Zinc oxide nanoparticles in the soil environment: dissolution, speciation, retention and bioavailability

Narges Milani

In the fulfilments of the degree of

Doctor of Philosophy

A thesis submitted to

Soil Science Group

School of Agriculture, Food & Wine

The University of Adelaide

December 2011

In God, the Greatest

To my beloved parents

Table of contents

Acknowledgments

It is a pleasure to thank those who made this thesis possible.

Foremost, I would like to express my sincere gratitude to my supervisor Prof. Mike McLaughlin for his continuous support of my project. His wide knowledge and logical way of thinking have provided a strong foundation for this project. His understanding, encouragement and guidance have been of great value to me.

I feel incredibly lucky to have a team of experts on my supervisory panel. I would like to thank Dr Jason Kirby for his considerable efforts in helping me to make the project work and his patience for my endless questions. I am truly grateful for the help and support provided by Dr Ganga Hettiarachchi throughout my candidature. Despite the geographical distance, she was always available by some means of communication to provide advice on pretty much everything. I would like to thank Dr Samuel Stacey for his willingness to help in development of experimental protocols and significant scientific contribution to this thesis. I would like to express my gratitude to Dr Douglas Beak who became one of my supervisors part-way through my candidature. Thanks to Doug for his patience and persistence in helping me deal with complicated XAS data. Many thanks must go to Dr Geert Cornelis who always had a practical solution for resolving problems in working with nanoparticles. Outside of my formal supervisory panel, I would like to thank Dr Therese McBeath, Dr Fien Degryse and Dr Sola Ajiboye who helped me with statistics and several aspects of chemistry. Their contribution greatly improved the science of this project.

I owe my deepest gratitude to Dr Lauren Bennet who appointed me as her visitor in the Department of Forest and Ecosystem Science in the School of Land and

Environment at the University of Melbourne and provided me with the perfect environment for writing up my thesis.

This research project would not have been possible without the technical support of many colleagues at the University of Adelaide and at the Commonwealth Scientific and Industrial Research Organization (CSIRO), namely Dr Peter Self and Adelaide microscopy staff, Mark Raven, Catherine Fiebiger, Gillian Cozens, Lester Smith, Caroline Johnston, Margaret Yam, Claire Wright, Michelle Smart, Andrew Wright and Waite Analytical services (WAS) staff. I am grateful to all of them for their time and patience.

I would like to acknowledge financial support for the work in this thesis which was provided by the CSIRO and the U.S. Department of Energy, Office of Science for use of Advanced Photon Source. I would like to extend my thanks to the Australian Soil Science Society Incorporated for financial support to attend a conference in Brisbane.

I feel privileged to have made many friendships during my candidature. There are too many people to name. A special thank must go to my lab buddies, Lakmalie, Ashlea, Jen, Jia, Margaret, Sarah and Kate for all friendly chats and wonderful memories.

My special thanks to my loving, supportive and encouraging husband, Mohesn who has been there through all the highs and lows. I never felt helpless with him around.

Finally and the most sincerely, my greatest thanks are reserved for my parents and family whose support and love gave me the strength and courage to continue. I truly believe I could not have done this without them.

Abstract

Zinc oxide nanoparticles (ZnO NPs) have unique physical and chemical characteristics which deviate from larger particles of the same material, due to their extremely small size, higher specific surface area and surface reactivity. The peculiar properties of ZnO NPs could potentially improve zinc (Zn) fertilizers for sustainable agriculture. This is based on the assumption that ZnO NPs provide a more soluble and bioavailable source of Zn in soil compared to micron- or millimetre- sized (bulk) ZnO particles currently used for Zn fertilizers in Zn deficient soils. However, a thorough understanding of the fate and reactions in soils and interactions of nanoparticles with plants of ZnO NPs is required prior to the recommendation for use of these novel materials. Therefore, there is a need to investigate dissolution, diffusion, transformation, partitioning and availability of manufactured ZnO NPs in soil to ensure safer and more sustainable application of ZnO NPs as a new source of Zn fertilisers for plants, and better management of their potential risks.

Given inclusion of Zn in macronutrient fertilizers is the common procedure for their field application, ZnO NPs and bulk ZnO were coated onto macronutrient fertilizers (monoammonium phosphate (MAP) and urea) and dissolution kinetics, diffusion and solid phase speciation of Zn from coated fertilizers were evaluated. Coating of ZnO on macronutrient fertilizers significantly affected solubility and dissolution kinetics of the ZnO sources, but nano-sized ZnO did not show any enhanced solubility over bulk ZnO. The low pH value of ZnO-coated MAP granules resulted in greater and faster dissolution of ZnO compared to ZnO-coated urea granules. However, interactions of ZnO particles with phosphate in MAP granules likely resulted in precipitation of Zn-phosphate species. The high pH and ionic strength of the dissolving

solution resultant from hydrolysis of urea likely promoted aggregation of any ZnO NPs released from coated urea granules and also hindered dissolution of ZnO.

To evaluate changes in Zn speciation with coating of the ZnO sources and after incorporation of the coated-fertilizers into an alkaline calcareous soil, synchrotron-based micro X-ray absorption fine structure (µ-XAFS) method was used. The findings confirmed precipitation of Zn-phosphate species at the surface of MAP fertilizer granules irrespective of the size of ZnO particles used for coating. For coated urea, the Zn remained as ZnO species for both nano-sized and bulk ZnO coatings. Solid phase speciation in the fertilized soil varied with distance from the point of fertilizer application. Significant amounts of $Zn(OH)_2$ and $ZnCO_3$ species were identified in the soil some distance from coated urea and MAP, respectively, indicating dissolution/precipitation processes were active. Moreover, limited and comparable diffusion of Zn from coated fertilizers with nanoparticulate or bulk ZnO into soil was observed using micro x-ray fluorescence mapping (µ-XRF). Transformation of Zn at the surface of MAP granules, mass flow of water towards the hygroscopic fertilizer granules or strong aggregation of ZnO nanoparticles released from urea granules could have been the mechanisms which restricted Zn diffusion. Given that coating of ZnO on macronutrient fertilizers markedly reduced Zn solubility, reactions of ZnO NPs and bulk ZnO in soil were studied when applied as the pure oxides.

Availability of Zn for durum wheat (*Triticum durum*) plants from nanoparticulate and bulk sources of ZnO was evaluated in an acidic and an alkaline soil using an isotopic dilution procedure (L value). Significant dissolution and plant acquisition of Zn from ZnO was observed (ca. 50 – 100 % of added), even with limited pre-incubation of soils with the Zn sources. However, no significant effect of particle size was observed on plant acquisition of Zn from the ZnO.

Retention and dissolution of ZnO NPs and dissolved Zn species from ZnO NPs was further investigated in five soils with diverse physical and chemical properties. Strong retention of ZnO NPs and/or dissolved Zn species from ZnO NPs was found in all soils especially in alkaline and calcareous soils. The adsorption affinity of ZnO NPs was generally greater than that of soluble Zn, which suggested ZnO NPs were retained more strongly than soluble Zn in soils. Soil pH and clay content of soil were the most important soil properties affecting retention, although the number of soils used was too small to draw firm conclusions as soil parameters co-varied.

Generally, nanoparticulate forms of ZnO appear to offer little advantage over bulk-sized ZnO as a source of fertilizer Zn to crops. Rapid dissolution of ZnO NPs and partitioning of dissolved Zn species derived from ZnO NPs and/or high retention of ZnO NPs in soils suggested that soil application of manufactured ZnO NPs would not appear to offer any benefits over bulk ZnO, whether applied in pure form or along with macronutrient fertilisers. However, from an ecotoxicological point of view, ZnO NPs would not be persistent in soil systems and hence their mobility in soil would be limited. Therefore the risks associated with application of ZnO NPs in soil would be similar to that of soluble Zn.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Narges Milani and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Narges Milani _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ **Date** _ _ _ _ _ _ _ _ _ _ _

List of Publications

Milani, N., M.J. McLaughlin, S.P. Stacey, J. Kirby, G. M. Hettiarachchi, D. Beak and G. Cornelis. 2012. Dissolution kinetics of macronutrient fertilisers coated with manufactured zinc oxide nanoparticles. Journal of Agricultural and Food Chemistry. 60: 3991-3998.

Milani, N., M.J. McLaughlin, G.M. Hettiarachchi, D.G. Beak, J.K. Kirby and S.P. Stacey. 2012. Fate of nanoparticulate zinc oxide fertilisers in an alkaline calcareous soil. Soil Science Society of America Journal. Submitted.

Milani, N., M.J. McLaughlin, J.K. Kirby, G. Cornelis and S. P. Stacey. 2012. Retention and lability of manufactured zinc oxide nanoparticles in natural soils. To be submitted

Milani, N., M.J. McLaughlin, G.M. Hettiarachchi, , D.G. Beak, J.K. Kirby and S.P. Stacey. 2010. Fate of nanoparticulate zinc oxide fertilisers in soil: Solubility, diffusion and solid phase speciation. *19th World Congress of Soil Science*, Brisbane, Australia, 1-6 August 2010 *(oral presentation)*

Milani, N., M.J. McLaughlin, G.M. Hettiarachchi, D.G. Beak, J.K. Kirby and S.P. Stacey. 2009. Zinc diffusion and speciation in an alkaline-calcareous soil treated with fertilisers coated with bulk and nanoparticulate zinc. *13th Australasian Society for Ecotoxicology Conference.* Adelaide, Australia, 20-23 Sep 2009 *(oral presentation)*

Thesis structure

The experimental chapters in this thesis have been written in manuscript format. Given manuscripts must be self-contained, there is some degree of repetition in this thesis.

Chapter 1 outlines the specific physical and chemical properties of manufactured nanoparticles. A review of the current literature is provided in this chapter on the fate and behaviour of manufactured nanoparticles in aquatic and terrestrial environments and uptake and interactions of nanoparticles with soil organisms and plants. This chapter highlights the need for new sources of Zn in fertilizers and the potential benefits of using ZnO NPs for Zn nutrition of plants .

