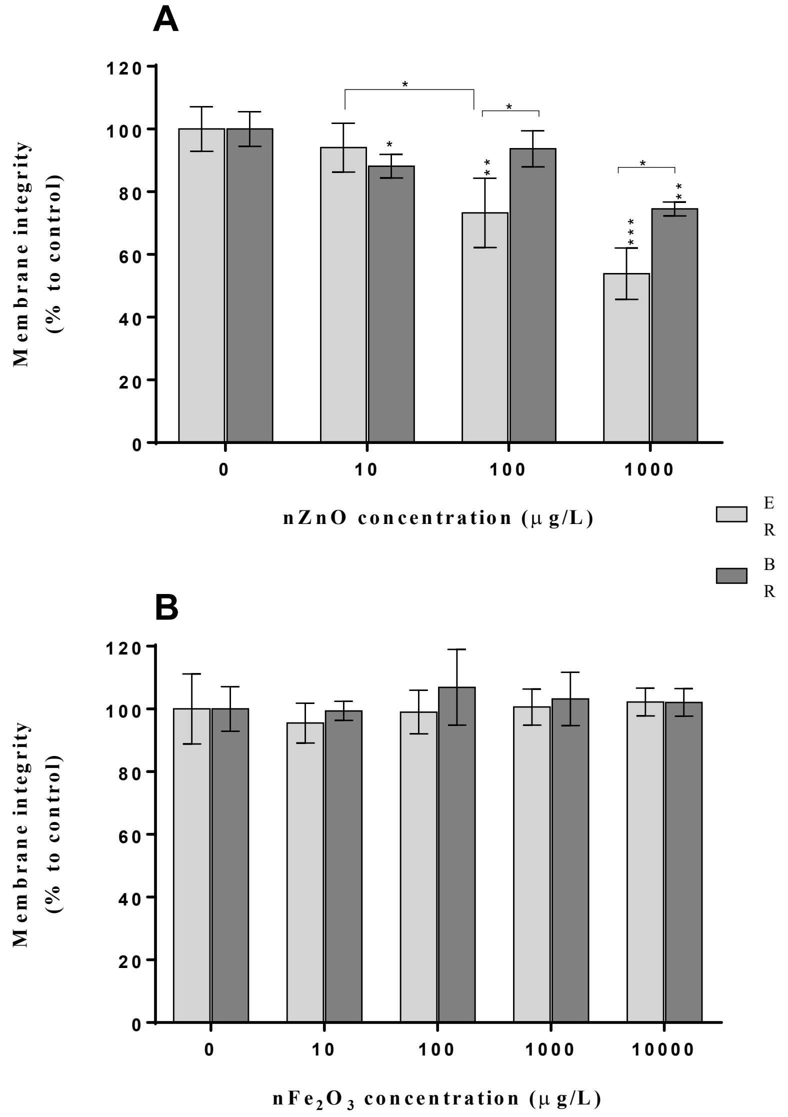

Figure 5-4: Effects of (a) nZnO and (b) nFe_2O_3 against *B. subtilis* cell membrane integrity in natural water. Data represents the average ± SD (n=3). Asterisks (*) represent significance levels from Tukey's *post hoc* tests in two-way ANOVA (*$p < 0.05$, **$p \leq 0.01$, * ≤ 0.001).**

The nFe_2O_3 showed no cell membrane integrity effects to *B. subtilis* irrespective of river water used at all concentrations (Fig. 5-4b). The results are in agreement with literature where no effects were observed at elevated concentrations of ≥100 mg/L (Auffan et al., 2008; Wang et al., 2016). The toxicity of ENPs could also come from surface contact with microorganisms (Kumar et al., 2011). To probe the likely bacteria-ENPs interactions, TEM was used to observe for exposure samples from ER water, and the micrographs of cells observed are shown in Fig. 5-5.

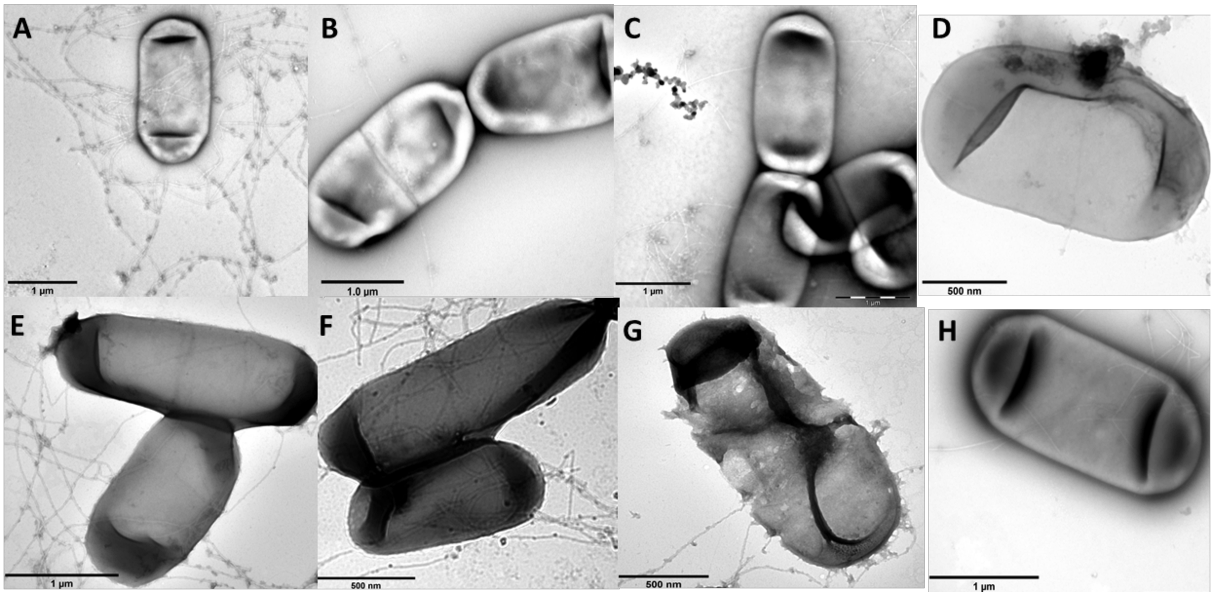

Figure 5-5: Transmission electron micrographs of *B. subtilis* following exposure in (a) 10, (b) 100, (c) 1000, (d) 10000 µg/L nFe_2O_3; (e) 10, (f) 100, (g) 1000 µg /L nZnO; and (h) control, in ERW.

Intact bacteria cells were observed, with minimal ENP-cell interactions for nFe_2O_3 nominal exposure concentrations (10-10 000 µg/L) as shown in Figs. 5-5 a-d. Raptured cells were observed, however, at higher concentrations (100 and 1000 µg/L) for nZnO (Fig. 5-5 f and g), whereas at lower concentrations (10 µg/L), the cells remained intact. Cross-sections of the cells following exposure to ENPs for qualitative assessment of membrane structures are shown in Fig. 5-6.

At lower concentrations of 10 and 100 µg/L nFe_2O_3 intact cells were observed (Fig. 3-6a and b), however, at both 1000 and 10 000 µg/L (Fig. 5-6c and d), a few cells with impaired cell walls were observed with ENPs in close proximity to the cells. For nZnO, evidently raptured cells were observed at 1000 µg/L (Fig. 5-6g), but whereas intact, unimpaired cells were observed at 10 and 100 µg/L concentrations (Figs. 5-6e and f).

There was minimum contact between the ENPs (nZnO and nFe_2O_3) and the cells, as observed from TEM results. As noted from the zeta potential results in both water samples (Table 5-1), the negative charges pointed to repulsive forces between ENPs and cells, and may partly account for the limited or no contact. Similarly, high aggregation reduced the contact between ENPs and cells, reducing the available surface area of the ENPs. Owing to the formation of large aggregates resulted to the sedimentation of nFe_2O_3. It should be pointed out that lack of observed ENP-cell contact should not be ruled out completely, particularly at higher concentrations, where evidence of membrane damage was observed.

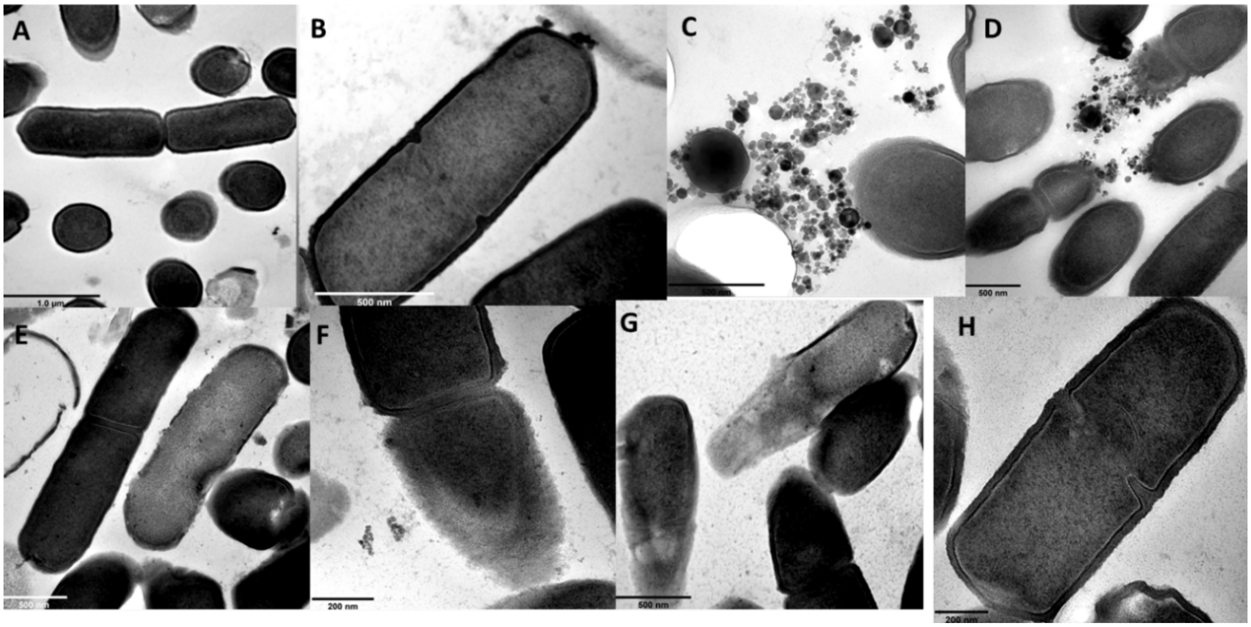

Figure 5-6: Cross-sections of transmission electron micrographs of *B. subtilis* following exposure to (a) 10, (b) 100, (c) 1000, (d) 10000 µg/L nFe_2O_3; (e) 10, (f) 100, (g) 1000 µg/L nZnO; and (h) control, in ERW.

There are likely interactions that will occur due to mechanisms such as hydrogen bonding, Van der Waals forces and receptor-ligand interactions, through liposaccharides attraction to nZnO, as suggested elsewhere (Jiang et al., 2009; Leung et al., 2016; Kadiyala et al., 2018). This is because the Zetasizer only measures the largest particles in solution, and does not report smaller sizes that may still collide with cells at high concentrations, due to the concentration effect. This remains to be examined further to provide exact mechanisms on ENPs toxicity to microorganisms. Released Zn^{2+} ions could also lead to membrane integrity loss through attachment to the cell membrane (Huang et al., 2014). Furthermore, Further, disruption of zinc homeostasis due to internalised Zn^{2+} ions may lead to cell rupture. In other studies, cell membrane integrity was reduced by between 10 and 20% on *Aeromonas hydrophila* and *E. coli due* to released ions by nZnO at 1000 µg/L. Thus, Zn^{2+} would likely contribute to observed effects by decreasing cellular functioning by pure chemical effect on mitochondrial functioning and lipid peroxidation (Anders et al., 2017).

5.3.4. ATP Production

The response of *B. subtilis* to nZnO and nFe_2O_3 was determined by analysis of ATP levels and the results are shown in Fig. 5-7. Declines in ATP levels following exposure to nZnO were observed at all nominal concentration as a function of time (Fig. 5-7), with more pronounced effects in ERW, on a concentration dependent manner (Fig. 5-7a). This results are consistent with results from Tong et al. (2015) where concentration-dependent reductions in ATP levels on *E.coli* and *A. hydrophila* by nZnO in lake water at concentrations of 250 and 1000 µg/L following 1 h incubation under dark conditions. Furthermore, significant decreases in ATP levels at 10 µg/L nZnO ($p < 0.05$) following 1 h incubation were observed. Previous studies by Wilke et al. (2017; 2018) have shown ATP level measurements to be a sensitive of bacterial stress due to ENPs even at concentrations as low as 10 µg/L. This correlation is highly plausible because ATP is a physiological assay since it only responds to live cells. Secondly, ions formed following the dissolution of ENPs may deactivate energy-dependent reactions in the cells as previously observed for zinc toxicity. The results herein showed ATP levels observed followed the dissolution patterns, and thus, were affected by released ions from nZnO as suggested in Tong et al. (2015).