Chapters 2 and 3 present the results of investigations on dissolution kinetics of Zn from ZnO NPs and bulk ZnO coated onto macronutrient fertilizers in sand columns as well as diffusion and solid phase speciation of Zn from coated fertilizers in an alkaline calcareous soil. The results presented in these chapters provide a good understanding of possible benefits of application of nanoparticulate source of ZnO over bulk ZnO in the coating of macronutrient fertilizers on Zn availability.

Given the reactions which reduced Zn availability when Zn was coated onto macronutrient fertilisers, Chapter 4 presents the data on application of pure ZnO NPs and bulk ZnO in soils (i.e. not associated with macronutrient fertilizers). The results of dissolution, partitioning of dissolved Zn and / or retention of ZnO NPs was compared to soluble Zn in 5 Australian soils with diverse chemical and physical properties. This chapter also compares lability of ZnO NPs for durum wheat (*Triticum durum*) plants in 2 soils with bulk ZnO and soluble Zn.

Chapter 5 summarizes and discusses the findings from this thesis and makes suggestions for future research arising from the experimental work presented.

1 Review of the Literature

1.1 Introduction

Nanotechnology is a multidisciplinary and rapidly growing field in science and technology which involves the manufacture, processing and application of nanometre scale assemblies of atoms and molecules (Tran et al., 2005). Nanomaterials are generally defined as materials with at least one dimension less than 100 nm (Powers et al., 2006). They have unique physical and chemical characteristics which deviate vastly from those of individual atoms or molecules and also the same material at a bulk scale (Banfield and Zhang, 2001). These differences are due to their extremely small sizes and accordingly higher specific surface area and reactivity, which enable them to have novel applications.

The unique properties of these materials along with the industrial ability to design nanoparticles with specific features allow the expansion of their applications in different sectors. As an example, in the period between March 2006 to August 2009, a 379% increase in nanotechnology-based products has been observed in the list of consumer products, totalling of 1025 items produced by 485 companies in 24 countries (Peralta-Videa et al., 2011). Current applications of nanotechnology products are focused in the fields of energy, electronics, medicine and environmental remediation (Aitken et al., 2006; Baruah and Dutta, 2009; Liu, 2006; Luther, 2004; Rickerby and Morrison, 2007; Schmid, 2001). The experiences gained from these fields as well as the rapid growth of nanotechnology due to innovations and developments in analytical and imaging technologies suggests that a large variety of other applications will be found. The use of nanoparticles in agriculture is a promising area which could potentially improve current crop management techniques in the long term. Already, nanoencapsulated pesticides have been successfully applied to release chemicals in a controlled and specifically targeted manner which provides a safer and easier control system for pests (Beddington, 2010; Nair et al., 2010). In the future nanotechnology may provide smart devices that are capable of soil monitoring which will enable early remedial actions and synchronization of delivering chemicals with plant needs (DeRosa et al., 2010; Nair et al., 2010). It is claimed that if nanotechnology meets current expectations of mass production and reducing costs, significant improvements can be expected following 2020 in more efficient usage of agricultural inputs in crop production (Dewick et al., 2004).

One potential application of nanotechnology in agriculture is to address issues related to micronutrient deficiency, which is one of the major problems in agricultural productivity. Zinc deficiency is the most important micronutrient problem which is found particularly in crops grown on alkaline and calcareous soils of arid and semi-arid regions of the world (Cakmak et al., 1996). Application of organic and inorganic Zn fertilizers is necessary to correct Zn deficiency and improve crop productivity in these soils. However, after Zn is added to soils, it transforms gradually from more active and available fractions into less available forms such as precipitates (i.e. zinc carbonate) and associations with oxide phases (e.g. iron (Fe), aluminium (A)l-oxides) (Ma and Uren, 2006). These transformations, which decrease the availability of added Zn, are more prevalent in calcareous soils (Xiang et al., 1995).

Designing new more efficient sources of fertilizer Zn for crops has been the focus of much research in the past and now there are opportunities for the application of nanotechnology to this field. The smaller size and higher reactivity of zinc oxide (ZnO) nanoparticles compared to micron- or millimetre sized (bulk) ZnO particles may affect their solubility, diffusion and mobility and hence their fate and behaviour in soil environments. If such nano-scale materials were applied to supplying plant Zn requirements, their effect on nutrient accessibility by plant roots could be significantly enhanced resulting in increased yields and Zn content of crops.

Although the use of ZnO nanoparticles for plant nutrition is an emerging and promising field, some unique properties of nanoparticles that make them useful are also likely to be properties that could impact the environment. Therefore, a proactive understanding of the environmental impact and fate of nanotechnology-based products in the early stages of their development is needed to ensure safe and sustainable use of nanoparticles in agriculture and better management of their associated risks (Bernhardt et al., 2010; Colvin, 2003; Klaine et al., 2008; Rickerby and Morrison, 2007; Thomas et al., 2011; Wiesner et al., 2006). As there have been few studies on the fate of nano-scale materials in terrestrial environments, it is necessary to conduct initial research on the dissolution, transformation, diffusion, mobility and availability of these materials in these complex systems. Accordingly, the focus of this thesis was to develop a better understanding of the reactions of ZnO nanoparticles in the soil in order to evaluate the possibility of their application as a source of Zn in agriculture to improve plant yields and micronutrient contents.

1.2 Nanomaterials

Nanomaterials are classified as materials with at least one dimension less than 100 nm (Wiesner et al., 2006). Thus, nanomaterials have dimensions between ions (10^{-10} m) and macroscopic materials (Banfield and Zhang, 2001) and may contain 20-15000 atoms (Liu, 2006). The lower limit of nanomaterials diameters that distinguishes the smallest nanomaterials from molecules is generally defined as being between 0.2 nm and 1 nm (Powers et al., 2006). The size range of nanoparticle in relation to other size categories of particulate materials in water and air is shown in Figure 1. The size range of nanoparticles is overlapping with colloidal (1 nm – 1 µm) and dissolved particles (operationally defined as compounds passing through a cut-off at 0.45 µm diameter) and their differentiation can be obtained through analytical methods that can distinguish between them without introducing artefacts during measurements (Lead and Wilkinson, 2006; Nowack and Bucheli, 2007).

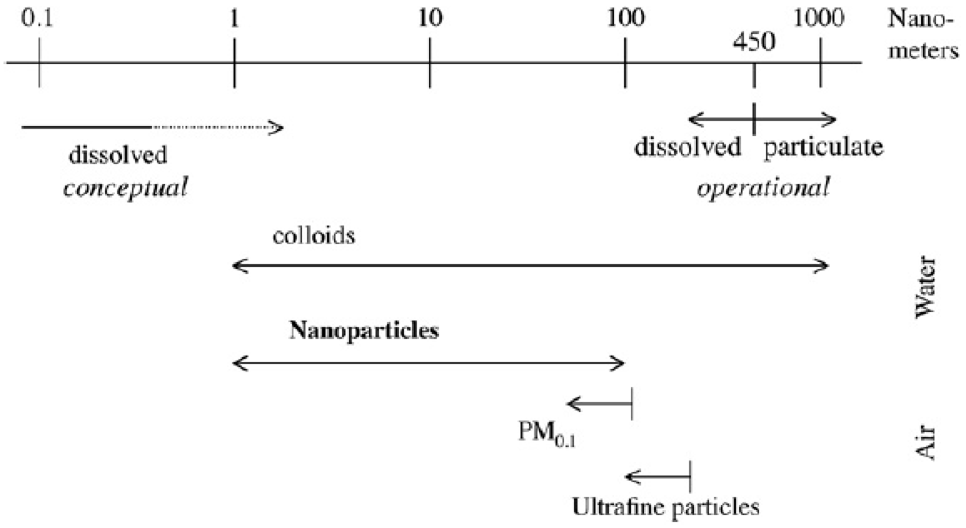

Figure 1- Definition of different size classes relevant to nanoparticles (Nowack and Bucheli, 2007).

Nanomaterials can be found in different forms: nanolayers that have one dimension in the nano-scale and are extended in the other two dimensions, nanowires and nanotubes which are nano-scale in two dimensions and extended in the third dimension, and also nanoparticles which are nano-scale in three dimensions (The Royal Society & Royal Academy of Engineering, 2004). Moreover, they can exist in fused, aggregated or agglomerated forms in the environment (Nowack and Bucheli, 2007).

Nanoparticles are of particular interest because of their novel chemical, physical, magnetic and optical properties. They exist widely in the environment and may form naturally through biotic or abiotic processes or through anthropogenic activities (Banfield and Zhang, 2001). Anthropogenic nanoparticles may be formed unintentionally as a result of human activities or produced intentionally due to their special properties (Nowack and Bucheli, 2007; Wigginton et al., 2007). Unintentional formation of natural nanoparticles may be the result of some adverse human activities (e.g. pollution of the natural resources, mining and mineral processing or nucleation waste generation and storage) or even some environmentally beneficial practices such as water purification or environmental remediation (Wigginton et al., 2007). Intentionally produced nanoparticles are usually referred as engineered or manufactured nanoparticles (Nowack and Bucheli, 2007).

1.2.1 Naturally occurring nanoparticles

Nanoparticles have been present on the earth for millions of years and can be found almost everywhere in the environment (Nowack and Bucheli, 2007). Atmospheric, geogenic or biogenic processes which generate super-saturation conditions may lead to the formation of natural mineral nanoparticles (Banfield and Zhang, 2001; Nowack and Bucheli, 2007).

Nucleation of key species in the atmosphere (sulphuric acid, nitric acid and organic gases) is the main process which results in the formation of natural nanoparticles in the atmosphere (Biswas and Wu, 2005). These nanoparticles may continue their growth by condensation of low volatile gases, coagulation with other nanoparticles and surface reactions which increases their particle mass and subsequently their lifetime in the atmosphere (Nowack and Bucheli, 2007).

Nanoparticles may be formed through physical and chemical weathering of rocks and minerals near the earth’s surface. These processes cause the unstable minerals under surface to dissolve or react with other phases (Banfield and Zhang, 2001). Enhanced concentration of these minerals due to evaporation provides a supersaturated condition which initiates the formation of nanoparticles such as amorphous silica (Si), hydrous aluminosilicates (e.g. allophone), clays (e.g. hallosite) and oxides (e.g. magnetite and hematite) (Nowack and Bucheli, 2007). Acid mine drainage, which is derived from weathering of metal sulphide-rich rocks, has a very low pH and contains high concentrations of dissolved ferrous and ferric Fe, Al and other metals which can form a variety of nanoparticles when mixed with natural waters due to changes in temperature, pH and higher oxygen concentrations (Banfield and Zhang, 2001; Nowack and Bucheli, 2007).

Microorganisms are known to contribute to the formation of nanoparticles in soils with properties similar to chemically synthesized nanoparticles (Gericke and Pinches, 2006). Microorganisms can directly produce nanoparticles as part of their metabolic requirements e.g. intercellular production of magnetite (Fe_3O_4) nanoparticles by magnetotactic bacteria which are required for their motility (Wigginton et al., 2007). Nanoparticles can be also formed indirectly as a result of microbial activity for production of metabolic energy through redox reactions (Banfield and Zhang, 2001; Wigginton et al., 2007). Changes in ion solubility during redox conditions provide suitable conditions for formation of nano-scale minerals such as Fe-oxides and Mn-oxides (Wigginton et al., 2007). Manceau et al. (2008) reported the transformation of copper (Cu) into metallic Cu nanoparticles in and near roots of common wetlands plants (*Phragmites australis* and *Iris psedoacorus*) as a detoxification mechanism for excess cationic Cu in soil. Moreover, the use of fungi and actinomycetes as sources of enzymes can catalyse reactions which lead to inorganic nanoparticle formation (Sastry et al., 2003). Recently, biosynthesis of nano-scale materials has led to a new area of research involving organisms such as bacteria, yeast, algae, fungi and actinomycetes and plants for the formation of inorganic nanoparticles (Mandal et al., 2006; Mohanpuria et al., 2008).

1.2.2 Manufactured nanoparticles

Manufactured nanoparticles do not occur naturally in the environment but are intentionally engineered for specific applications. The U.S. Environmental Protection Agency (2005) has grouped manufactured nanomaterials into four types: (a) carbon (C)-based nanoparticles that are composed entirely of C; (b) metal-based materials such as quantum dots, nano-Zn, nano-Al, and nano-scale metal oxides like titanium dioxide (TiO_2), ZnO and alumina (Al_2O_3); (c) dendrimers, which are nano-sized polymers built from branched units capable of being tailored to perform specific chemical functions; and (d) composites, which combine nanoparticles with other nanoparticles or with larger, bulk-type materials.

1.2.3 Manufactured metal oxide/metal nanoparticles

Metal oxide/metal nanoparticles such as ZnO and metallic silver (Ag) are receiving increasing attention in material science and nanotechnology-based industries (Aitken et al., 2006). This increased attention is due to changes in physical and chemical properties such as optical properties, melting points, magnetic properties, surface energies and consequently surface reactivity and specific heats at the nano-scale (Schmid, 2001). These unique properties have led to commercial applications of nanoparticles in different fields in industry which can be categorized into (a) electronic and magnetic, (b) energy and catalysts, (c) cosmetics, biomedical and pharmaceutical, (d) colorant and pigments and (e) environmental applications.

Metal nanoparticles are now widely being employed in the area of electronics, optoelectronics and magnetics. The use of metallic nanoparticles in semiconductors changes the way in which electronics and optoelectronics are designed and made (Parker, 2001). Nanocrystal semiconductors are generally composed of group II and VI elements (e.g. cadmium selenide (CdSe), cadmium sulfide (CdS) and cadmium telluride (CdTe)) at their core and a shell of zinc sulfide (ZnS) or cadmium sulfide (CdS) (Liu, 2006) which can be applied in flat panel display technologies such as computer monitors and televisions or in colour-changing fabrics for decorative applications (Parker, 2001). Moreover, nanocrystalline silicon dioxide (SiO_2) is used in electronic manufacturing as both semiconductors and electrical insulators (Adams et al., 2006). Magnetic nanoparticles are formed from materials like Fe, nickel (Ni), cobalt (Co) and alloys of a mixture of magnetic materials can be strongly attracted by magnets or be magnetized (Liu, 2006). Application of Fe nanoparticles in information storage has led to production of magnetic disks which have 100 times the storage capability of previous disks (Parker, 2001).

Metallic nanoparticles can provide resource savings through improvements in energy storage devices and more efficient use of renewable energy resources (Rickerby and Morrison, 2007). The use of nanostructured electrodes in rechargeable lithium batteries has been proven to be effective and safe (Klabunde, 2001). In this regard, nanostructured materials such as Co oxide, Fe fluoride, Co chloride, rubidium oxide and Ni phosphate can be employed (Rickerby and Morrison, 2007). Metal/metal oxide nanoparticles have the potential for the production of more efficient solar cells (Klabunde, 2001). Nanoporous silicon has been successfully applied to manufacture more effective solar cells; moreover, inexpensive organic solar cells have been developed using TiO_2 nanoparticles coated with an organic dye (Rickerby and Morrison, 2007). Metal oxides catalysts have an important role in the production of petrochemicals and fuel cells (Rickerby and Morrison, 2007) and it is claimed that heterogeneous catalysis would be achieved by the application of metal nanoparticles (Klabunde, 2001). Nanoporous Al is the most common metal nanoparticle that is being served as a supporting material for car exhaust catalysts (Luther, 2004). Gold nanoparticles are also used for their catalytic activities (Nowack and Bucheli, 2007).

In the range of cosmetics, metal oxide nanoparticles (e.g. TiO_2 and ZnO nanoparticles) are increasingly being used in sunscreens (Luther, 2004). Application of nanoparticles in medicine is an emerging area which can improve drug delivery, diagnosis and imaging (Nel et al., 2006). Silver and ZnO nanoparticles can be used as antibacterial agents (Becheri et al., 2008; Zhang et al., 2007).

Nanoparticles can enhance the functionality and durability of paints and coatings (Rickerby and Morrison, 2007). Gold nanoparticles can be used to give paints a metallic radish colour due to their special optical properties (Klabunde, 2001). Titanium dioxide and ZnO nanoparticles can be used as pigments in paints (Adams et al., 2006). Titanium dioxide nanoparticles have been used to manufacture self-cleaning paints and glasses (Becheri et al., 2008; Klabunde, 2001; Tran et al., 2005).

Environmental application of nanoparticles can be categorized into three fields: environmental monitoring, remediation and resource saving through production of sustainable products (Rickerby and Morrison, 2007; Tratnyek and Johnson, 2006). Nanocrystalline metal oxide thin films of indium, tin, ZnO and tungsten oxide have been used to manufacture faster gas sensors with higher spatial resolution and lower running costs for monitoring air pollution (Rickerby and Morrison, 2007). Iron oxide nanoparticles have been used to reduce contaminants such as chlorinated hydrocarbons (Burleson et al., 2004) and arsenic (Hartley et al., 2004) in groundwater. Incorporation of metal/metal oxide nanoparticles in manufacturing better batteries and solar cells may be examples of their application for increasing efficient use of natural resources and energy (Rickerby and Morrison, 2007). Future application of manufactured nanoparticles in improving the efficiency of fertilizer products could have positive effects on farm costs and the environment (e.g. decrease energy use and decreased fertilizer application). Nanofertilizers could be tailored to synchronize the release of the nutrients with plant demands and prevent interactions of nutrients with soil, water and microorganisms which may immobilize nutrients (DeRosa et al., 2010).

1.2.4 Production methods of metal oxide/metal nanoparticles

There are a wide variety of methods that are used to produce nanoparticles. The fundamental approaches in nanoparticle fabrication can be categorized into two groups: bottom-up methods and top-down methods. However, hybrid techniques using both of these methods are under exploration.

Top-down methods

Top-down methods reduce macroscopic particles to nano-size scale by high energy ball milling, mechano-chemical processing, etching, electro-explosion, sonication, sputtering or laser-ablation (Luther, 2004). These methods usually are not suitable for generating uniformly shaped nanoparticles (Schmid, 2001). The nanoparticles produced may or may not have properties different from those of the bulk material from which they were developed (USEPA, 2005; Zhang, 2003). Metal oxanes are examples of a top-down procedure in which a mineral is cut into smaller parts by an organic acid in aqueous solution (Wiesner et al., 2006).

Bottom-up methods

Bottom-up methods represent constructing nanomaterials from basic building blocks such as atoms or molecules (Tavakoli et al., 2007) and usually include aggregation of atoms or molecules in solution or in the gas to form particles with distinctive size, shape and structure (Schmid, 2001). The nanoparticles produced have properties different from those of the bulk material. Synthesis of metal nanoparticles using bottom-up methods can be achieved through approaches identified as gas phase or chemical phase synthesis (Schmid, 2001).

In gas-phase synthesis, metals are vaporized using a combustion flame, laser beam or plasma torch as the heat source. Then, the vaporized metals are rapidly condensed to form nanoparticles. A feature of this method is high agglomeration of metal nanoparticles produced. To overcome this, reduced pressure environments, inert gas condensation, or surface chemical coverings have been employed (Schmid, 2001). Therefore, chemical synthesis of nanoparticles which will be described later has been identified as the method of preference.