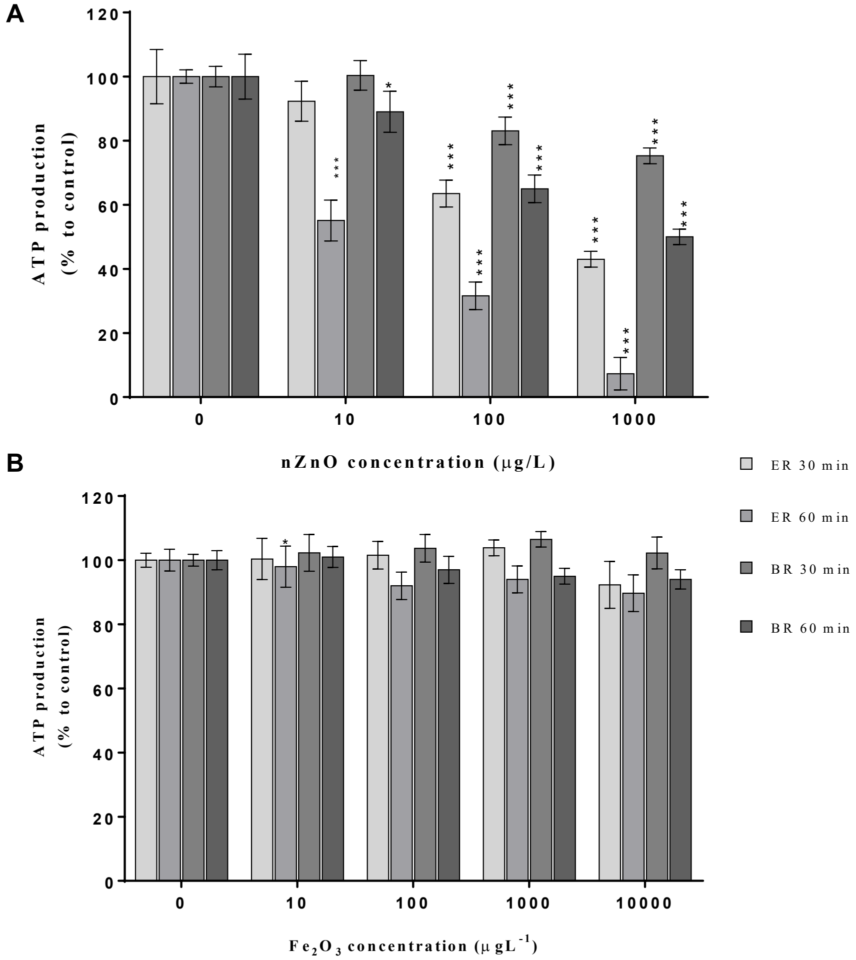

Figure 5-7: Effects of (a) nZnO and (b) nFe_2O_3 on *B. subtilis* ATP levels in Elands and Bloubank river waters. Percentage of bacterial ATP was normalized to that of the control (no exposure to ENPs). Asterisks (*) represent significance levels from Tukey's *post hoc* tests in two-way ANOVA (*$p < 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

There were no observed changes in ATP levels following exposure to nFe_2O_3 being insignificant irrespective of nominal exposure concentration and water chemistry (Fig. 5-7b). To the authors' knowledge, this is for the first time ATP levels on bacteria exposed to nFe_2O_3 in the natural water samples were observed (10-10 000 μg/L). The lack of effects observed by ATP measurements, even at 10 mg/L, show that nFe_2O_3 may not pose any undesirable effect on microorganisms in aquatic systems, particularly at current predicted concentrations of 28 ng/L (Wang et al., 2016). The findings from ATP measurements illustrate influence of ENPs type was evident as results in Fig. 5-7b show relative ATP levels due to fate and behaviour of the two ENPs in the two water systems.

5.3.5. ROS production

ROS production is considered an important mechanism in ENP toxicity. In this study, we evaluated intracellular ROS production following exposure to nZnO and nFe_2O_3 (Fig. 5-8). There was no observed increase in intracellular ROS levels on *B. subtilis* compared to the control for both ERW and BRW (Fig 5-8) under visible light conditions. Numerous studies have shown linked observed toxicity from nZnO to oxidative stress due to released Zn^{2+} ions and the particulate (Kumar et al., 2011; Von Moos et al., 2014; Leung et al., 2016).

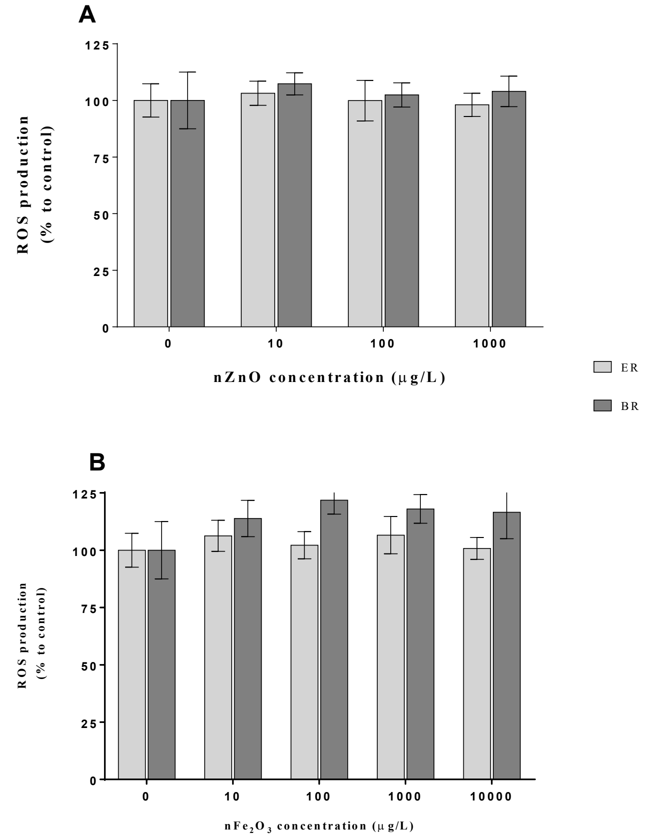

Figure 5-8: Effects of (a) nZnO and (b) nFe_2O_3 on ROS levels in natural water. Data represents the average ± SD (n=3). Asterisks (*) represent significance levels from Tukey's *post hoc* tests in two-way ANOVA (*$p < 0.05$, **$p \leq 0.01$, *≤ 0.001).**

However, oxidative stress from ROS has been found to not contribute to observed toxicity in a number of studies (Dasari et al., 2013; Rago et al., 2017; Kadiyala et al., 2018). For instance, fluorescence levels from ROS were found to have no significant difference to controls for bacteria exposed to 0.1 and 1 mg/L nZnO under both dark and light (sunlight) conditions. Similarly, Rago et al. (2017) showed ROS levels were negligible and count not be linked to the cytotoxic effects observed on bacteria. In the present study, observed effects and membrane damage to the organism due to nZnO exposure in both river water samples could not be accounted by ROS production due to non-significant ROS level (Fig. 5-8). It is important to highlight that the results from this study were obtained under visible conditions, and only results exposure under those conditions.

5.3.6. Mixtures Toxicity

Dose-response effects of nZnO, nFe_2O_3 and TCS on *B. subtilis* were assessed in freshwater, and the results of dose response curves at different concentrations of nZnO, and triclosan are shown below (Fig. 5-9 and 5-10).

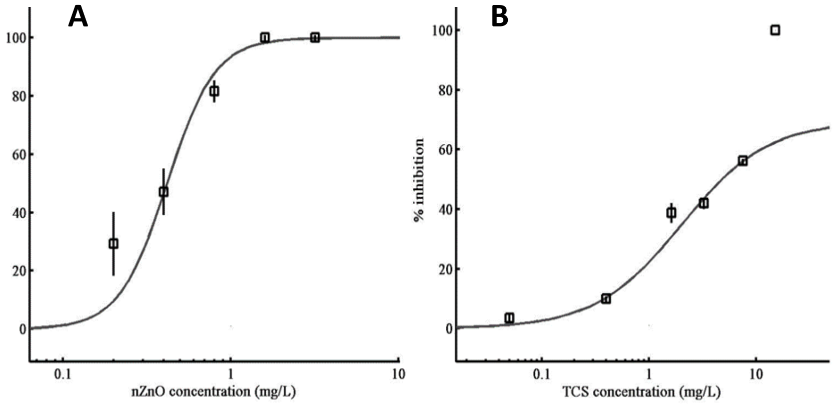

Figure 5-9: The concentration-response relationships of (a) nZnO and (b) TCS to *B. subtilis* in ER water following 2 h exposure under visible light.

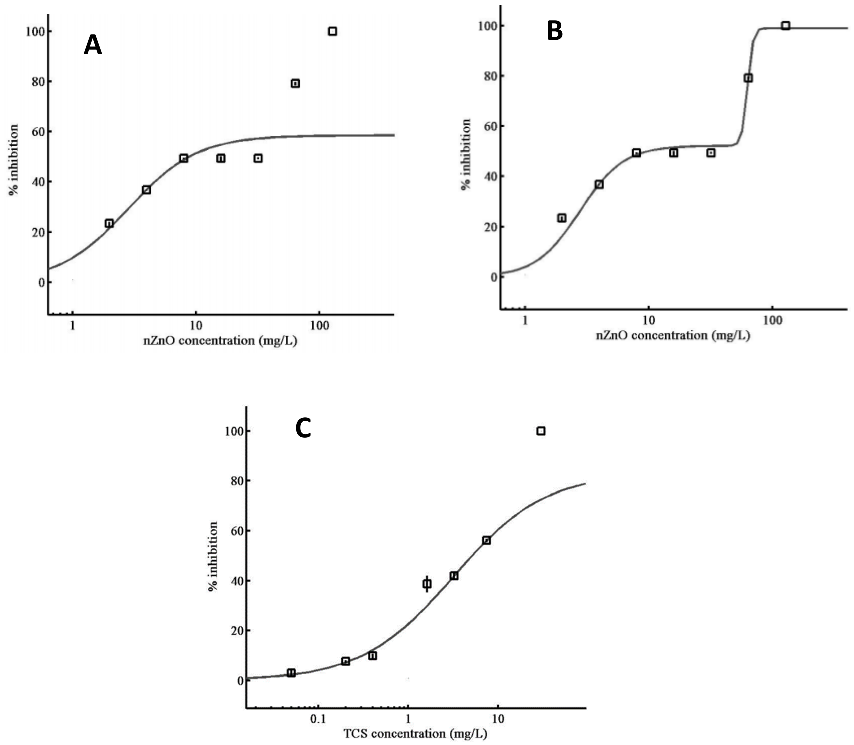

Figure 5-10: Concentration-response relationships of: (a) nZnO using the standard Hill model; (b) nZnO using the biphasic model with points of inflexion; and (c) TCS to *B. subtilis* in BR water after 2 h exposure.

Water chemistry of the two rivers had no influence on the TCS toxicity as both EC_{50} values were closely similar. The EC_x values of nZnO in the ER and BR water were variant, with EC_{50}, the median effective concentration that causes 50% of growth inhibition with respect to a non-treated control, being 0.418 and 8.8 mg/L, respectively (Table 5-3 and 5-4). Low EC_{50} values < 1 mg/L previously have been reported in freshwater for *B. subtilis* (Li et al., 2010). In this study, low EC_{50} value < 1 mg/L for nZnO were observed in ER water; but a high magnitude effect in BR water was observed at 10 mg/L. The differences in nZnO toxicity between the river waters was attributed to transformation processes (aggregation and dissolution behaviour) associated with differences in physicochemical parameters (Table 5-1).

Thwala et al. (2013) reported that test media properties had significant influence on nZnO solubility and toxicity, and in this study, a similar trend was observed. In comparison to nZnO, the EC_x values for TCS were not significantly different in the two river systems where, for example, EC_{50} values were 4.78 and 5.14 mg/L in ER and BR, respectively. These values were several orders of magnitude higher than those reported for bacteria value of 0.25 mg/L (Tatarazako et al., 2004), 0.28 mg/L (La Farre et al., 2008), and 0.73 mg/L (Villa et al., 2014) for *Vibrio fischeri*. EC_{50}. Notably, EC_x values for nFe_2O_3 could not be established at the tested concentrations (≤ 1 000 mg/L), Conversely, Wang et al. (2016) recently obtained EC_{50} value of *ca* 288.87 mg/L on *Vibrio fischeri* using the 15 min bioluminescence assay.

5.3.6.1. Prediction of joint effects of chemical mixtures

Predictive models aid to estimate an EC_x-value based on observed EC_x-values of constituent components in a mixture (Backhaus, 2012; Park et al., 2017). The mixture toxicity of nZnO and TCS in both river waters using CA and IA prediction models were done, and results are summarised in Tables 5-3 (ER) and 5-4 (BR). The CA model predicted lower concentrations compared to the IA model for ER. The CA model is applied where toxicants have similar mechanisms of toxicity on the same target site whereas IA method assumes the individual toxicants in a mixture have different target sites (Heys et al., 2016). The CA and IA model are only used as a general guideline for the assessment of mixture toxicity. These concepts do not consider the test organism's biology, i.e. its catabolic enzymes, uptake routes, and mechanisms (Backhaus & Faust, 2012).

Table 5-3: Single and predicted mixture toxicity EC_{xi} values for TCS and nZnO on *B. Subtilis* in Elands river water.

EC_{mix}	[nZnO] mg/L	[TCS] mg/L	Mixture toxicity (CA)	Mixture toxicity (IA)	Toxic Unit (TU_x)*
10	0.2030	0.3840	0.2935	0.5090	3.4072
20	0.2650	0.8630	0.5640	0.8993	1.7730
30	0.3160	1.5500	0.9330	1.3762	1.0718
40	0.3660	2.6500	1.5080	2.0461	0.6631
50	0.4180	4.7800	2.5990	3.1999	0.3848

* TU < 0.8, synergism; TU >0.8 but <1.2, simple addition; TU > 1.2, antagonism (Broderius et al., 2005; Wang et al., 2014

Table 5-4: Single Toxicity exposures and predicted mixture EC_{xi} values for TCS and nZnO on *B. subtilis* in BR water.

EC_x	[nZnO] mg/l	[TCS] mg/l	Mixture toxicity (CA)	Mixture toxicity (IA)	Toxic Unit(TU_x)*
10	1.02	0.307	0.6635	1.01386	1.5072
20	1.85	0.822	1.336	1.1513	0.7485
30	2.92	1.62	2.27	-0.1904	0.4405
40	4.64	2.9	3.77	-5.916	0.2653
50	8.8	5.14	6.97	-31.292	0.1435

* TU < 0.8, synergism; TU >0.8 but <1.2, simple addition; TU > 1.2, antagonism (Broderius et al., 2005; Wang et al., 2014)

Thus, both CA and IA models could possibly quantify combined effects of the individual toxicants, thus providing for the prediction of the interaction between components in a mixture (e.g. synergism, antagonism, or additive action) (Wang et al., 2014; Park et al., 2017). In this study, TCS/nZnO mixtures were established to be synergistic or simple addition in both river water systems, at their EC_{30} to EC_{50} values based on the estimated TU_x based on the mixture ECx values (Table 5-3 and 5-4). However, for EC_{10} and EC_{20}, the mixtures results revealed antagonistic effects which was at their lower concentrations. The difference in the mixture effects shown by the nZnO/TCS mixtures was likely due to the concentration-combination of the components and the inherent properties of water chemistry on nZnO under the mixture conditions. Further work on the fate and behaviour of the mixtures compared to the individual toxicants is warranted to clear delineate differences of the observed effects. Overall, given the concentrations of nZnO are expected to be low due minimal concentrations incorporated in consumer products, these results may imply their likely effects will be minimal owing to other organic pollutants. Nonetheless, this scenario may change over time as the concentrations of these chemicals increase in the aquatic systems.