The chemical routes for the manufacture of nanoparticulate metals are based on the reduction of positively charged metal atoms by chemical reductants or decomposition of organometallic precursors with extra energy to form atoms followed by aggregation of atoms (Schmid, 2001; Tavakoli et al., 2007). A simplified scheme of the chemical synthesis of metal nanoparticles is shown in Figure 2. Molecular hydrogen, citrate, alcohol, borohydrides, hydroxylamine hydrochloride, formaldehydrate, carbon monoxide and many other reducing agents have been used as chemical reductants (Schmid, 2001). Moreover, the energy required for decomposition of metal precursors can be supplied through thermal energy, electricity, photoenergy (ultraviolet and visible light) or sonochemical energy (Tavakoli et al., 2007). A vital step in production of metal nanoparticles, independent of the method used, is stabilising their growth and dispersion. Surface ligands such as organic polymers (poly vinyl pyrrolidone, poly vinyl alcohol or poly methyl ether) or surfactants provide stabilizing agents that control nanoparticle growth and solubility, prevent aggregation and limit surface oxidation of nanoparticles (Lin and Samia, 2006; Schmid, 2001).

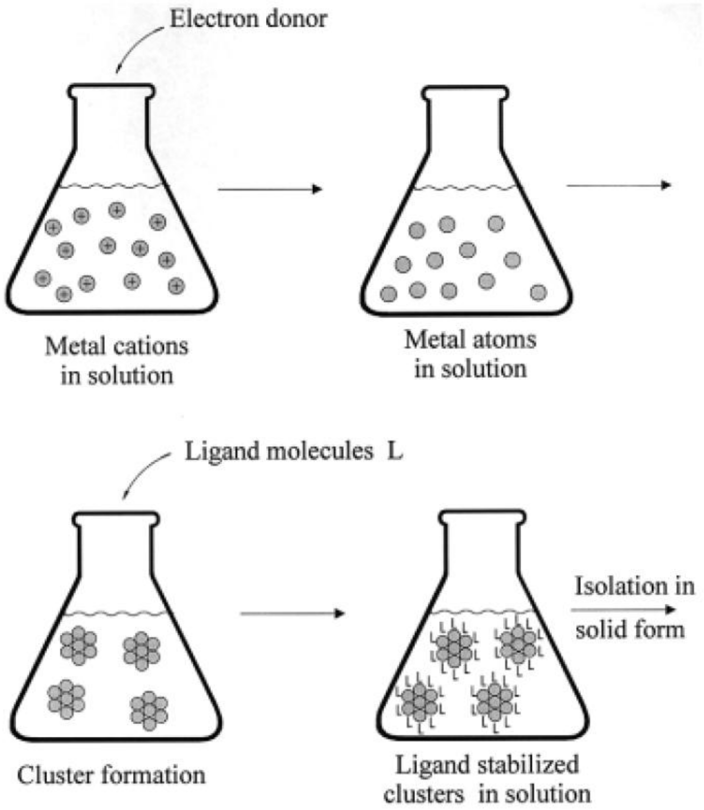

Figure 2- Schematic procedure of production of metal nanoparticles using chemical approaches (Schmid, 2001).

1.3 Physico-chemical characteristics of nanoparticles

The chemical and physical properties of nanoparticles are vastly different from those of their bulk materials as a result of their small size, chemical composition and surface structure (Nel et al., 2006). Nanoparticles exhibit unique properties such as high specific surface area and surface reactivity, particle aggregation and agglomeration, photoemission, high conductivity and catalytic activities (Liu, 2006). Understanding these unique properties as well as the processes that play key roles in the fate of nanoparticles is an essential step to assess their efficacy and environmental impact. This information may enable the modification of the characteristics of the nanoparticles in order to avoid their possible adverse effects, and hence lead to more sustainable application in the environmental and industrial sectors.

1.3.1 Specific surface area

Reactions of particles mainly occur at their surfaces; therefore the molecules and atoms at the surface play an important role. Generally, surface atoms and molecules are different from those in the bulk materials because they may be unsaturated or bonded to the surrounding species such as solution species, gas molecules or near solid materials rather than to the bulk atoms (Burleson et al., 2004).

In the case of nanoparticles, as the particle size decreases, the proportion of atoms and molecules at the surface or near-surface regions increases (Nel et al., 2006). Exposure of a greater proportion of atoms and molecules at the surfaces leads to a considerable enhancement in specific surface area (Wigginton et al., 2007). Nanoparticles may have specific surface areas in the hundreds of square meters per gram (Powers et al., 2006), as shown for nano-sized Fe in Figure 3.

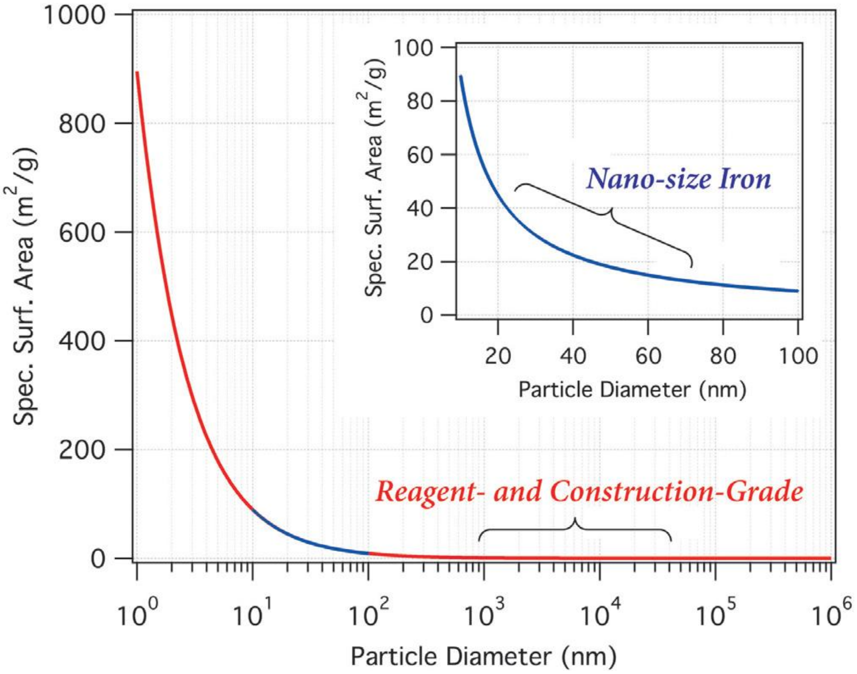

Figure 3 – Specific surface area as a function of particle diameter (Tratnyek and Johnson, 2006).

Accordingly, the surface free energy of the particle will change as a function particle size and the thermodynamics of chemical reactivity will be influenced (Wigginton et al., 2007). An abrupt and significant increase in the particle reactivity is observed as particle size decreases toward atomic clusters or individual atoms. Therefore, it can be speculated that chemical and biological reactivity of nanoparticles per given mass is more than that of larger particles of the same material (Wigginton et al., 2007). The enhanced activity of nanoparticles may be positive and responsible for desirable reactions such as antioxidant activity and drug delivery through cellular barriers, or may be negative by causing toxicity, induction of oxidative stress or cellular dysfunction (Oberdorster et al., 2005).

1.3.2 Surface charge

The surface charge of particles is an important feature which determines their dispersion/stability and affects their mobility in the environment (Darlington et al., 2009; Powers et al., 2006). The surface charge of metal oxide particles is known to be dependent on the pH, composition and ionic strength of the surrounding solution and adsorbed species on the particle surface (Degen and Kosec, 2000; Illes and Tombacz, 2006; Powers et al., 2006). Recent studies have shown that surface charge density of metal oxide nanoparticles is highly size-dependent (Abbas et al., 2008; Vayssieres, 2009). Theoretical modelling of the surface charge for the spherical metal oxide nanoparticles in the size range of 1-100 nm showed a considerable increase in the surface charge of nanoparticles as the particle size decreased to diameters less than 10 nm (Abbas et al., 2008). Experimental investigations on spherical maghemite nanoparticles with average sizes of 3.5, 7.5 and 12 nm also confirmed higher surface charge of particles with smaller size (Vayssieres, 2009). Their results demonstrated a shift in the pH value of the point of zero charge (PZC where the net surface charges is zero) toward higher pH values with a decreasing particle size. Although fundamental reasons for this change are not fully understood, it is speculated that that surface curvature of metal oxide nanoparticles may affect their surface charge density. This hypothesis is based on the assumption that with decreasing size of particles into the nano-range, the radius of curvature of interference increases, which may lead to decrease in electrostatic repulsion between surface charged sites and consequently an increase in the number of charged sites at the surface of the nanoparticle (Abbas et al., 2008; Vayssieres, 2009). The higher PZC for smaller particles also can be attributed to desorption of more protons from smaller particles at a negatively charged medium which decreases the acidity of the surface (Vayssieres, 2009). The size-dependant charge of metal oxide nanoparticles may significantly affect their sorption behaviour (Qafoku, 2010). Therefore, mobility and bioavailability of nanoparticles can be largely different compared to bulk particles from the same material.

1.3.3 Aggregation and agglomeration

Aggregation and agglomeration of nanoparticles due to the presence a liquid-solid interface is one of the features which distinguish nanoparticles from their analogous soluble ions. Nanoparticles can readily form aggregates/agglomerates because smaller particles have relatively higher surface energy and the process of particle attachment can minimize free enthalpy of the system by decreasing the surface area at constant volume (He et al., 2008; Vayssieres, 2005). Although the terms agglomeration and aggregation are frequently used indiscriminately, they have distinct meanings in particle science. Nichols et al. (2002) described agglomerate as an assembly of particles that are rigidly bound by fusion sintering or growth, while aggregates are loosely bound particles that are readily dispersed. The state of aggregation or agglomeration has a significant impact on the nanoparticle reactivity and hence their fate, mobility and bioavailability in the environment. Nevertheless, studies on the environmental fate and behaviour of nanoparticles are mostly focused on aggregation state and disaggregation or dispersion as a result of biological or environmental activities (Nowack and Bucheli, 2007).

For nanoparticles, long-range forces between particles are believed to be controlled by Brownian motion which may bring nanoparticles in contact and result in the attachment of particles (Hotze et al., 2010). On the other hand, thermodynamics of surface interactions in the short range are attempted to be described by the classical DLVO model (Derjaguin, Landau, Verwey and Overbeek model) initially developed for aggregation of colloidal particles. It is assumed that as the size range of colloids overlap with the size range of manufactured nanoparticles, the same principals could apply (Vayssieres, 2005). In DLVO theory, named after Derjaguin and Landau (1941) and Verwey and Overbeek (1948), the aggregation and stability of particle dispersions is described in terms of potential energy curves, which are determined by the sum of the attractive and repulsive forces between individual particles (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948). The attraction between particles is due to the van der Waals force (V_A). The interaction of the electrical double layer surrounding each particle is called the electrostatic repulsive force (V_R). At very small distances repulsion between atomic electronic clouds is also effective which is called Born repulsion (Stumm and Morgan, 1996). As it is illustrated in Figure 4, particles can have a net attraction at a primary or secondary minimum. Particles at the distance of the primary minimum are considered to aggregate irreversibly, while particles at the secondary minimum are reversibly aggregated and can be separated if shear forces are exerted (e.g. stirring, sonication) (Hotze et al., 2010). It has been suggested that DLVO forces alone are not sufficient to predict aggregation behaviour of manufactured nanoparticles. For example, surface coatings (e.g. adsorbed polymers or polyelectrolyte coatings or natural organic matter) are features of manufactured nanoparticles which introduce steric repulsion forces and therefore these coated nanoparticles may have only a net attraction at the secondary minimum. Therefore, an extended DLVO model has been proposed which considers additional short range forces such as steric, bridging, osmotic, hydrophobic Lewis acid-base and magnetic forces (Hotze et al., 2010).

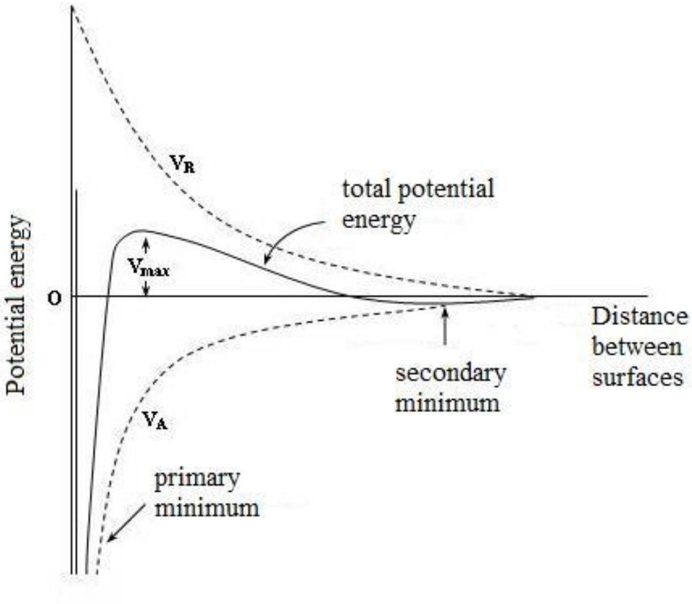

Figure 4– The physical DLVO model for colloid stability. Curve of the total potential energy of interaction corresponding to van der Waals attraction (VA) and electrostatic repulsive force (V_R) as a function of distance between surfaces.

It has been found that similar factors to those controlling aggregation of colloids also affect nanoparticle aggregation. These factors include pH, ionic strength, solution composition, particle size and surface chemistry (He et al., 2008). In metal oxide nanoparticle suspensions, at the pH where net surface charge is zero (i.e. pH = PZC), the highest rate of aggregation occurs at low ionic strength (Guzman et al., 2006; Illes and Tombacz, 2006). At pH values far from the PZC, particles have strongly repulsive charged double layers and therefore, overall particle interactions are repulsive which results in a stable suspension of metal oxide nanoparticles (Guzman et al., 2006).

However, as the ionic strength increases there is a decrease in the diffuse double layer around nanoparticles, aggregation will be enhanced and larger aggregates will be formed at any pH (Brant et al., 2005; Illes and Tombacz, 2006). The solution composition may also affect degree of aggregation. Since the tendency of cations to compress the diffuse double layer increases in the order of $Na^+ \leq K^+ \leq Mg^{2+} \leq Ca^{2+}$, divalent cations may reduce the thickness of diffuse double layers more than monovalent cations and consequently lead to an decrease in electrostatic repulsion between particles, causing aggregation.

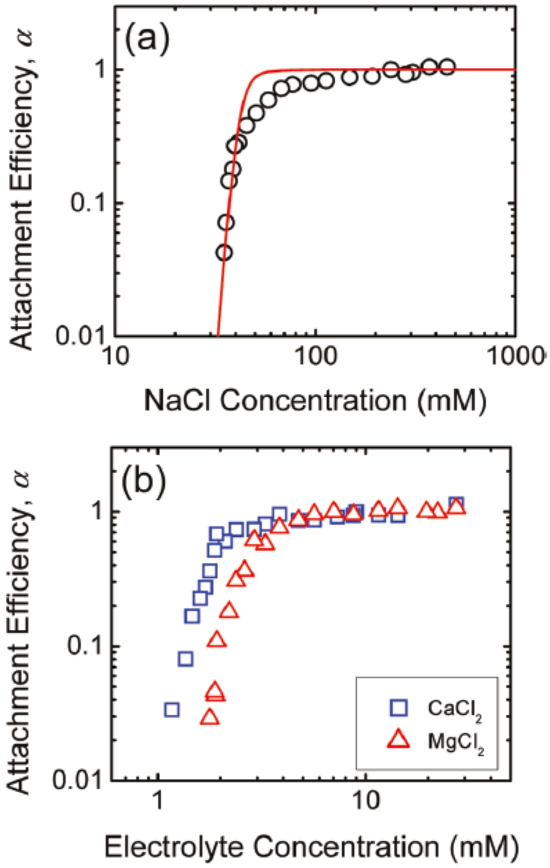

Figure 5- Attachment efficiency of Ag nanoparticles as a function of ionic strength in (a) NaCl and (b) $CaCl_2$ and $MgCl_2$ solutions at pH 7 showing the influence of the presence of divalent cations in the electrolyte solution enhancing aggregation of Ag nanoparticles compared to solutions containing monovalent cations (Huynh and Chen, 2011).

He *et al.* (2007) investigated the effect of particle size on nanoparticle aggregation and reported that at the same ionic strength and pH conditions, different sizes of particles tended to aggregate differently. At the same ionic strength, smaller particles had higher aggregation rates due to the different surface energy of smaller particles compared to larger materials (He et al., 2008). Moreover, surface ligands or surfactants provide stabilizing organic shells that prevent nanoparticle aggregation (Lin and Samia, 2006). Adsorption of highly charged, macromolecular organic matter on the metal oxide nanoparticle surfaces leads to an enhanced electrostatic and steric stabilization of nanoparticles (Illes and Tombacz, 2006). The presence of natural organic matter and surfactants in the natural environment can alter the aggregation behaviour of nanoparticles upon release into the environment. Therefore, a thorough understanding of the aggregation behaviour of nanoparticles may help to predict mobility and availability of nanoparticles in the environment.

1.3.4 Dissolution

Solubility and dissolution kinetics of particles are size dependent (Borm et al., 2006). As mentioned earlier, a decrease in the size of particles leads to higher surface area and consequently larger free energy at the surface of nanoparticles compared to bulk materials. For particles less than 1 µm, the surface free energy becomes very large which may affect the thermodynamics of dissolution (Stumm and Morgan, 1996). This excess energy at the surface of particles is expressed as surface tension. In theory, the solubility of solids depends on the surface tension, which is correlated with the specific surface area and thus also with the particle size (Stumm and Morgan, 1996). A number of studies have been made early in the 20th century to measure solubility of finely divided solids as a function of particle size. The resulting expression for excess solubility of small spherical particles which was developed from the Gibbs-Thompson equation for liquid-vapour systems was proposed by Ostwald (1900) and further refined by Freundlich which is referred to as the Ostwald-Freundlich equation (Freundlich, 1926; Ostwald, 1900):

$$\frac{RT\rho}{V_m} \, ln \frac{S}{S_0} = \frac{2\gamma}{r}$$

where, S is the solubility of small particles with size r (mol kg^{-1}), S_0 is solubility of bulk particles (mol kg^{-1}), R is the gas constant (8314.5 mJ mol^{-1} K^{-1}), T is the absolute temperature (K), V_m is molecular weight of solid in solution (mol m^{-3}), ρ is the density of solid (kg m^{-3}) and γ is the surface tension (mJ m^{-2}). According to the Ostwald-Freundlich equation, the relative solubility of spherical particles of the same material increases as the particle size decreases in the solid-liquid system. Dissolution is a dynamic process which has been attempted to be explained by thermodynamically based Ostwald-Freundlich equation. Experimental findings on particles have been found not always to follow these thermodynamic considerations proposed by the Ostwald-Freundlich equation. Tang et al. (2004) described a “self inhibition” phenomenon for sparingly soluble salts in which decrease in dissolution and eventually dissolution suppression was observed as the particle size reached within 50r of the critical value (r is the critical value) which is calculated as:

$$r = \frac{2\,\gamma_{SL}\,\Omega}{KT\, lnS}$$

where, γ_{SL} is the surface tension, Ω is volume occupied by each growth unit, K is Boltzman constant and S is the supersaturation term which represents ratio of the solute concentration to saturated value (dimension less). Nanoparticles fall into this critical value and therefore dynamically stabilized suspension of nanoparticles in an under-saturated solution can be found despite their thermodynamically unstable state.