5.4. CONCLUSIONS AND RECOMMENDATIONS

The toxicity of nZnO and γ-nFe_2O_3 in natural water samples with varying physicochemical parameters were investigated using a battery of multiple endpoints, including traditional and sublethal endpoints, were used to ascertain the effects of ENMs on *B. subtilis*. In addition, investigations on the toxicity of the ENMs among themselves and TCS as mixtures were also undertaken. Results from the single ENMs exposures revealed diminished cell viability, cell membrane integrity, and ATP production in Elands River compared with Bloubank River water for nZnO exposures, however, γ-nFe_2O_3 induced very low or no cytotoxicity to microorganisms at exposure concentrations using in this work. The toxicity of the ENMs was found to be dependent on the water chemistry properties. Primarily, the ionic strength and NOM in both river systems influenced the aggregation and dissolution of ENMs, (i) either mitigating the toxicity of nZnO in BR and/or γ-nFe_2O_3 in both water samples, (ii) enhancing the toxicity of nZnO in ER water.

ROS production was observed to be negligible for both ENPs; thus ruling out oxidative stress as the cause of observed toxicity. Moreover, no interactions of nZnO and bacteria were observed, suggesting that the toxicity of nZnO were likely driven by release of Zn^{2+}, which in turn, the water chemistry was the key determinant as evidenced by the differences in dissolution between the two river water samples. Use of several endpoints to assess the toxicity of ENPs showed insights on discrete effects even in cases where there are no apparent responses, for example, at whole organism such as cell viability. For instance, although viability results revealed no cytotoxic effects of nZnO, however, cell membrane damage and ATP production demonstrated sub-lethal effects of ENPs on bacteria. The findings indicated a correlation between nZnO released ions and observed effects in the two river water sources. The released ions likely affect metabolic pathways and cell membrane structures that lead to observed outcomes on organisms, however, we cannot rule out the nanoparticulate effects.

Overall, the study findings herein highlighted the complexity and variations in natural water chemistry, and the reason why they should be considered when establishing the toxicity of ENPs to bacteria. The aggregation effects on nFe_2O_3 reduced its toxicity on *B. subtilis*, whereas toxicity was more than additive for TCS and nZnO in freshwater systems, at concentrations above their individual EC_{20}, while they were antagonistic at concentration below the EC_{20} value. These values were based on the individual dose-response values of the toxicants. Although our TCS results were lower than reported results in literature, the findings herein are from natural aqueous media and were conducted in controls conditions. Factors such as light which transforms TCS, should are to be taken in consideration for future work. A comparison of the calculated and experimental results of the toxicant mixtures, as well as the behaviour and toxicity mechanisms are still warranted to fully understand interactions of pollutants in environmentally relevant media.

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CHAPTER 6 : AGGREGATION AND DISSOLUTION OF SINGLES AND BINARY MIXTURES OF METAL OXIDE NANOPARTICLES AND TRICLOSAN IN FRESHWATER SYSTEMS

Astone Nanja, Ndeke Musee

6.1. INTRODUCTION

In recent years, unregulated contaminants commonly referred to as emerging contaminants (ECs) have attracted increasing attention with respect to environmental safety as they are generically outside the provisions of existing legislative framework (Petrović et al., 2003). ECs are incorporated in various products for industrial and domestic use such as pharmaceuticals, personal care products (PCPs) and industrial additives (Yan et al., 2009; Musee, 2011; Matsoukas et al., 2015; Musee, 2018). Among the ECs of concern are engineered nanoparticles (ENPs) and organic pollutants such as triclosan (TCS). For example, today there is wide commercial production and use of ENPs in consumer and industrial applications (Roco, 2011; Piccinno et al., 2012). As a result, ENPs presence in the environmental systems has increased as attested by both modelling and field measurements (Musee, 2011; Muller and Nowack, 2008; Bhuvaneshwari et al., 2016; Zakaria et al., 2018). For instance, metal- and metal-oxide based ENPs render them of urgent concern because of their wide spread use in various commercial and domestic products (Wong and Kurma, 2008; Lewis et al., 2010; Das et al., 2013; Le Van et al., 2016). In addition, once in the environment, ENPs undergo physical and chemical transformations as well as interactions with macromolecules through processes such as aggregation, dissolution, dispersion, adsorption, complexation to mention a few that may influence their fate and behaviour (Levard et al., 2012; Lowry et al., 2012, Thwala et al., 2016; Wang et al., 2016). To date, numerous studies have investigated the fate and behaviour of ENPs in different exposure aqueous media systems (Romanello et al., 2013; Musee et al., 2014; Wang et al., 2016); with key focus on single ENPs.

Aluminium oxide nanoparticles (nAl_2O_3) and copper oxide nanoparticles (nCuO) are among metal based ENPs that have attracted attention because of their widespread applications. nAl_2O_3 are widely used for commercial products such as high-performance ceramics, cosmetic fillers, packing materials, polishing materials, semiconductor materials, paints, composite materials and resins, wear-resistant reinforcement and advanced waterproof materials, catalyst, and catalyst carriers (Defriend et al., 2003; De Luca et al., 2005; Khanna, 2008; Landry et al., 2008; Wong and Kurma, 2008; Lewis et al., 2010). nCuO are applied in a lot of areas which includes semiconductors, cosmetics, textiles, catalysts, e.g. in rocket propellant

(Applerot et al., 2012; Das et al., 2013; Zhao et al., 2013; Le Van et al., 2016). Cosmetics, paints, catalysts, catalyst carriers, and textiles among others will readily release ENPs into different environmental compartments. Once In the environment, ENPs interact with other contaminants including organic contaminants such as TCS due to similar areas of application such as in PCPs.

However, all these compounds have been classified as emerging contaminates (ECs). For example, nCuO have been shown to have negative effects on the survival and growth of organisms (Zietz et al., 2003; Nations et al., 2011) while nAl_2O_3 have been reported to be toxic towards bacteria, algae, nematodes and other species in the environment (Wang et al., 2009; Ji et al., 2011). TCS is known to be toxic to a range of aquatic organisms such as Daphnia magna and fish (Orvos et al., 2002) and that it may cause adverse environmental effects (Brausch and Rand, 2011; Gardner et al., 2012).

Moreover TCS is widely used as antimicrobial compound that is found in a variety of consumer products such as soaps, deodorants, skin creams, toothpastes, acne creams, fabrics, and plastics and is often employed together with ENPs in the same products such as PCPs (Daughton and Ternes, 1999), and more details on uses and implications are provided in Chapter 3 of this volume, and therefore, will not be repeated here.

In the environmental systems, ENPs co-exist as mixtures either with other ENPs or other classes of pollutants ubiquitous in the environment. Hence, results for studies investigating the effects and fate of individual pollutants do not reflect the likely actual fate and toxicity of their mixtures in the environmental systems. For example, to date knowledge on the effects and fate of ENPs as mixtures among the same classes as well as with other classes of pollutants remain poorly accounted for (Tong et al., 2014; Iswarya et al., 2015; Chen et al., 2018). This is because fate results for individual contaminants have been shown to be limited to predict that of mixtures (Heys et al., 2016). This implies that mixtures would likely exhibit different fate and behaviour and as well as characteristic toxicity potential to organisms as opposed to that of constituent chemical components. Numerous toxicological studies have indicated that contaminant mixtures including organic-organic mixtures (Laetz et al., 2008), metal-metal mixtures or rather inorganic-inorganic mixtures (Preston et al., 2000; Chu and Chow, 2002) and organic-inorganic mixtures (Wang et al., 2009), exert toxic effects that differ markedly from those of constituent contaminants. Even though the toxic effects of individual contaminants may be well established, the environmental risks posed by their mixtures may not be predicted based on this knowledge.

Moreover, bioavailability of toxicants depends on their fate in aquatic systems; which in turn influences their toxicity potential within a given environment of focus. To date, few studies have reported on the interactions of ENPs mixtures in freshwater systems (Tong et al., 2014; Iswarya et al., 2015; Wilke et al., 2016). Tong et al. (2014) investigated the interactions of nZnO and $nTiO_2$ in natural aqueous medium and observed the adsorption of Zn^{2+} from nZnO on $nTiO_2$. In addition, the mixture aggregates were larger than those of individual ENPs. Iswarya et al. (2015) investigated the interactions between anatase and rutile crystalline phases of titania in freshwater medium under UV irradiation; where heteroaggregation was observed in the mixture. Wilke et al. (2016) examined the interactions between nAg and $nTiO_2$, where adsorption of Ag^+

onto the surface of $nTiO_2$ was evident. Thus, from these studies, it is likely the co-existence of ENPs in the freshwater may alter fundamental fate of individual ENPs as available data suggests. To the best of our knowledge, there are no reports on fate and behaviour of binary mixtures of nAl_2O_3, nCuO and TCS in freshwater systems. Hence, herein the aim of the current study was to investigate the fate and behaviour of single ENPs and the interactions between binary mixtures in freshwater systems at low environmentally relevant concentrations. The results reported herein are envisaged to contribute towards increasing our collective understanding on the stability of mixtures of ENPs and organic pollutants with specific reference to natural freshwater systems.

6.2. MATERIALS AND METHODS

6.2.1. Materials and characterization

6.2.1.1. Characterisation of ENPs

Copper oxide (nanopowder, <50 nm, CAS No 1317-38-00; Aluminium oxide nanoparticles (30-60 nm, 20 wt% in water, CAS No 1344-28-10); Triclosan (CAS No 3380-34-5) and molar mass 289.54 g/mol were all purchased from Sigma-Aldrich (Johannesburg, South Africa). All materials were used as received from the supplier. The ENPs were characterised using transmission electron microscopy (TEM) (JEM 2010F, JEOL Ltd., Japan) for size and morphology. Malvern Zetasizer Nano (Model ZEN 3600; made in UK) was used to track changes in hydrodynamic diameter (HDD) and Zeta potential (ζ-potential) in freshwater media.

6.2.1.2. Water sampling and preparation

Freshwater samples were collected in May 2018 from two river systems Bloubank River (BR) (26°01'20.3"S 27°26'31.6"E) in North West Province and Elands River (ER) (25°32'58.4"S 28°33'53.4"E) in Gauteng Province of South Africa. The samples collected from ER had a temperature of 22 ℃ and pH 8.1 where those from BR were at temperature of 18 ℃ and pH 7.9. All samples were filtered through a 0.2 μm pore size standard filter (Millipore) and stored in the fridge at 4 ℃ prior to use. The water samples were sent for characterisation at a certified laboratory and the results are presented in Table 6-1.

6.2.1.3. Interactions of ENPs in mixtures

Stock suspensions of nAl_2O_3 or nCuO were prepared to achieve a concentration of 10 mg/L. The suspensions were sonicated for 25 min at 25 ℃ to ensure homogeneity. Further dilutions were done to obtain lower concentrations of 1 and 0.1 mg/L nAl_2O_3 or nCuO. Stock concentrations of 100 and 1000 ng/L TCS were also prepared. Binary mixtures of ENPs were investigated at ratios of 10:1 (1, 0.1 mg/); 1:1 (1, 1 mg/L), and 1:10 (1, 10 mg/L) where the values in the parenthesises are concentrations of nAl_2O_3 and nCuO, respectively, in a given ratio. Whereas binary mixtures of ENPs and TCS were investigated at ratios of (1000 : 1); (100 : 1); (10 000 : 1) and (1000 : 1) at concentrations of (0.1 mg/L ENPs, 100 ng/L TCS); (0.1 mg/L ENPs, 1000 ng/L TCS); (1 mg/L ENPs, 100 ng/L TCS) and (1 mg/L ENPs, 1000 ng/L TCS), respectively. The freshwater samples from BR and ER were used as exposure media. Samples were loaded into cuvettes to measure HDD and ζ-potential. All measurements were done in triplicates over 48 h.

Table 6-1: Characterisation of freshwater used as media sourced from two river systems

Parameter	Unit	Elands river	Bloubank river
pH		8.1	7.9
IS	mM	2.45	4.94
K^+	mg/L	4.24	3.13
Na^+	mg/L	15.6	22.4
Ca^{2+}	mg/L	14.0	36.0
Cl^{-1}	mg/L	17.1	12.9
SO_4^{2-}	mg/L	9.03	6.77
Mg^{2+}	mg /L	9.82	31.0
NO_3^-	mg/L	0.33	0.20
PO_4^{3-}	mg/L	0.57	1.23
NH_4^+	mg/L	4.27	3.40
Cu_{tot}	mg/L	<0.002	<0.002
Al_{tot}	mg/L	<0.002	<0.002
Fe_{tot}	mg/L	<0.004	<0.004
Zn_{tot}	mg/L	0.008	0.010
DOC	mg /L	5.51	8.25
Alkalinity	mg $CaCO_3$/L	75.6	217
Electrical conductivity (EC) @ 25℃	mS/m	19.6	39.8

2. DOC – dissolved organic carbon; Al_{tot} – aluminium; Fe_{tot} – total iron; Zn_{tot} – total zinc; Cu_{tot} – total copper

6.3. RESULTS AND DISCUSSION

6.3.1. Characterisation of nanoparticles

ENPs were characterised using TEM to obtain their size and morphology properties. TEM images of nAl_2O_3 and nCuO presented in Fig. 6-2 showed nAl_2O_3 had spherical and hexagonal shapes and an average size range of 30-60 nm. These size values were in agreement with those provided by the manufacturer. nCuO were a mixture of hexagonal, rods and spheres with an average size distribution of < 50 nm as specified by the manufacturer.

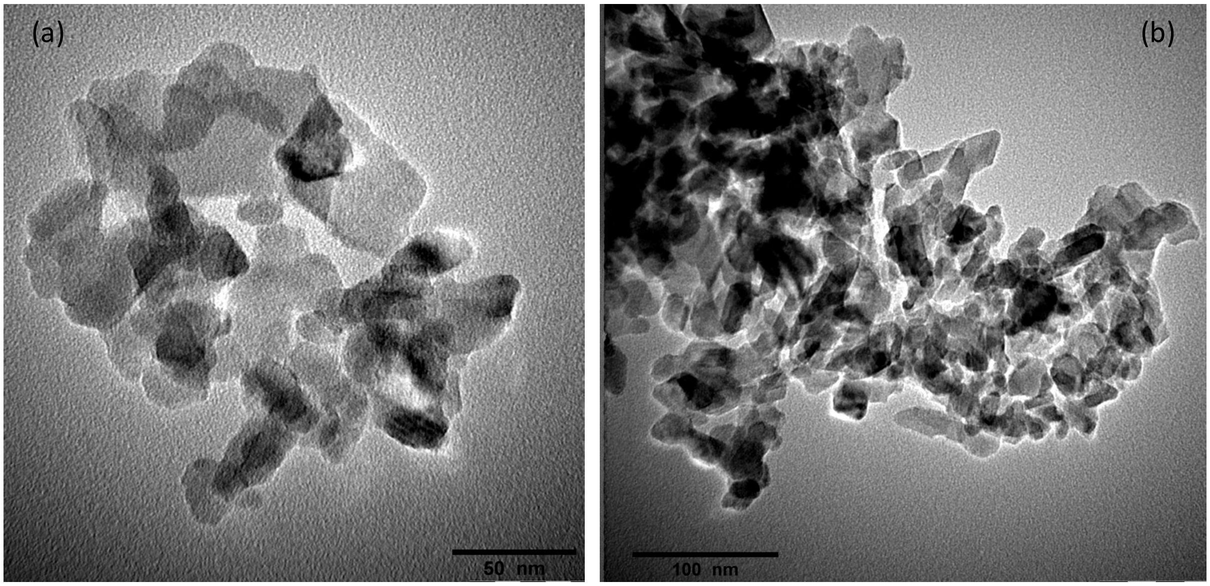

Figure 6-1: TEM images of pristine nCuO (a) and nAl_2O_3 (b) in deionised water.

6.3.2. Aggregation of individual ENPs in freshwater

In both river systems, the HDD of nCuO over 48 h was in the range of 200 to 300 nm, 300 to 400 nm, and 400 to 600 nm, for 0.1, 1 and 10 mg/L, respectively (Fig. 6-2b and d). nAl_2O_3 were in the range, 300 to 400 nm, 300 to 500 nm and 500 to 900 nm, for 0.1, 1 and 10 mg/L, respectively (Fig. 6-3b and d). These results indicate that aggregation of ENPs were concentration dependent and increased as the concentration increased (with 10 mg/L having the highest HDD, and least at 0.1 mg/L). Overall, the low HDD observed in this work may be associated with adsorption of NOM onto the ENPs, which in turn, induced a net negative surface charge thus increasing interparticle repulsions leading to stabilization of ENPs via electrostatic and/or steric repulsion mechanisms (Omar et al., 2013; Wang et al., 2014).

Although ionic strength is known to promote aggregation, however, in this case may have been outweighed by the stabilization effect of NOM. Similar observations have been reported elsewhere (Romanello and de Cortalezzi., 2013) where results indicated that the aggregation of titanium oxide nanoparticles ($nTiO_2$) in the presence of humic acid (HA) – a surrogate of NOM at various concentrations of monovalent (Na^+) and divalent (Mg^{2+} or Ca^{2+}) electrolytes using ultra-pure type 1 water (resistivity 18 MΩ) as exposure media – observed the stabilization effect of NOM. As such, the NOM effect counteracted the destabilization effect of both mono and divalent cations at concentrations lower than 1.5 mM. However, above 1.5 mM, Ca^{2+} promoted aggregation due to presence of specific NOM and Ca^{2+} interactions leading to formation of NOM–Ca^{2+} complexes as a result of calcium bridges. Thus, in our case due to likely large NOMs found in the environment as opposed to the simple HA and pure water system, may account for the lower aggregation observed.

No statistically significant differences were observed for HDD over 48 h. However, there was rapid aggregation and disaggregation over the first 6 h. ζ-potential increased with increasing concentration of ENPs from 0.1 to 10 mg/L as shown in Figs. 6-2a and c, and Figs. 6-3a and c. Hence, based on the current results on stability of ENPs at the lowest concentration of 0.1 mg/L, ENPs exhibit higher residence time in the water column and may cause adverse effects to water dwelling organisms such as Daphnia magna and fish.

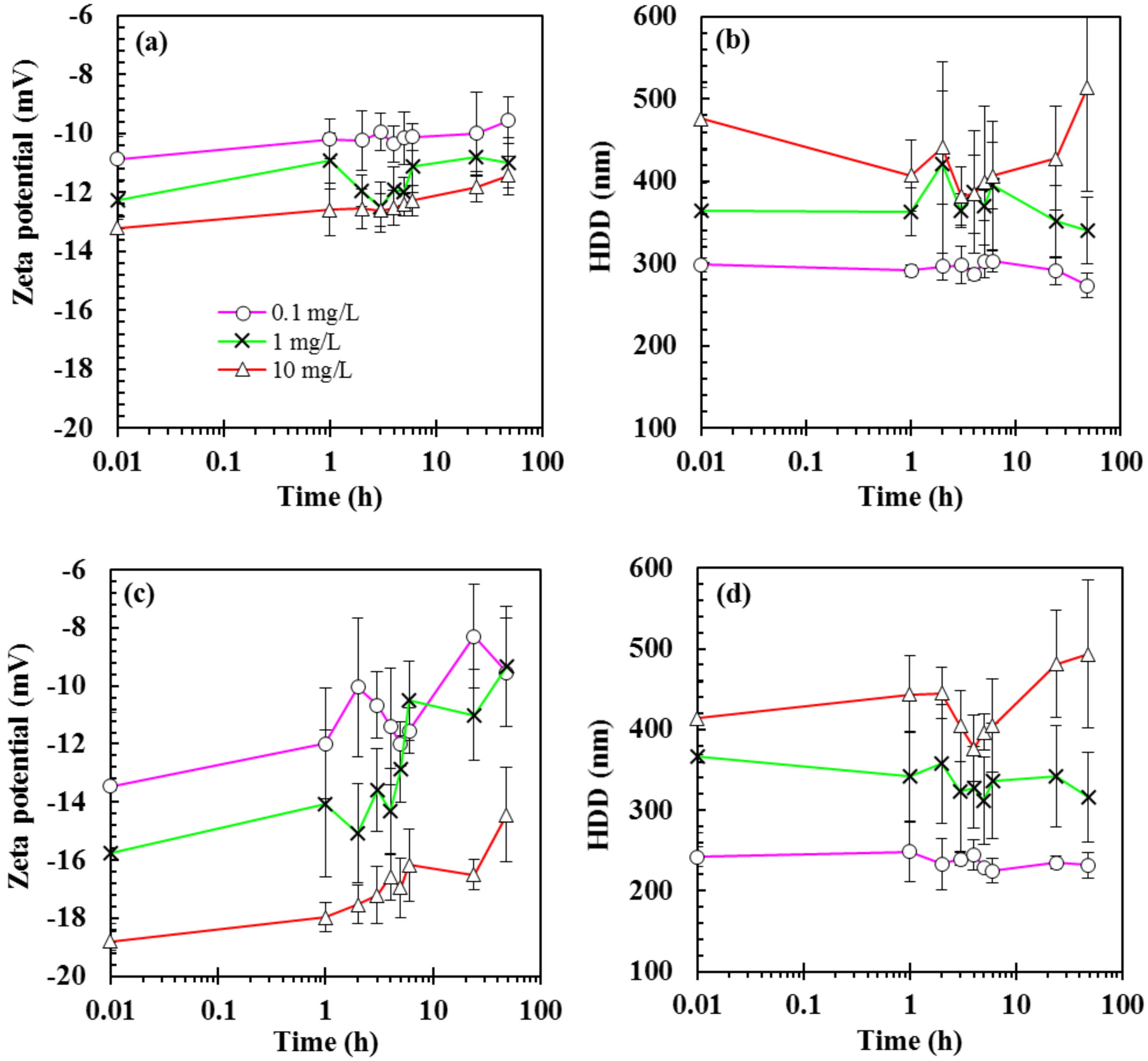

Figure 6-2: nCuO ζ-potential (a) and HDD (b) in BR and ζ-potential (c) and HDD (d) in ER over 48 h. The error bars represent standard deviation of three replicates.

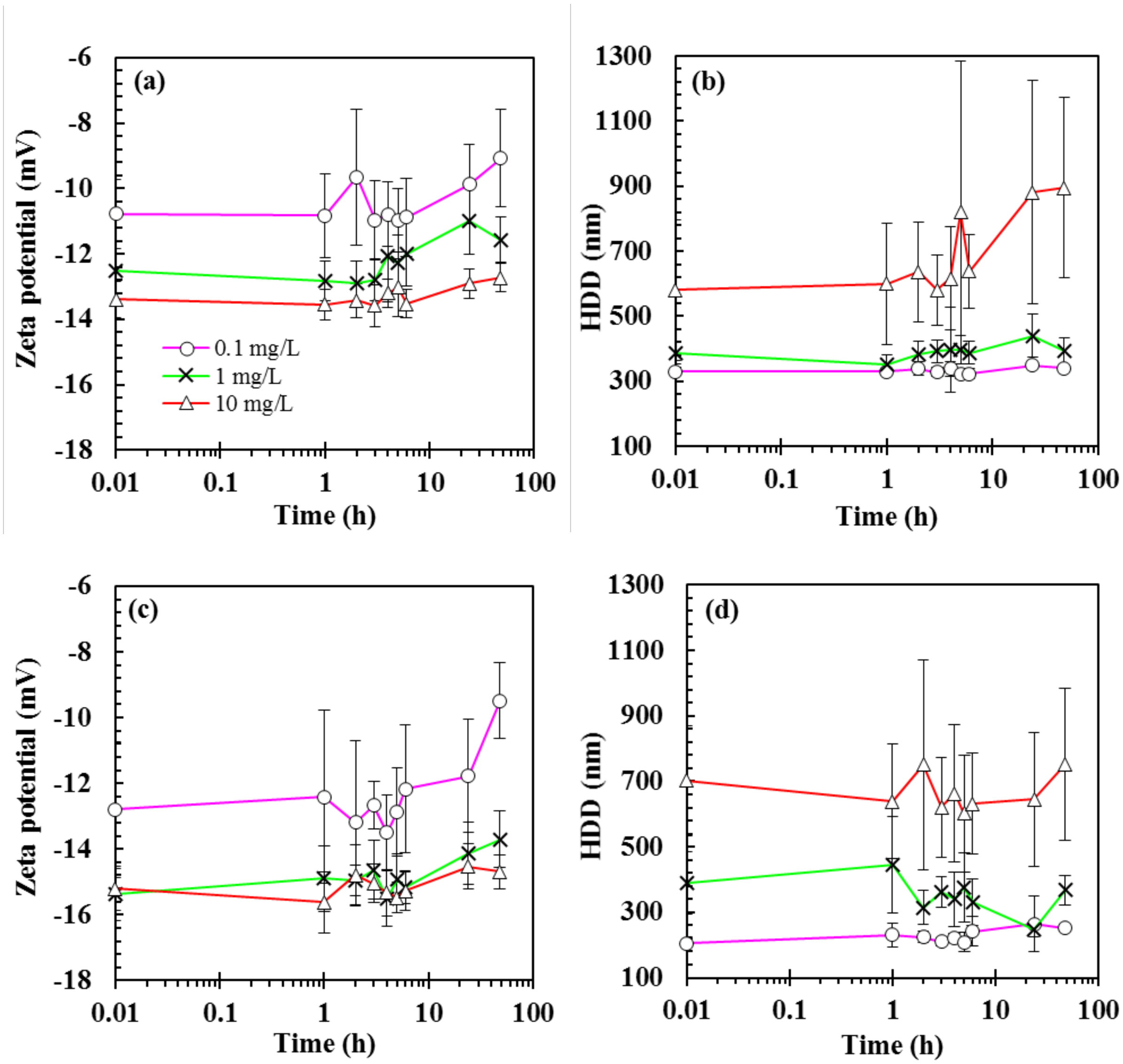

Figure 6-3: nAl_2O_3 ζ-potential (a) and HDD (b) in BR and ζ-potential (c) and HDD (d) in ER over 48 h. The error bars represent standard deviation of three replicate

6.3.3. Dissolution of ENPs in freshwater

6.3.3.1. Dissolution of ENPs of nAl_2O_3 in freshwater

Following the introduction of nAl_2O_3 to freshwater, very high dissolution (almost 100%) was observed within the first 2 h at lower concentration of 0.1 mg/L in ER. In BR however, at the same concentration dissolution increased from *ca* 50% after 2 h to *ca* 70% at 48 h see Fig. 6-4a. The results of high dissolution in ER may account for the low HDD observed in ER (≤ 200 nm) at 0.1 mg/L compared to results of BR (≤ 300 nm) as shown in Figs. 6-3. b and d, respectively. As the ENPs concentration increased from 0.1 to 1 mg/L dissolution was found to decrease. In ER dissolution was *ca* 10% after 2 h and increased slightly to *ca* 16%

after 48 h. Conversely, in BR it was *ca* 2% after 2 h and increased to only *ca* 4% after 48 h as seen in Fig. 6-4b. The difference in dissolution was considered to be linked to differences in water chemistry between the two rivers. Based on these findings, at low concentrations of ENPs in the environment, organisms are likely to be exposed to ionic species rather than their particulates; and this will largely depend on differences in water chemistry.

The dissolution observed in this study at 1 mg/L of nAl_2O_3 was less compared to earlier findings of Bhuvaneshwari et al. (2016) over 24 h in lake water. The low dissolution at 1 mg/L especially in BR was likely due to higher concentration of NOM as it has been reported to impend dissolution even in the case of highly soluble ENPs such as zinc oxide nanoparticles (nZnO) (Miao et al., 2010).The adsorption of NOM onto ENPs provides a coating impending dissolution (Ghosh et al., 2008; Pakrashi et al., 2012). In addition, the released ions of Al^{3+} may undergo chelation with components of the fresh water such as algal making them less available for detection (Colin et al., 1998).