The dissolution kinetics (rate) of a particle has been found to be directly proportional with the surface area because a relatively larger interface for dissolution is available which promotes diffusion of dissolved ions away from the particles (Borm et al., 2006; Sasson et al., 2007). The relationship between dissolution rate and the surface area of particles is expressed by the Noyes-Whitney equation (Sasson et al., 2007):

$$\frac{dC}{dt} = \frac{DA\,(C_S - C_B)}{h}$$

where, A is surface area of the solid (cm^2), h is diffusion layer thickness (cm), Cs is the saturation solubility of the bulk particles ($\mathrm{mg\ kg}^{-1}$), C_B is the concentration of solid in the bulk dissolution medium ($\mathrm{mg\ kg}^{-1}$) and D is the diffusion coefficient of the solid ($\mathrm{cm}^2\ \mathrm{s}^{-1}$).

In addition to particle size and surface area, surface morphology and curvature can affect the dissolution rate of particles (Tang et al., 2004). Borm et al. (2006) reviewed the effect of morphology, agglomeration and adsorbed species on particle solubility and concluded that smaller particles with more convex features, and therefore a thinner diffusion double layer, have faster dissolution rates compared with concave features existing in a particle.

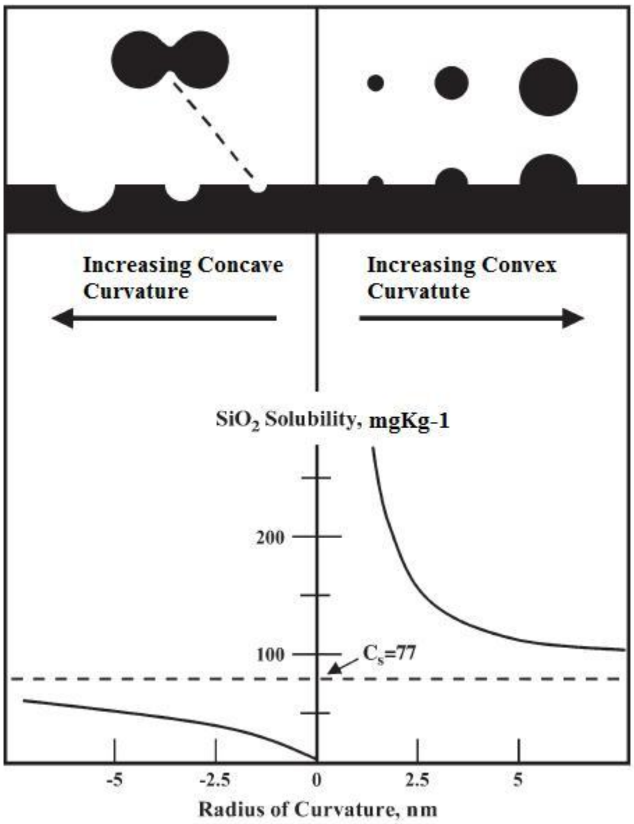

Figure 6– Representation of the effect of curvature features on solubility of amorphous Si which has saturation solubility equal to 77 mg kg^{-1} (Borm et al., 2006).

The unusual dissolution behaviour of zirconium oxide nanoparticles reported by Voglesberger et al. (2008) can be explained by growth of particles in supersaturated suspension of nanoparticles. In their experiments, a high dissolution of oxide nanoparticles was observed at the beginning of the dissolution process when the saturation concentration was exceeded, followed by a decrease in dissolution rate (Vogelsberger et al., 2008). The supersaturated suspension resulting from the high dissolution of nanoparticles may undergo Ostwald ripening or coalescence to form particle aggregates which reduced the dissolution rate.

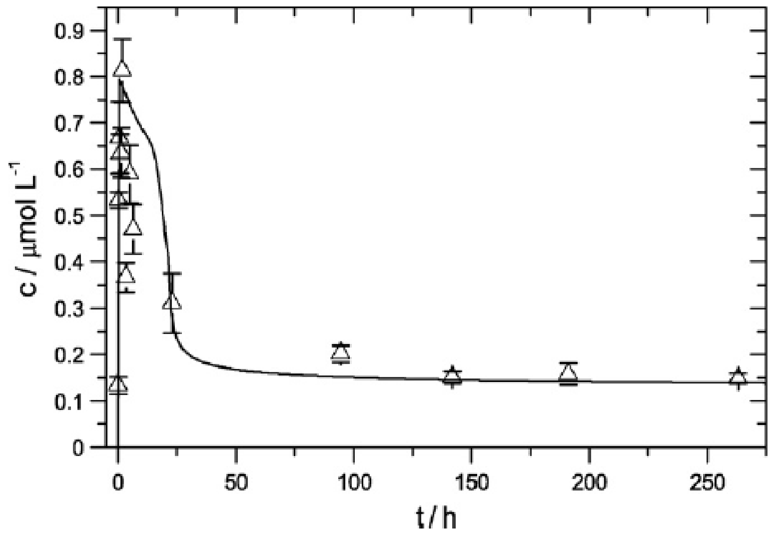

Figure 7- Unusual dissolution kinetics of zirconium oxide (with radius of 21.3 nm) in water which shows high solubility at the beginning of the dissolution process and a decrease in dissolution over time. In this graph, concentration of dissolved zirconium (c) is plotted as a function of time (t) (Vogelsberger et al., 2008).

1.3.5 Deposition

Deposition is defined as the transport of particles to an immobile surface followed by attachment (Lecoanet et al., 2004). Two main processes may cause deposition of colloids - and therefore nanoparticles - in soil. Firstly, straining or physical filtration where nanoparticles are larger than the pore size and are trapped; and secondly, true filtration (Nowack and Bucheli, 2007). In true filtration, nanoparticles are removed from the solution by three mechanisms: interception, which is the contact of a particle moving across the streamline with the solid phase due to its infinite size; gravitational sedimentation as a result of settling of particles with greater density than the fluid; and Brownian diffusion, which can induce contact with the solid phase (Tufenkji and Elimelech, 2004) (Figure 8). In the case of nanoparticles, gravitational sedimentation is negligible (Guzman et al., 2006) and collisions with the solid phase are predominantly through Brownian motion (Lecoanet et al., 2004; Nowack and Bucheli, 2007).

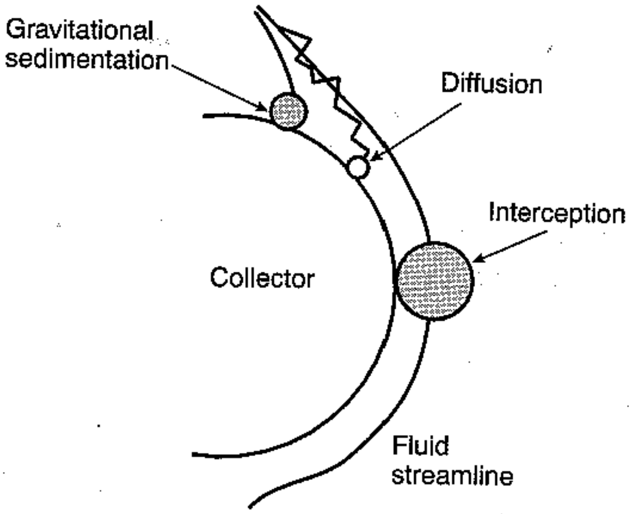

Figure 8 – Three mechanisms of the attachment of particles on the solid phase (Logan, 1999).

There are different hypotheses about the influence of particle size on the deposition process. One view argues that, based on electric double layer interactions, the deposition rate is dependent on particle size, and nanoparticles undergo collisions with the collector more frequently than larger particles due to Brownian motion (Hydutsky et al., 2007). However, Elimelech and O'Melia (1990) concluded that collision efficiency is independent of particle size and therefore deposition concentration is insensitive to particle size. The other view suggested by Lecoanet et al. (2004), who investigated the attachment efficiency for Si particles and found that deposition rate for the larger Si particles was one order of magnitude larger than for smaller Si particles. These contradictory views on particle deposition suggest results in porous media indicate that further work is needed to determine the effect of particle size on the deposition rate of nanoparticles in soil systems.

1.4 Behaviour of nanoparticles in aquatic environments

The predicted increase in the production and use of manufactured nanoparticles will likely lead to release of a portion of these materials into the environment and increasing exposure to humans and organisms will likely occur. The ultimate sinks of nanoparticles which have been released into the environment are soils and water/sediments, although they may first be released into the atmosphere (Nowack and Bucheli, 2007). The deliberate application of nanoparticles to remediate contaminated soils and groundwater, or potential use as fertilizers to deliver nutrients to crops, may release large amounts of manufactured nanoparticles into the environment. The interactions of nanoparticles with environmental media can affect their physical and chemical properties, such as size and surface chemistry (Darlington et al., 2009). Therefore, it is important to examine the fate and reactions of these nanomaterials in aquatic and terrestrial environments to understand their potential mobility, speciation, availability and effects on organisms and ecosystems. Much is already known regarding the behaviour of naturally occurring nanoparticles in the environment (Buffle, 2006; Lead and Wilkinson, 2006) which will help us understand the consequences of dispersing manufactured nanoparticles in the environment. However, the environmental behaviour of manufactured nanoparticles may be different due to their higher mobility and reactivity.

Current studies have found that the mobility and reactivity of nanoparticles in the aquatic environment are mainly dependent on the stability of their suspension, degree of aggregation or their interaction with other particles (Chen and Elimelech, 2007; Nowack and Bucheli, 2007). Moreover, surface area, surface charge, coating, purity and solubility of nanoparticles may influence their fate and reactivity in aquatic environments (Franklin et al., 2007). Surface properties of nanoparticles play a dominant role in their sorptive properties in aquatic systems (Nowack and Bucheli, 2007). Consequently, they may mediate and facilitate the transport of contaminants, especially metals and radionuclides in these systems (Wigginton et al., 2007).