6.3.3.2. Dissolution of ENPs of nCuO in freshwater

In systems where NOM is present such as freshwater, runoff and waste water systems, the dissolution of nCuO is impeded due to adsorption of NOM on ENPs surface owing to the formation of a coating (Conway et al., 2015; Miao et al., 2016). In the two freshwater systems (ER and BR) investigated, dissolution of nCuO was observed to be low. Dissolution was higher at lower concentration of 0.1 mg/L than at higher concentration of 1 mg/L as seen in results summarised in Figs. 6-4c and d, respectively. At 0.1 mg/L nCuO, no apparent change was observed in dissolution from 2 to 48 h. However, at 1 mg/L dissolution increased from 2 to 48 h. In both freshwater systems, the detected concentration of Cu^{2+} were $\leq$ 1% of the exposure concentrations (0.1 and 1 mg/L). The lower concentrations of Cu^{2+} detected could also be due to chelation effect with other components present in freshwater, e.g. sulphates, phosphates and NOM (Adeleye et al., 2014; Conway et al., 2015). For instance, copper has a high affinity for sulphur atoms (Lowry et al., 2012), and tend to undergo disproportionation reactions with the production of Cu^0, Cu^{1+} and Cu^{2+} which are necessary precursors for the formation of precipitation complexes such as Cu_2S (Conway et al., 2015). Copper is also known to precipitate as a hydroxide in the pH range 6.6 to 7.8 with ligands present in freshwater (Dimpka et al., 2015) – a value close to the pH of freshwaters (7.9 and 8.1) investigated in this study. Dissolution of nCuO decreases with increasing pH such that at pH >7.7 it is very low (less than 3%) (Odzak et al., 2014). The pH of both freshwater samples was >7.7, hence the low dissolutions may also be attributed to the high pH. As such, the low dissolution of nCuO in freshwater systems implies their particulates may be more likely to interact with aquatic organisms as opposed to their ionic forms.

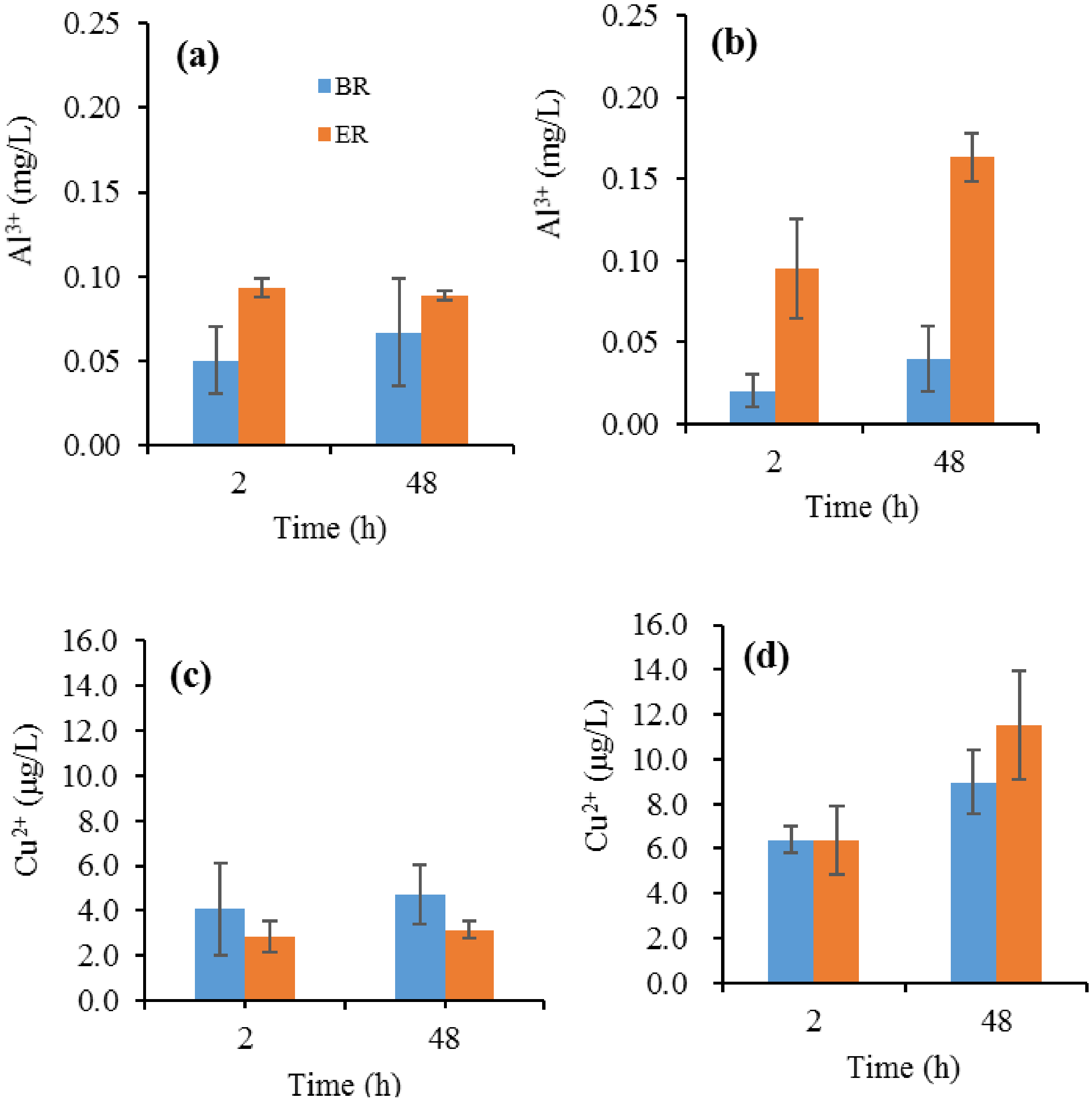

Figure 6-4: Dissolution in BR and ER after 2 and 48 h for Al^{3+} from nAl_2O_3 at 0.1 mg/L (a) and 1 mg/L (b); and Cu^{2+} from nCuO at 0.1 mg/L (c) and 1 mg/L (d). The error bars represent standard deviation of three replicates.

6.3.4. Aggregation of binary ENPs mixtures in freshwater

6.3.4.1. Influence of nCuO on aggregation of nAl_2O_3

The influence of concentration of nCuO at fixed concentration of nAl_2O_3 in both freshwater samples were investigated. In BR water, no apparent changes at variant concentrations of nCuO to 1 mg/L of nAl_2O_3 were observed on ζ-potential (Fig. 6-5a), but HDD decreased as the nCuO concentration increased as depicted in Fig. 6-5b. Further, the HDD under any mixture ratio considered (Fig. 6-5b) was found to be higher than observed at 1 mg/L of nAl_2O_3 alone, or at various concentrations of nCuO (0.1, 1 and 10 mg/L) in BR (Fig. 6-2b). For the mixture ratio of 10:1 for nAl_2O_3 (1 mg/L) and nCuO (0.1 mg/L), HDD ranged from 900 to 1

200 nm whereas for the ratio of 1:10 of nAl_2O_3 (1 mg/L) and nCuO (10 mg/L), were lower (600 to 900 nm). Hence, larger HDD were observed in mixtures when compared to results of individual ENPs at corresponding concentrations. The aggregation of nAl_2O_3 increased with decreasing amount of nCuO in BR (Fig. 6-5b) whereas no apparent difference was observed in ER (Fig.6-5d). Therefore, individual ENPs maybe more stable than mixtures in freshwater matrix depending on exposure media chemistry characteristics. The lower HDD for mixtures in ER at fixed nAl_2O_3 as nCuO concentration increased was plausibly due to the higher dissolution of ENPs in ER compared to BR as seen in Fig. 6-4.

Since BR had a pH 7.9, and both ENPs were negatively charged with ζ-potential values of -9 to -11 mV for 0.1 mg/L nCuO (Fig. 6-2a), and -10 to -13 mV for 1 mg/L nAl_2O_3 (Fig. 6-3a); Despite the negative ζ-potential on both ENPs, higher HDD was observed from the mixture than from the individual ENPs. Higher HDD may be accounted by higher collision frequency per unit volume in the nAl_2O_3/nCuO mixture where heteroaggregation was more thermodynamically favoured. Hence resulting in formation of larger aggregates. Similar observations on higher HDD in mixtures compared to individual ENPs have been reported elsewhere (Tong et al., 2014: Iswarya et al., 2015). Tong et al. (2014), for example, studied the interactions of nZnO and $nTiO_2$ in Lake Michigan water (LMW) as exposure media where the results showed $nTiO_2$/nZnO mixtures were larger than those of the individual component ENPs. For ER, no significant differences were observed for both ζ-potential and HDD at all mixture combination ratios (Figs. 6-5. c and d). However, the reasons for this observation remains unclear and warrants further investigations.

Overall, for nCuO in both river systems, ζ-potential and HDD increased with increasing concentration as shown in Fig. 2; however, an opposite effect was observed in BR for the mixtures as evidenced by a reduction in HDD at higher concentration of nCuO (Fig. 6-4b). But, no changes in HDD were observed by increasing concentration of nCuO to the mixture in ER (Fig. 6-5d). The reason for this trend remains unclear as well as its implications to the environment, and thus, calls for further interrogation.

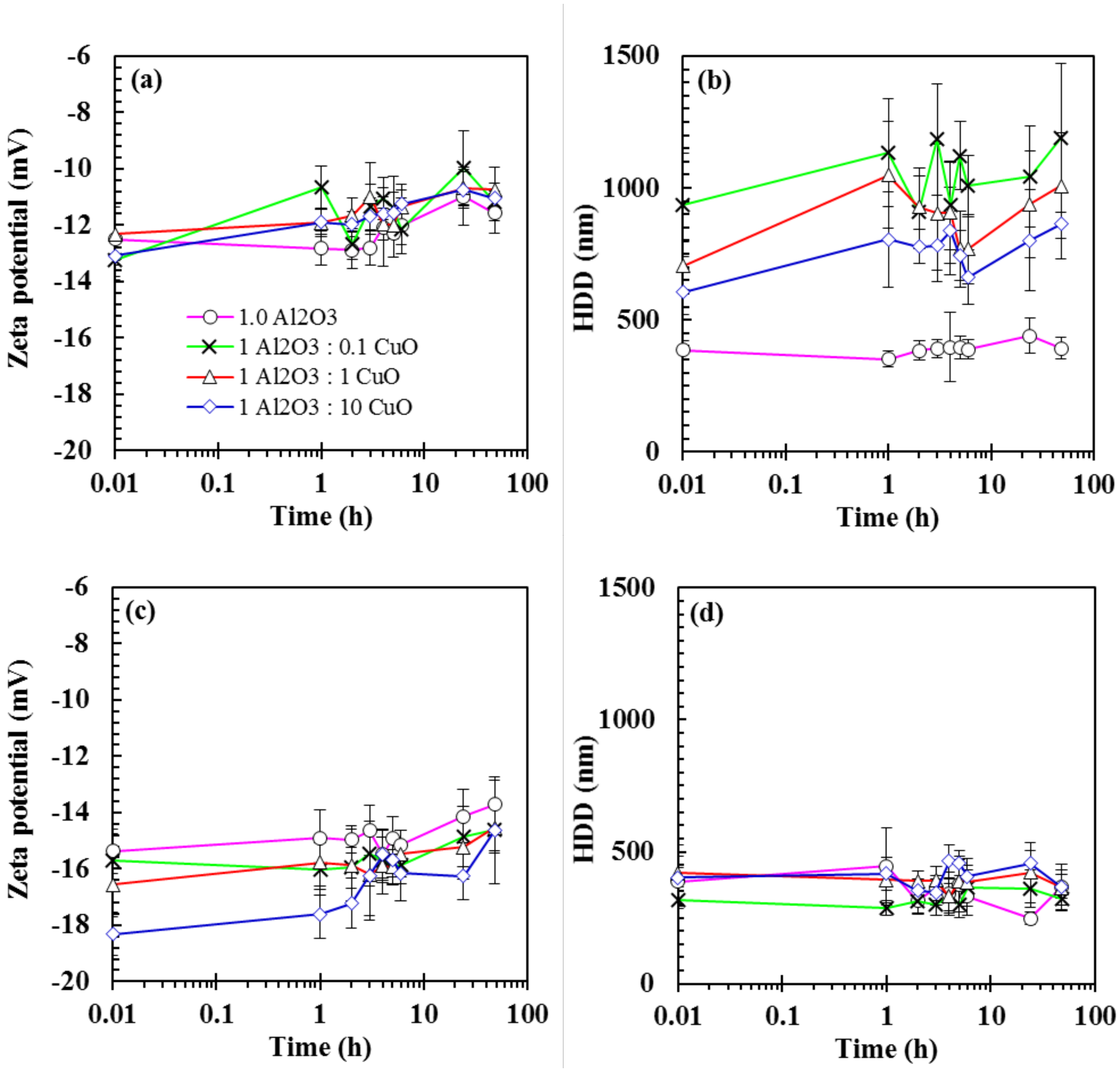

Figure 6-5: nAl_2O_3 and nCuO: ζ-potential (a) and HDD (b) in BR; ζ-potential (c) and HDD (d) in ER over 48 h. The error bars represent standard deviation of three replicates.

6.3.4.2. Influence of nAl_2O_3 on aggregation of nCuO

In BR, at fixed concentration of 1 mg/L nCuO, higher HDD was observed from mixtures compared to that from individual ENPs and it increased as the concentration of nAl_2O_3 increased in the mixture (Fig. 6-5b). For example, HDD for mixture ratio of 10:1 for nCuO (1 mg/L) to nAl_2O_3 (0.1 mg/L), HDD ranged from 600 to 900 nm whereas for the ratio of 1:1 of nCuO (1 mg/L) to nAl_2O_3 (1 mg/L) ranged from 700 to 1100 nm HDD. In light of these results, nCuO was considered to be stabilised by lower concentrations of nAl_2O_3. In ER, for the mixture ratio of 10:1 for nCuO (1 mg/L) to nAl_2O_3 (0.1 mg/L), HDD decreased from 783 ± 65nm at 0 h to 619 ± 92 nm after 48 h most likely due to increase in dissolution over time (Fig. 6-4d). However, for 1:1 of nCuO (1 mg/L) to nAl_2O_3 (1 mg/L) and 1:10 mg/L of nCuO (1 mg/L) to nAl_2O_3 (10 mg/L) HDD increased with 1:10 of nCuO (1 mg/L) to nAl_2O_3 (10 mg/L) reaching a maximum of 1 682 ± 322nm at 48 h

(Fig. 6-6b). In ER, HDD results for the mixture were comparable to those of nCuO except in the case of 1:1 mixture where higher HDD was observed in comparison to that of nCuO at 1 mg/L (Fig. 6-6d); but less than observed at 10 mg/L nAl_2O_3 (Fig. 6-3d). There was no significant difference in ζ-potential as the concentration of nAl_2O_3 increased at fixed 1 mg/L of nCuO in both river waters. Thus, our findings suggest that the fate of ENPs mixtures in a given exposure media (particularly freshwater environments) are distinctive from those of individual ENPs. ENPs mixtures may agglomerate and sediment in the aquatic system and interact with benthic organism or maybe stable and interact with organisms within the water column as influenced by the exposure media chemistry as the results in this study illustrates.