Batley and McLaughlin (2010) summarized the main physicochemical pathways that govern the fate of nanomaterials in the aquatic environment (Figure 9). Aggregation and subsequent sedimentation, dissolution, adsorption to particulates and other solid surfaces, binding to natural dissolved organic matter, and stabilisation via surfactants are major reactions of nanoparticles in aquatic environments. Natural organic matter present in aquatic systems, as well as concentration and composition of dissolved salts and pH of the water, may affect these pathways.

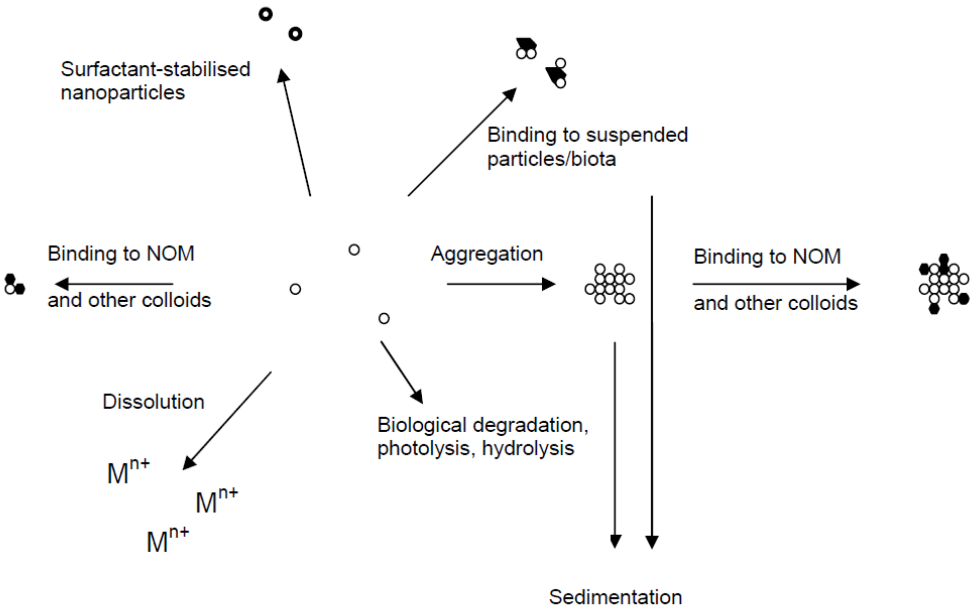

Figure 9– Possible reactions of metal oxide nanoparticles released into the aquatic environments (Batley and McLaughlin, 2010). *With author's permission*.

Dissolved natural organic matter (e.g. phenolic compounds, humic and fulvic acids) in natural waters is a heterogeneous mixture of mainly acidic products which is rich in acidic functional groups (Illes and Tombacz, 2006). Complex formation between organic functional groups and active sites on the surface of metal oxide nanoparticles may lead to adsorption of natural organic matter on the nanoparticle surfaces and consequently production of negatively charged nanoparticle surfaces, which reduce nanoparticle aggregation through combined steric and electrostatic repulsions (Espinasse et al., 2007; Franklin et al., 2007; Illes and Tombacz, 2006). Specific groups of natural organic matter, particularly polysaccharides, which can be produced by algae or bacteria in natural waters, may cause the opposite phenomena (e.g. increased aggregation) by a binding to nanoparticles (Buffle et al., 1998; Espinasse et al., 2007). Therefore, the nature of the organic matters in aquatic environments may affect aggregation and reactivity of nanoparticles.

Dissolved salts in natural waters may affect the aggregation of metal-based nanoparticles in aquatic systems. High concentrations of ions in natural waters (which are typically greater than 0.001 mol L^{-1}) may reduce electrostatic repulsion between nanoparticles as a result of the compression of electric double layers and therefore large aggregates may be formed (Brant et al., 2005; Sun et al., 2007). Large aggregates may be immobilized by settling out of suspension which may reduce the toxicity and exposure of these materials (Brant et al., 2005; Zhang et al., 2008). He et al. (2008) found an increase in the hydrodynamic diameter of Fe oxide nanoparticles with increasing NaCl concentration (ionic strength) (Figure 10). At the same ionic strength, smaller nanoparticles produced larger aggregates (He et al., 2008). However, such predictions may be complicated by interactions between natural organic matter, such as fulvic and humic acids, in natural waters with nanoparticles. Surface charge of nanoparticles can be neutralized by adsorption of natural organic matter and aggregation process would be reduced (Baalousha et al., 2008; Tso et al., 2010).

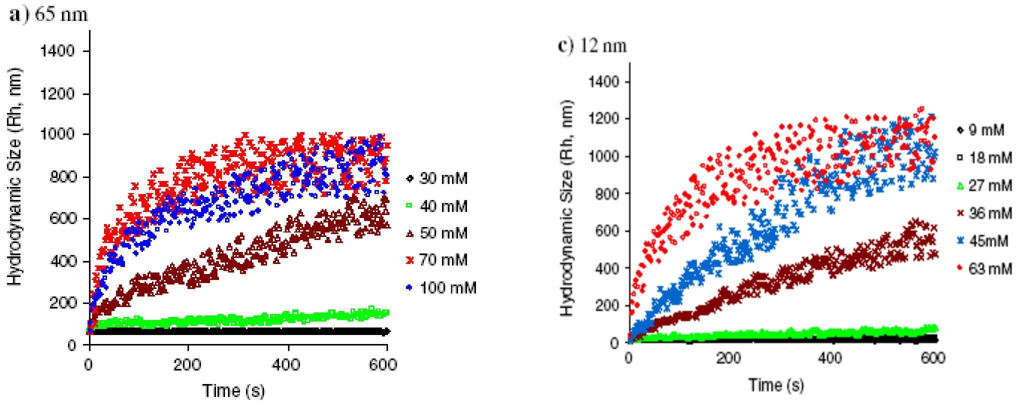

Figure 10- Effect of electrolyte concentration (NaCl) on aggregation of Fe oxide nanoparticles with different particle sizes (12 and 65nm) at pH 5.7 (He et al., 2008).

The composition of natural waters may influence the behaviour and activity of nanoparticles in aquatic systems. Studies on the effect of monovalent (NaCl) and divalent ($MgCl_2$ and $CaCl_2$) electrolyte solutions on aggregation of metallic nanoparticles have revealed more effective destabilization of the suspension of nanoparticles with increase in the valence of electrolyte through compressing the diffuse double layer and increased aggregation (Huynh and Chen, 2011; Tso et al., 2010). Although natural organic matter in aquatic environments is able to reduce aggregation of nanoparticles, it cannot effectively stabilize nanoparticles at the presence of high concentrations of Ca^{2+} (Domingos et al., 2009; Huynh and Chen, 2011; Liu et al., 2011). This effect may be due to aggregation of humic acids macromolecules via bridging with Ca which subsequently aggregates the nanoparticles (Liu et al., 2011). Huynh and Chen (2011) investigated the aggregation kinetics of coated Ag nanoparticles in the presence and absence of humic acids in different concentrations of $CaCl_2$ electrolyte. The attachment efficiency which is defined as aggregation rate constant in the solution of interest to the rate constant obtained under favourable (nonrepulsive) aggregation condition was calculated to quantify the aggregation kinetics of Ag nanoparticles. The results showed that at $CaCl_2$ concentrations lower than 9 mM, the attachment efficiency of Ag nanoparticles in the presence of humic acids was smaller than in the absence of humic acids due to electrostatic repulsion which aids stabilization of nanoparticles (Figure 11). However, at higher concentrations of $CaCl_2$, the attachment efficiency of Ag nanoparticles in the presence of humic acids exceeded unity and was enhanced with increasing the concentration of $CaCl_2$ in the electrolyte.

The other important factor in the behaviour of metallic nanoparticles in aquatic environments is solution pH. Guzman et al. (2006) examined the effect of solution pH on the aggregation of TiO_2 nanoparticles. The authors showed that solution pH changed the surface energy and consequently the size of the aggregates. As the pH approached the PZC, nanoparticles were highly aggregated and aggregate sizes increased (Guzman et al., 2006).

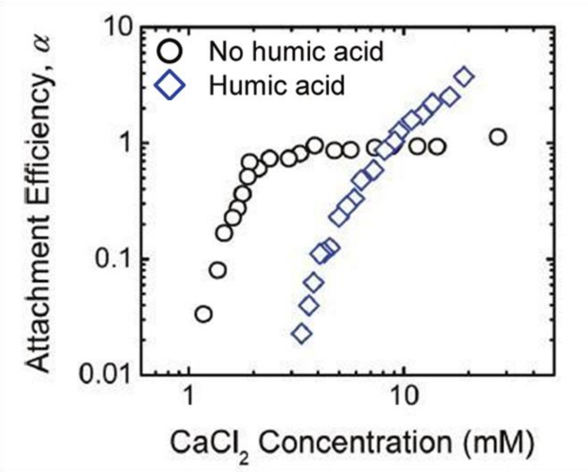

Figure 11- Attachment efficiencies Ag nanoparticles in the absence and in the presence of humic acid (1 mg/L TOC) as functions of $CaCl_2$ concentration at pH 7 (Huynh and Chen, 2011).

1.5 Nanoparticles in terrestrial environments

Although terrestrial environments are among the most significant pathways by which nanoparticles may enter the food chain, information about the fate, mobility and transformations of nanoparticles in these environments is limited (Klaine et al., 2008). The complexity of the soil matrix and the difficulties associated with nanoparticle detection in the presence of natural nanoparticles in soils presents a significant challenge to increasing our understanding of their fate and behaviour in the terrestrial environment (Klaine et al., 2008). Limitations in the detection of nanoparticles originally added to soil or sediments make it difficult to distinguish between natural soluble metal and particles and direct effects of manufactured nanoparticles and indirect effect of components released from manufactured nanoparticles (Bernhardt et al., 2010). The most important reaction pathways which may affect fate and behaviour of nanoparticles in terrestrial environments are dissolution, aggregation, partitioning between solution and solid phase and nanoparticle mobility (Figure 12).