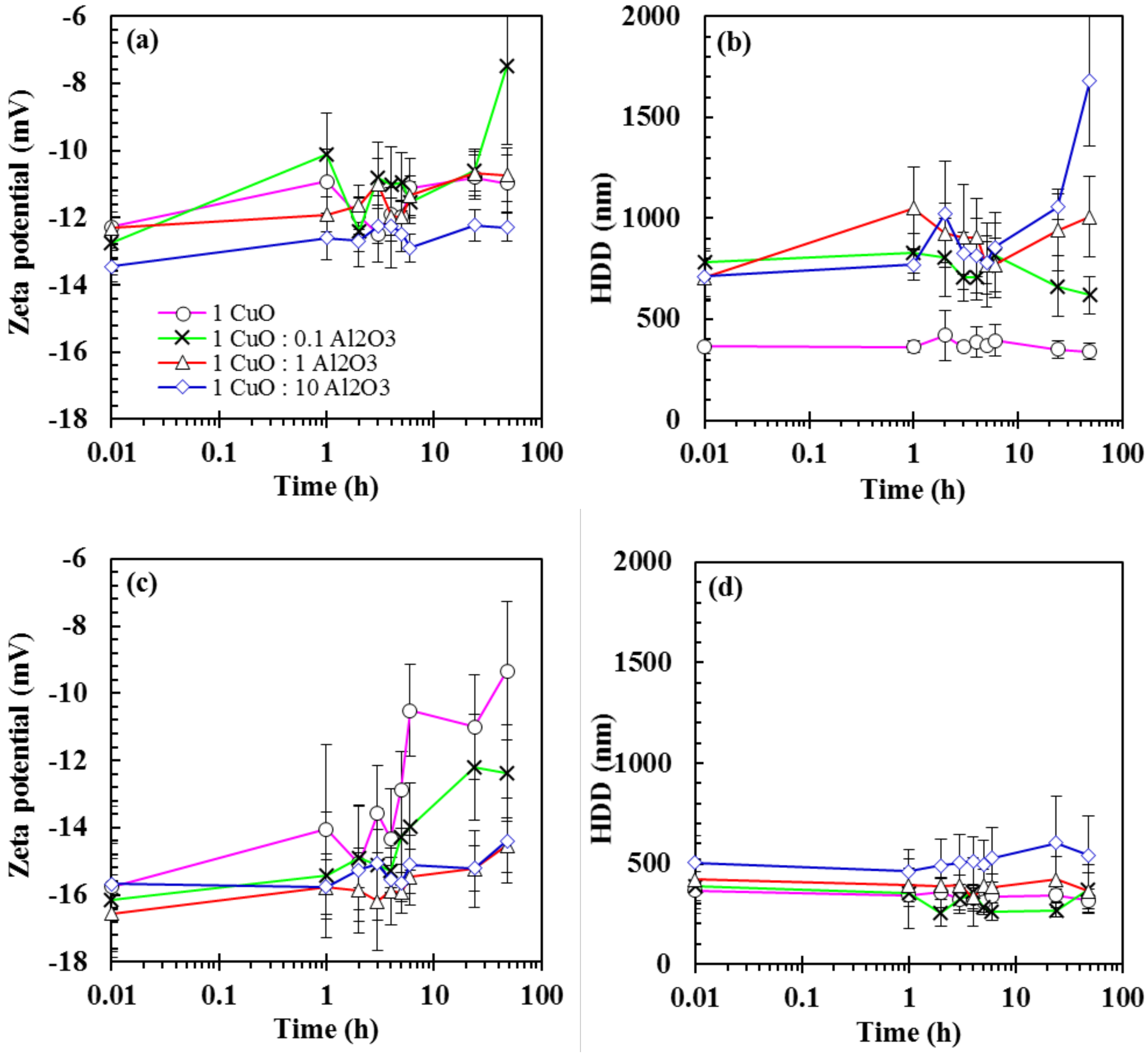

Figure 6-6: nCuO and nAl_2O_3 mixtures: ζ-potential (a) and HDD (b) in BR; ζ-potential (c) and HDD (d) in ER over 48 h. The error bars represent standard deviation of three replicates.

6.3.5. Aggregation of binary mixtures of ENPs and TCS in freshwater systems

6.3.5.1. Aggregation of nAl_2O_3 in freshwater in presence of TCS

Results of HDD for 0.1 mg/L nAl_2O_3 ranged from 300 to 350 nm in BR whereas in ER, a lower range of 200 to 265 nm was observed (Figs. 6-7. b and d). Increasing the concentration to 1 mg/L nAl_2O_3, HDD ranged from, 350 to 450 nm in BR whereas in ER it was in the range 250 to 450 nm (Figs. 6-8. b and d). In BR, following the introduction of TCS to 0.1 mg/L nAl_2O_3, HDD ranged from 300 to 440 nm and 400 to 570 nm at 100 and 1000 ng/L TCS, respectively (Fig. 6-7b). The ζ-potential varied within a narrow range of -9 to -14 mV without any statistically significant difference as seen in Fig. 6-7a. Increasing the concentration of nAl_2O_3 to 1 mg/L in the presence of TCS, HDD varied within the same range comparable to nAl_2O_3 alone (Fig. 6-8b). The ζ-potential varied within a narrow range -12 to -17 mV without any statistically significant difference (Fig. 6-8a). According to the results, at lower concentration, nAl_2O_3 was destabilised with increasing concentration of TCS whereas at higher concentration no significant effect of TCS was observed on stability of nAl_2O_3 despite a slight increase in ζ-potential. In ER, at 0.1 mg/L nAl_2O_3, HDD ranged from 170 to 190 nm and 175 to 180 nm at 100 and 1000 ng/L TCS, respectively (Fig. 6-7d). At 1 mg/L nAl_2O_3, HDD increased slightly within the range 180 to 210 nm and 190 to 210 nm at 100 and 1000 ng/L TCS, respectively (Fig. 6-8d). ζ-potential varied within a narrow range -14 to -18 mV with a slight increase in the presence of TCS but no statistically significant difference (Fig. 6-8c). ENPs were stabilised by TCS and the stabilization was independent of the concentration of both nAl_2O_3 and TCS as shown in Fig. 6-7d and Fig. 6-8d. Altogether, these results point to either stabilization or destabilization of ENPs by organic pollutants, and the extent of these effects are dependent on the water chemistry of the exposure media. In ER, for example, nAl_2O_3 was stabilised by TCS and the degree of stabilization was not dependent on concentration of TCS as shown in Fig. 6-7d, and similar trend was observed for destabilization effect in Fig. 6-7b. The stabilization of ENPs in ER was most likely due to adsorption of TCS onto ENPs as evidenced by the slight increase in ζ-potential. However, the reasons for the destabilisation of ENPs at lower concentration by TCS in BR remain unclear.

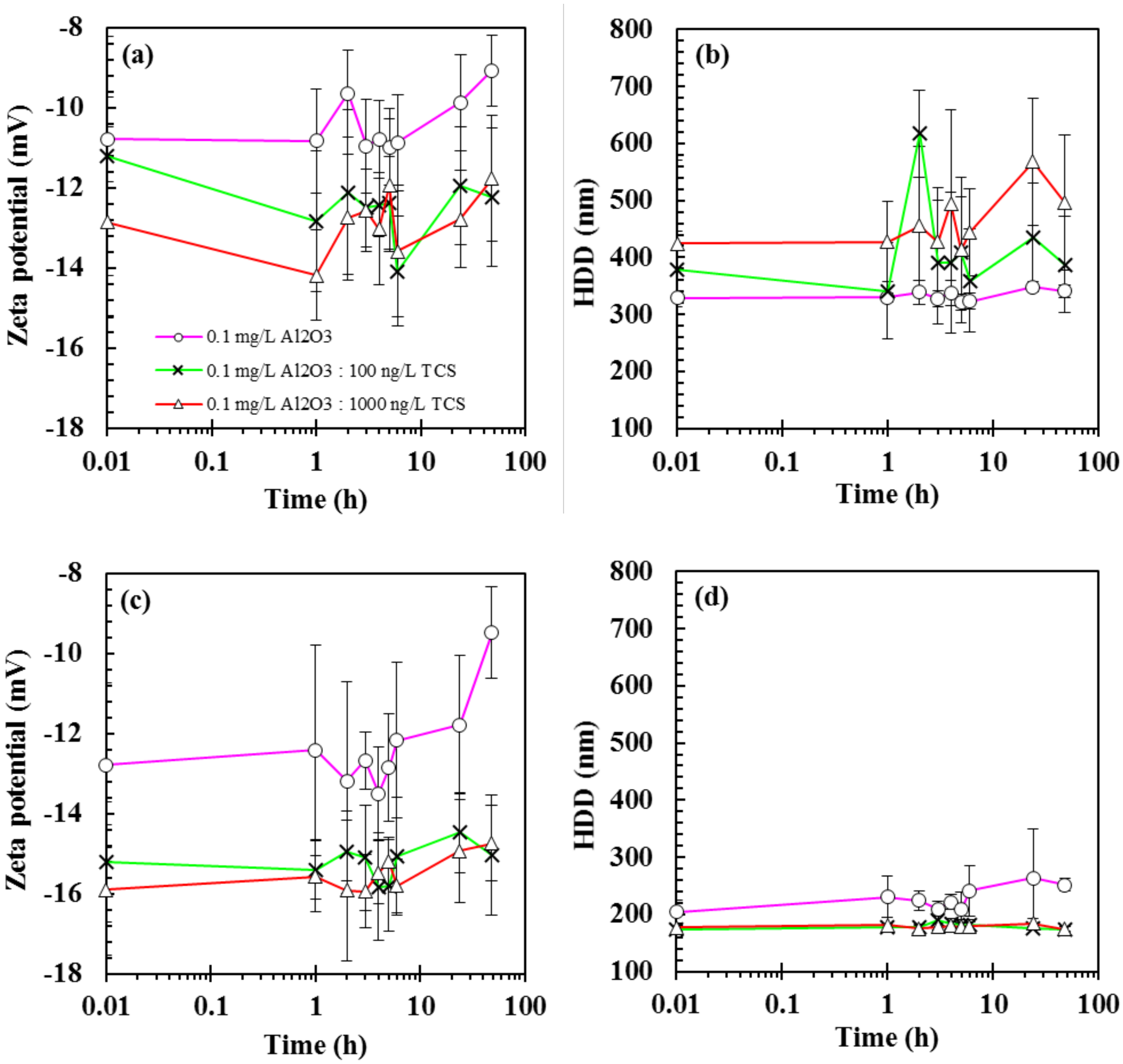

Figure 6-7: Aggregation of 0.1 mg/L nAl$_2$O$_3$ ζ-potential (a) and HDD (b) in BR; ζ-potential (c) and HDD (d) in ER over 48 h in absence and in presence of TCS. The error bars represent standard deviation of three replicates.

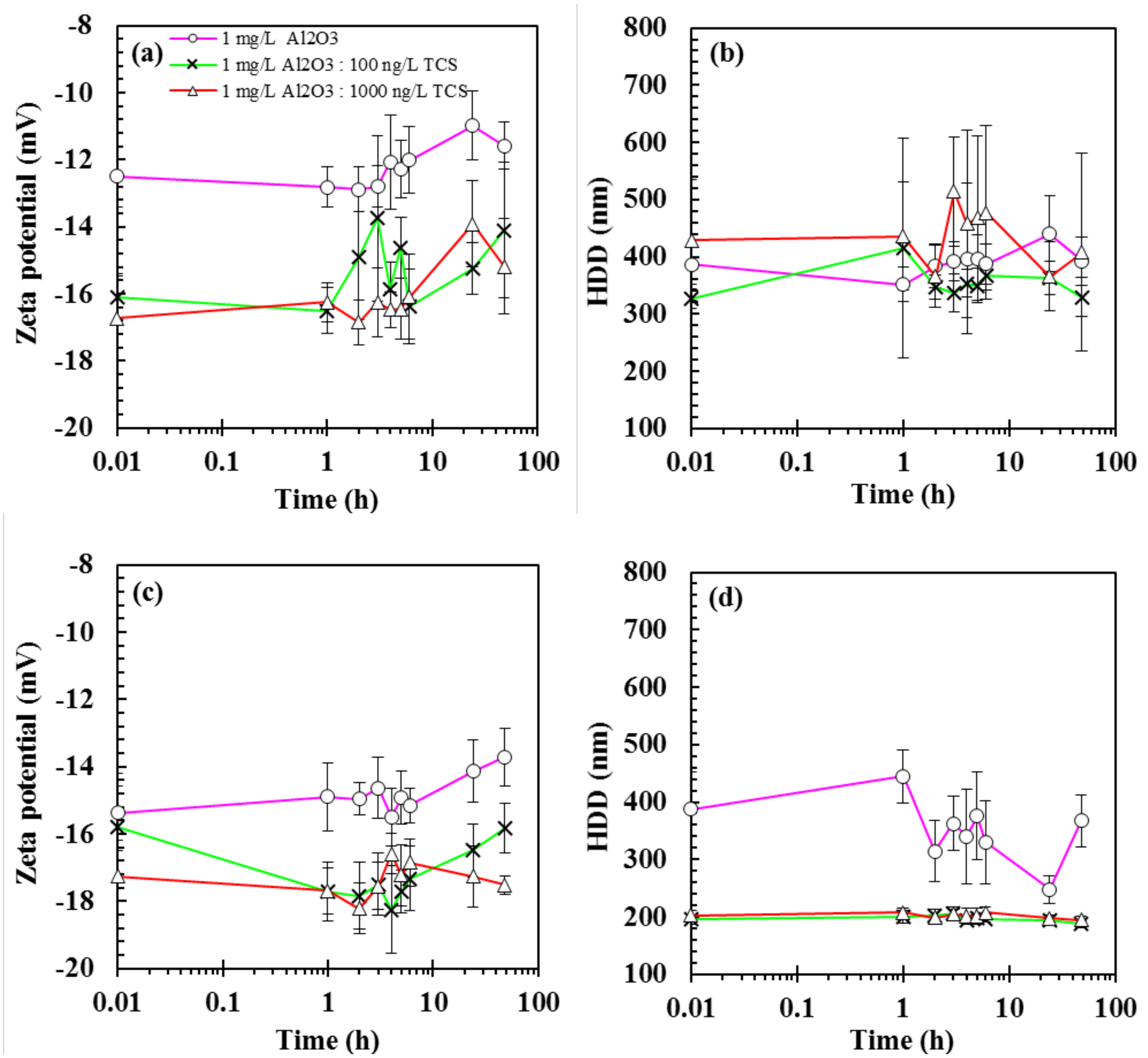

Figure 6-8: Aggregation of 1 mg/L nAl_2O_3 ζ-potential (a) and HDD (b) in BR; ζ-potential (c) and HDD (d) in ER over 48 h in absence and in presence of TCS. The error bars represent standard deviation of three replicates.

6.3.5.2. Aggregation of nCuO in freshwater in presence of triclosan

In BR, HDD was in the range, 270 to 300 nm whereas in ER it was in the range 200 to 250 nm for 0.1 mg/L nCuO (Figs. 6-9. b and d). Increasing concertation of nCuO to 1 mg/L, HDD ranged from 300 to 420 nm in BR whereas in ER it ranged from 300 to 400 nm (Figs. 6-10. b and d). In BR, for 0.1 mg/L nCuO, HDD ranged from 300 to 700 nm in the presence of either 100 or 1000 ng/L TCS (Fig. 6-9b) and ζ-potential varied within a narrow range of -9 to -12 mV without any statistically significant difference (Fig. 6-9a). Increasing the concentration of nCuO to 1 mg/L, HDD ranged from 400 to 650 nm and 400 to 800 nm in the presence of 100 and 1000 ng/L TCS, respectively (Fig. 6-10b) and ζ-potential varied within a narrow range of -10 to -16 mV without any statistically significant difference Fig. 6-10a. In BR, ENPs were destabilised by TCS and the destabilisation was independent of the concentration of both nCuO and TCS.

In ER, for 0.1 mg/L nCuO, HDD ranged from 150 to 180 nm at both 100 and 1000 ng/L TCS (Fig. 6-9d) whereas ζ-potential varied within a narrow range of -9 to -17 mV without any statistically significant difference under different concentrations of TCS Fig. 6-9c. Increasing the concentration of nCuO to 1 mg/L, HDD ranged from 160 to 250 nm at both 100 and 1000 ng/L TCS (Fig. 6-10d) whereas ζ-potential varied within a narrow range -9 to -19 mV without any statistically significant difference under various concentrations of TCS (Fig. 6-10c). In ER, nCuO were stabilised by TCS and the degree of stabilization was independent of concentration of TCS but dependant on the concentration of nCuO as lower concertation was more stabilised than the higher concentration as shown in Figs. 6-9d and 6-10d. The stabilization of nCuO in ER is most likely due to adsorption of TCS onto ENPs as evidenced by the slight increase in ζ-potential. Similar observations have been reported during investigations on the interaction of TCS and copper nanoparticles (Chen et al., 2018). However, the reasons for the destabilisation of ENPs by TCS in BR remain unclear as well as its implications and require further interrogation. No statistically significant differences were observed in HDD over 48 h experimental time.

The influence of TCS on stability of different types of ENPs in identical exposure media is very similar. For example, both nAl_2O_3 and nCuO where stabilised to within a narrow range of 150 to 250 nm at various concentrations of ENPs and TCS in ER. The stabilisation effect of TCS is more significant at lower concentration of ENPs. In the current study, 0.1 mg/L was more stabilised than 1 mg/L ENPs under identical concentration of TCS and exposure media. Based on the current experimental results, the effects of TCS on stability of ENPs in freshwater are more distinctive under low concentration of NOM in the exposure media. For example, the effect of TCS was more apparent in ER with lower concentration of NOM than BR. However, it is worth noting that the stabilization or destabilization effect observed on ENPs as a result of organic pollutants, and the extent of these effects are dependent on the water chemistry of the exposure media in which the net effect is as a result of various effects from all influencing factors within the complex exposure media.

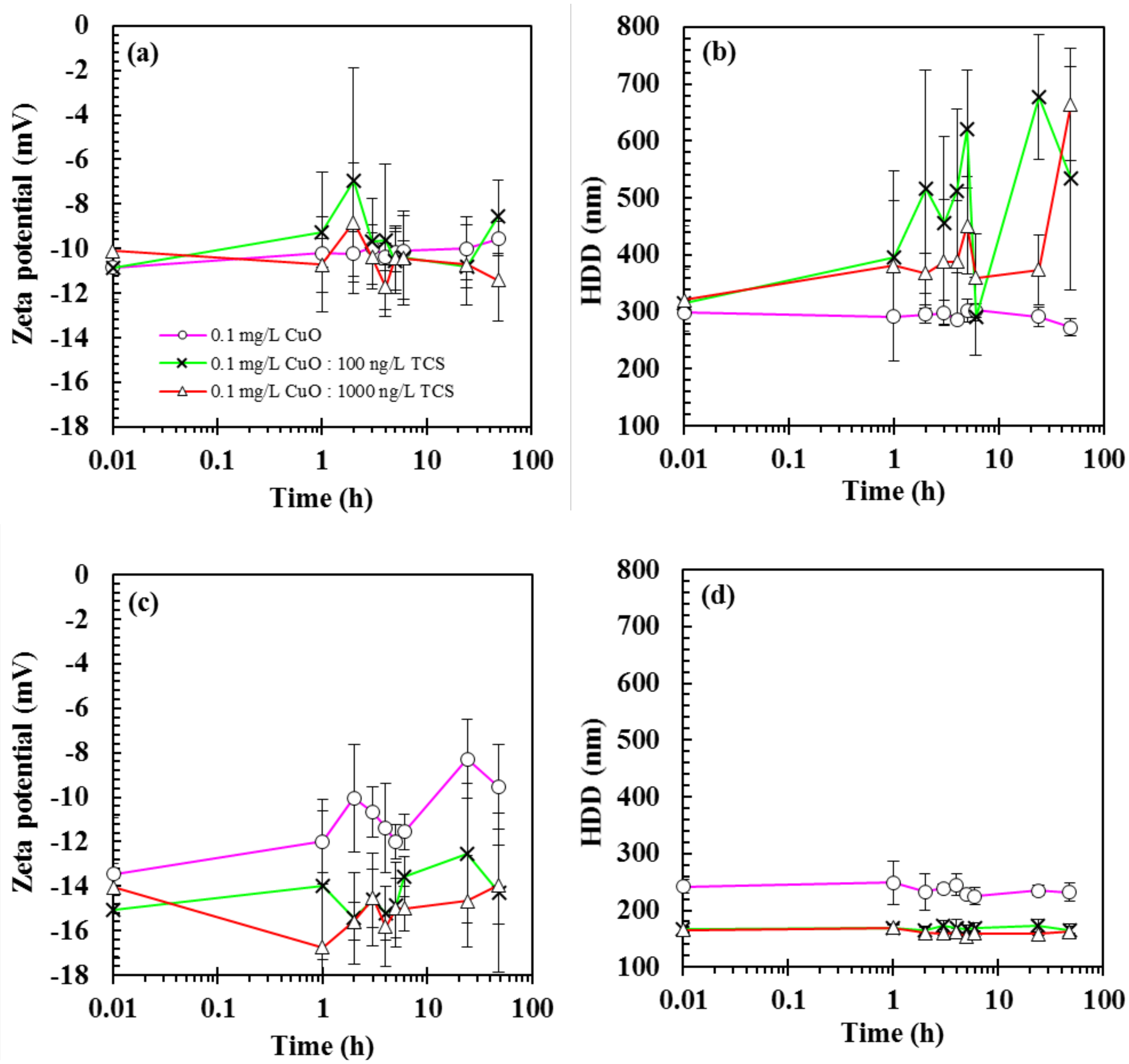

Figure 6-9: Aggregation of 0.1 mg/L nCuO ζ-potential (a) and HDD (b) in BR; ζ-potential (c) and HDD (d) in ER over 48 h in absence and in presence of TCS. The error bars represent standard deviation of three replicates.

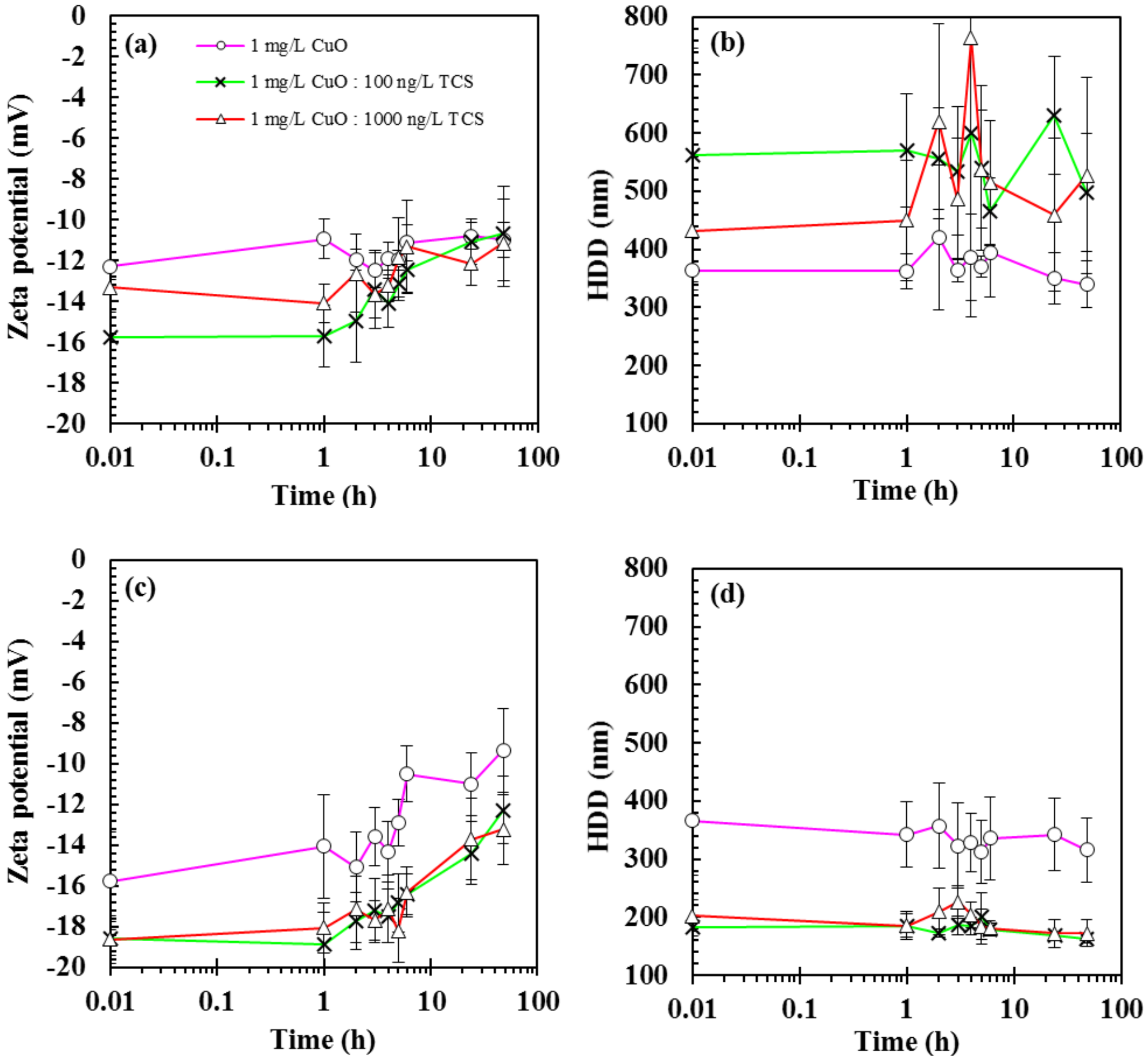

Figure 6-10: Aggregation of 1 mg/L nCuO ζ-potential (a) and HDD (b) in BR; ζ-potential (c) and HDD (d) in ER over 48 h in absence and in presence of TCS. The error bars represent standard deviation of three replicates.

6.4. CONCLUDING REMARKS

The findings from the singles and mixtures of commercially available nAl$_2$O$_3$, nCuO and TCS showed that aggregation was dependent on the water chemistry. Both single and mixture ENPs had higher surface charge in ER compared to BR river water as a result both were more stable in ER than BR. Whereas, both single and mixture ENPs showed negative ζ-potential at all concentrations, these had a concentration-dependent increase for singles, which were also evident in the aggregation results. Despite the increase in ζ-potential the HDD increased with increasing concentration of ENPs for the singles. This means concentration effects outweighed the repulsive effects of ENPs. However, for the mixtures in BR, maximum HDD up to µm range was observed from the lowest concentration ratio. The dissolution of ENPs was influenced by NOM which provides a coating and the influence was higher at lower than at higher concentration of ENPs. In freshwater systems with extremely complex composition, the fate and behaviour of ENPs is not determined by a single factor. This is because of the combined factors counteracting each other to yield the observed effects. BR had a higher concentration of both monovalent and divalent cations which have been proved to promote aggregation of ENPs. Hence, ionic strength effects outweighed the stabilization effects of NOM and TCS. The effect of TCS on aggregation of ENPs is similar and is more distinctive under low concentration of NOM in the exposure media. Each mixture has a unique behaviour in each exposure media and the behaviour of a given mixture in one chemical environment may not be used to predict the behaviour of a similar mixture in a different chemical environment. These results also suggest that the behaviour of mixtures of ENPs and TCS is different from the behaviour of individual components. The observed stabilization or destabilization of ENPs as a result of organic pollutants, and the extent of these effects are dependent on the water chemistry of the exposure media. In the environment, ENPs mixtures may form agglomerates and undergo sedimentation which may cause toxic effects to benthic organisms in the aquatic system or maybe stable and interact with organisms within the water column.

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CHAPTER 7 : APPENDIX

Human Capacity Development summary for WRC Project K5/2509/1

Herein is a summary of human development activities since the inception of the project from April 2016.

Table 7-1: Various types of collaborations together with current and planned activities.

Collaborations	Country	Organization	Department	Areas of collaborations	Status	Comments
National	SA	UP	Chemical Eng. (AIMs)	Fate and behaviour Modelling	Active	Co-supervision of PhD, and co-publication, co-supervision of undergraduates projects
		CSIR	Natural Resources and Environment	Effects and toxicity	Active	Co-supervision of MSc, and co-publication, hosts for two PhD students. PhD thesis in finalization phase
		DWS	Resource Quality Information Services	Effects and toxicity	Active	Host for MSc student
		MINTEK	Advanced Materials Division		Active	ENMs characterization, and planning co-s supervisions of postgraduate students/ postdocs
		UW	Process Services	Effects, toxicity, fate and behaviour	Active	Co-supervision of PhD and MSc (registered since Feb 2018)
International	BOT	BIUST	Biology Sciences and Biotechnology	Effects and toxicity	Active	Co-supervision of PhD (registered and UP), co-application for postdoc funding, and co-hosting of students
	FRA	AMU	CNRS	Fate and behaviour, nanowastes	Active	Exchange visits completed to both particles, co-publication currently in draft form

*BIUST: Botswana International University of Science of Technology, UEM: Universidade Eduardo Mondlane (UEM), CNRS – Aix-Marseille University, France, SA: South Africa, FRA: France, BOT: Botswana, AUT: Austria; UW: Umgeni Water

Table 7-2: List of international visits by the collaborating researchers

Dates	Visiting scientist/ students	Home institution, Country	Visited institution, Country	Activities
10-14 Nov 2016	N. Musee	UP, SA	CNRS, FRA	Presentation/ facilities tour, discussions on possible areas of research, and exchange of students and researchers
10 Aug 2017	L. Kebaabestwe	BIUST, BOT	UP, SA	Discussions of projects and areas of research. Tour of Chemical Engineering research facilities
26-28 Sep 2017	N. Musee	UP, SA	BIUST, BOT	Undertake experimental studies/design of programme for co-supervision of PhD and postdocs between institutions
26-28 Sep 2017	N. Mahaye, S. Leareng. A. Nanja	UP, SA	BIUST, BOT	Carried out experiments/ exposure to state-of-art equipment, and exchange of ideas with counterpart students
4-6 Dec 2017	J. Rose	CNRS, FRA	UP, SA	Discussions on current projects, likely timing for visit of students to France, possible funding streams
16-20 Jul 2018	N Musee	UP, SA	Umgeni Water, SA	Initiation of 5 year project, postgraduate students projects design, and discussions with institution's executive on collaborative research and partnerships
3-4 Sep 2018	L. Kebaabestwe	BIUST, BOT	UP, SA	Discussions of first collaboration work outputs, viz.: review paper based on co-supervised PhD student, and book chapter currently under preparation

*BOT: Botswana, FRA: France, SA: South Africa

Table 7-3: List of MScs and PhDs students in the ECERA Group from 2015/16 AY to 2018/19 AY.

Initial, Surname	Nat/Gen	Degree	Project title
M. Makofane	SA (F)	MSc	Effects of binary and ternary mixtures of silver and zinc oxide nanoparticles and atrazine to *Daphnia magna*
E. Bekker	SA (M)	MSc	Risk estimation for emerging contaminants mixtures using modelling techniques to aquatic systems
S. Leareng	BOT (M)	PhD	Effects of binary and ternary mixtures of iron and zinc oxide nanoparticles and triclosan to microorganisms in aquatic systems
N. Yalezo	SA (M)	PhD	Knowledge-based modelling of engineered nanomaterials risks in the aquatic systems
A. Nanja	ZAM (M)	PhD	Elucidating the fate and behaviour of engineered nanoparticles (ENPs) and triclosan in freshwater as binary and ternary mixtures.
N. Mahaye	SA (F)	PhD	Bioaccumulation, uptake and genotoxicity of Au and CeO_2 engineered nanoparticles to aquatic organisms
N. Nyangiwe	SA (M)	PhD	Modelling the interactions of engineered nanoparticles with natural organic matter in aquatic systems using in silica techniques.

*Abbreviations: NAT/GEN: nationality/gender, SA: South African, BOT: Botswana, ZAM: Zambia, F: female, M: male. Students registering for the first time in 2018/19 AY are not included in the table.

Table 7-4: Capacity building: undergraduate students' projects conducted over the last three years.

Initial, Surname	Gender	Project Title	Year	Status
M. Mukwevho	F	The effect of pH and humic acid on stability of positively charged 20 nm BPEI coated gold nanoparticles	2016	Completed
T. Modisakeng	F	Effect of pH and humic acid on the stability of citrate coated gold nanoparticles	2016	Completed
L. Nxiweni	F	K-Means document clustering: a case of risk information for ENMs in aquatic systems	2016	Completed
B. Laurens	M	Automated platform for metadata collection and analysis: a case of ENMs risk assessment	2016	Completed
G. Radebe	F	Effect of pH and humic acid on 5 nm BPEI coated positive gold nanoparticles in Hoagland's aqueous medium	2016	Completed
W. Tsotestsi	M	Preliminary inventory of nanoproducts and industrial applications of ENMs in South Africa	2016	Completed
S. Dlamini	M	Effects of zinc oxide nanoparticles on Bacillus Subtilis under the influence of pH and ionic strength	2017	Completed
J. Erasmus	M	Substance flow analysis of triclocarban in Gauteng province, South Africa	2017	Completed
R. Grundlingh	M	Fate and Behaviour of Cerium Oxide nanoparticles (CeO_2 NPs) in an aqueous media	2017	Completed
S. Mbungele	F	Influence of pH and ionic strength on aluminium oxide nanoparticles fate	2017	Completed
R. Pottinger	M	Influence of water chemistry on the toxicity of Iron (III) oxide	2017	Completed

Initial, Surname	Gender	Project Title	Year	Status
K. Viljoen	F	Simulated mass flow analysis of Triclosan from consumer products in Gauteng, South Africa	2017	Completed
S. Sithole	M	ZnO Engineered Nanoparticles: pH and Ionic Strength Effects on Aggregation over time.	2017	Completed
N. Nzimande	F	Study on the influence of pH and ionic strength on iron (III) oxide engineered nanoparticles (Fe_2O_3 ENPs) stability in an aqueous media	2017	Completed
M.S. Seotlo	F	Probability density functions to estimate the flows of Triclosan and Triclocarban from personal care products	2018	Completed
M.H.M Frederico	F	Probability density functions to estimate the flows of Triclosan and Triclocarban from personal care products	2018	Completed
S. K. Zwane	M	Behaviour of a binary mixture copper oxide nanoparticles and triclosan in fresh water	2018	Completed
L. K. Motaung	F	Binary mixtures effects of nZnO and Triclosan on Bacillus subtilis in freshwater systems	2018	Completed
S. Z. Zondi	F	Toxicity of binary mixtures of iron oxide ENPs and triclosan on Bacillus subtilis in freshwater	2018	Completed
I. A. Joäo	F	Genotoxicity effects of gold engineered nanoparticles to algae, *Raphidocelis subcapitata*	2018	Completed

*Abbreviations: F: female, M: male. Notes: all these students were registered for BSc Chem Eng degree

Knowledge Dissemination Summary for WRC Project K5/2509/1

Herein is a summary of the knowledge dissemination outputs and activities since the inception of the project

1. Publications

1.1. Published articles

1. Musee, N., Environmental risk assessment of triclosan and triclocarban from personal care products in South Africa, Environmental Pollution, 242, 827-838, 2018.
2. Musee N., Comment on risk assessments show engineered nanomaterials to be of low environmental concern, Environmental Science and Technology, 52, 6723−6724, 2018.

1.2. Articles submitted and under peer review

1. Leareng, S.K., Ubomba-Jaswa, E., Musee, N. Zinc oxide and iron oxide engineered nanoparticles toxicity on *Bacillus subtilis* in river water systems. *Environmental Science Nano*
2. Nanja, A.F., Focke, W., Musee, N. Stability and aggregation of aluminium oxide and copper oxide nano-particles in natural aqueous matrixes. *Environmental Science and Pollution Research*

1.3. Papers in preparation

1. Leareng S.K. Joint toxicity of Zinc oxide, Iron oxide and Triclosan on *Bacillus subtilis* in river water systems, *Chemosphere*
2. McDowell et al. Interactions of binary and tertiary mixtures of Fe2O3 and ZnO engineered nanoparticles and organic triclocarban in aqueous media, *Water SA*
3. Musee, N. et al. A review on engineered nanomaterials mixtures in the environment: current knowledge, data gaps, and future perspectives, *Environmental Science and Technology*
4. Yalezo and Musee, N. Exposure assessment of metal-based ENMs in the aquatic systems: Part (I): Knowledge acquisition and method development, *Journal of Hazardous Materials*
5. Yalezo N and Musee, N., Exposure assessment of metal-based ENMs in the aquatic systems: Part (II): Intelligent decision support systems, *Journal of Hazardous Materials*

6. Nanja AF, Focke WW, Musee N. Binary interactions of aluminium oxide and copper oxide nanoparticles in freshwater systems, *Chemosphere*
7. Bekker EH, Musee N. Probabilistic material flow analysis and risk quantification of nanomaterials in aquatic systems, Gauteng Province, South Africa, *Environment Pollution*
8. Bekker EH, Musee N. Deterministic estimation of nanomaterial flows based on nano products in retail commerce in South Africa, *Environmental Toxicology and Chemistry*

1.4. Popular articles

1. Musee, N., Nanotechnology: numerous benefits with unknown risks, Public Service SA Magazine, November 2017, pp. 17. Link: http://publicservicesa.co.za/magazines/2017/November/PSSA November2017.pdf
2. Musee, N., Thoola, M., Mazibuko, S., Maharaj, L., Thompson, P. Umgeni Water and University of Pretoria research collaboration on emerging contaminants, Flowmeter, September 2018.

2. Conference/symposiums contributions, and presentations

2.1. Conference presentations

1. Musee, N., Engineered nanomaterials-containing waste streams classification: a country with economy in transition perspective. In: 5th Nanosafe International Conference: Health and safety issues related to nanomaterials for a socially responsible approach, Minatec, Grenoble, France, 7-10 November, 2016.
2. Leareng, S. K., Ubomba-Jaswa, E., Musee, N. Binary mixture toxicity of metal oxides nanoparticles and triclosan on Bacillus subtilis in freshwater systems. In: 6th Nanosafe International Conference: Health and safety issues related to nanomaterials for a socially responsible approach, Minatec, Grenoble, France, 5-9 November 2018.
3. Musee, N., Leareng S. K., Mahaye, N., Nanja, A. F., Ernst Bekker, E. H., Yalezo, N. Trends on environmental transformations and effects of nanomaterials mixtures in aquatic systems: an overview. In: 6th Nanosafe International Conference: Health and safety issues related to nanomaterials for a socially responsible approach, Minatec, Grenoble, France, 5-9 November 2018.
4. Nanja, A. F.., Focke, W., Musee, N. Binary interactions of aluminium oxide and copper oxide nanoparticles mixture in freshwater systems. In: 6th Nanosafe International Conference: Health and

safety issues related to nanomaterials for a socially responsible approach, Minatec, Grenoble, France, 5-9 November 2018.

5. Yalezo, N., Musee, N., Assessment of metal oxide-based nanoparticles stability in the aquatic systems using fuzzy logic. In: 6th Nanosafe International Conference: Health and safety issues related to nanomaterials for a socially responsible approach, Minatec, Grenoble, France, 5-9 November 2018

2.1. Plenary presentations

1. Musee, N., Sustainable nanotechnology: need for ecological risk assessment considerations; in 7th International Conference on Nanoscience and Nanotechnology in Africa NanoAfrica 2018, 22-25 April 2018.

2.2. Invited lectures/presentations

1. Musee, N., A seminal presentation in the context of the Labex SERENADE and the European Program PROSAFE framework titled: "A decade of nanotechnology risk assessment initiatives with specific reference to South Africa: past, present and future directions, CNRS – Aix-Marseille University, Aix en Provence, France, 14 November 2016.

2. Musee N., Implications of engineered nanoparticles to the environment: in WRC Water Quality and Health Series: Emerging contaminants workshop – Microplastics and engineered nanoparticles in the environment: hype or real health risk? University of Pretoria, South Africa, Hatfield Campus, 31 August 2017.

3. Musee, N., Implications of emerging contaminants to natural and technical environmental systems, Presentation to Senior and Executive Management, Umgeni Water, Pietermaritzburg, 17 July 2018

4. Musee, N., Nanomaterials in the environment: status quo, challenges, and future perspectives, an Invited Lecture at the NIC 10 year Anniversary Workshop, CSIR International Convention Centre, Pretoria, South Africa, 14-16 October 2018

2.3. Keynote presentations

1. Musee, N., Engineered nanoparticles in the environment: hype or health risks. Invited Speaker in: Water Research Commission Symposium 2017, Adaptation to the New Normal, Johannesburg, South Africa, 18-20 September 2017.
2. Musee, N., Nanotechnology – a key driver for industrialization and potential threat to the environment. Invited Keynote speaker in: Africa Unity for Renaissance Conference, St Georges Hotel, Pretoria, South Africa, 23-25 May 2018.

3. Seminars presentations/workshops organized

1. ECERA Group, Chemical Engineering, UP hosted Dr. Jérôme Rose from CNRS Aix-Marseille University, France where a workshop with postgraduate students was held, and also Research Managers from the WRC were also in attendance. His visit was of the EU delegation on the "Strengthening EU-SA collaboration in the safety of nanomaterials", 6 December 2017. PhD students presented their research and benefited from Dr Rose’s valuable insights and academic critique.

2. Stakeholder workshop: addressing the impacts of chemical mixtures (micro- and nano-sized) in environmental systems, University of Pretoria, Hatfield Campus, 6 April 2017. Students and researchers presented the project proposals and up-date of the work done.

3. Organized in collaboration with Water Research Commission and North West University a workshop on WRC Water Quality and Health Series: Emerging contaminants workshop – Microplastics and engineered nanoparticles in the environment: hype or real health risk? University of Pretoria, Hatfield Campus, 31 August 2017