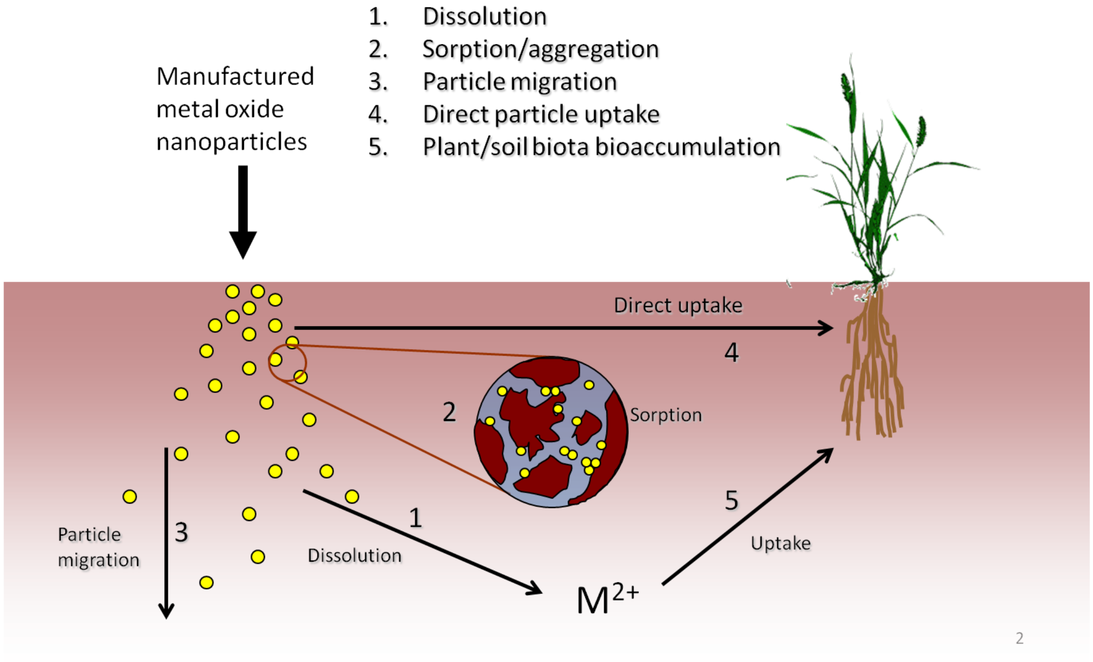

Figure 12- Key pathways affecting the fate of nanoparticles in soils. *Reproduced from* (Klaine et al., 2008).

1.5.1 Dissolution

Dissolution of nanoparticles in the terrestrial environment is a critical process determining the bioavailability and possible toxicity of nanoparticulate metals (Lin et al., 2010). Thermodynamics of dissolution mentioned earlier suggests greater and faster dissolution for nanoparticles compared to bulk particles from the same material. However, investigations showed that the properties of the media such as pH, ionic strength or presence of organic matter would affect the dissolution of nanoparticles through their affect on aggregation (Bian et al., 2011; Guzman et al., 2006; He et al., 2008; Huynh and Chen, 2011; Tso et al., 2010) and therefore introduction of a kinetic hindrance to the diffusion process (Borm et al., 2006). Hence, solubility and dissolution kinetics of nanoparticles in soil solution can effectively be influenced by soil properties such as pH, ionic strength, organic matter content and adsorbing minerals.

The acidic soil pH values which are far from PZC of metal oxide nanoparticles can promote dissolution of nanoparticles by direct proton attack to the surface of nanoparticles and formation of hydrolysis species in soil solution (Bian et al., 2011). However, as the soil pH reaches the PZC of the manufactured nanoparticles, the greatest aggregation occurs (Illes and Tombacz, 2006) which may be followed by deposition and reduction in the dissolution of nanoparticles. High concentration of dissolved salts (high ionic strength) and composition of cations (e.g. high percentages of divalent cations) in soil solution may also affect dissolution of nanoparticles through their impact on facilitating the aggregation process of nanoparticles which limits dissolution. Moreover, aqueous ligands (e.g. Cl, phosphate) in soil solution might enhance dissolution of nanoparticle by formation of surface complexes which subsequently might increase release of ions from the surface of particles (Stumm, 1987). In soil systems, a vast variety of organic matter (fulvic and humic acids, polysaccharides, etc.) and plant-derived compounds are present which may affect dissolution behaviour of nanoparticles depending on their charge and rigidity of functional groups (Ghosh et al., 2008). Coagulation of metal nanoparticles with clays also reported to be responsible for reduced dissolution of nanoparticles in soils containing high clay contents (Cornelis et al., 2010; Cornelis et al., 2011).

Dissolution of nanoparticles in soils can depend on the type of nanoparticles and their surface properties (e.g. modification with surface capping agents such as citrate, polymers or surfactants). Gimbert et al. (2007) showed that ZnO nanoparticle stabilized in sodium dodecyl sulphate (SDS) surfactant did not undergo dissolution for two weeks (Gimbert et al., 2007). In contrast, complete dissolution of ZnO nanoparticles without surface modification was reported in soil pore water so that free nanoparticle or aggregates of manufactured ZnO nanoparticles were not detected (Kool et al., 2011).

In general, a complex combination of different soil parameters would affect the dissolution behaviour of each type of nanoparticle in soil. Therefore, mechanistically understanding of parameters influencing solubility and dissolution kinetics of nanoparticles in terrestrial environments may help to modify nanoparticles in order to reduce their environmental risks and to benefit their advanced features in different applications.

1.5.2 Aggregation

Aggregation is probably a common phenomenon in soils affecting solubility and deposition of nanoparticles. The only direct evidence of aggregation of nanoparticles in soil was reported by Fang et al. (2009). They investigated the transport of TiO_2 nanoparticles through saturated homogenous soil columns. Comparison of the particle size of TiO_2 suspension introduced to soil columns and size distribution of TiO_2 in the outflow of the soil columns revealed significant increase in aggregate size (Fang et al., 2009). Interactions of cerium oxide (CeO_2, nominal size 20 nm) nanoparticles with the soil matrix and heterocoagulation of nanoparticles with clays in soils due to electrostatic attraction of surfaces with opposite surface charges was also reported by Cornelis et al (2011). These results might also explain the higher retention of Ag nanoparticles (10nm nominal size) in soils containing high clay contents in an earlier investigation by the same authors (Cornelis et al., 2010).

The role of different soil properties in aggregation of manufactured nanoparticles would likely be the same as their influence on aggregation and deposition of naturally occurring nanoparticles in soil. Moreover, experiments conducted in porous media have provided indications of the important factors in aggregation of nanoparticles in soils. Soil parameters such as high ionic strength, composition of soil solution (e.g. presence of Ca^{2+} or anionic ligands such as Cl^-, PO_4^{3-} in the case of positively charged nanoparticles) (Jiang et al., 2010; Lin et al., 2010; Perdrial et al., 2010), soil pH values close to pH_{PZC} of nanoparticle (Guzman et al., 2006; Kanel and Al-Abed, 2011), Presence of polysaccharide based natural organic matter and low content of humic-like substances in soil (Espinasse et al., 2007) can promote aggregation of nanoparticles in soils. Aggregation of nanoparticles can greatly restrict their transport and mobility in soils by deposition of aggregates through straining onto the surface of soil particles (Klaine et al., 2008; Lin et al., 2010).

1.5.3 Partitioning

Solid solution partitioning is one of the key processes which determines the potential mobility and bioavailability of nanoparticles in soils. High surface area and charge of manufactured nanoparticles may result in strong adhesion of nanoparticles to the reactive surfaces of soils (i.e. soil minerals and organic matter) (Klaine et al., 2008).

This may effectively reduce the direct contact of soil biota with nanoparticles and influence their bioavailability. However, sorption/retention of nanoparticles in soils is scarcely investigated. This may be due to lack of effective methods to distinguish natural soluble metal and particles from manufactured nanoparticles and their dissolution products, which may be relevant especially for metal oxide nanoparticles such as ZnO (Kool et al., 2011). With advances in filtration technology, which enable separation of individual nanoparticles or small aggregates of nanoparticles from dissolved species passing through 0.45 µm filters, a study on retention of nanoparticles in soils has been recently reported. Cornelis et al. (2010) developed a method for determination of the retention of metal oxide nanoparticles in soil which can distinguish retention of nanoparticulate materials from solute ions resultant from their dissolution. Application of the method to five soils showed that retention behaviour of Ag and CeO_2 nanoparticles is different from partitioning of bulk and soluble sources of the same materials (Cornelis et al., 2010). The method enabled the study of the effect of different soil factors (such as pH, ionic strength, clay minerals), addition of phosphate, citrate or biosolids in the soil, on retention of CeO_2 nanoparticles in sixteen soils with diverse physicochemical properties (Cornelis et al., 2011). An increase in the spike rate of Ag and CeO_2 nanoparticles resulted in higher retention of nanoparticles, suggesting that interaction with the soil surfaces was not electrostatic, but due to enhanced collision efficiency and aggregation which may deposit nanoparticles at the surface of the soil solid phase (Cornelis et al., 2011). Similar investigations are urgently needed in order to evaluate the retention of nanoparticles in soils and to identify soil properties that control retention. The influence of capping agents or functional groups at the surface of nanoparticles introduced to soils, different exposure rates, and effects of dissolved organic matter in biosolids are some of the main factors requiring evaluation in order to predict the fate of manufactured nanoparticles in soils.

1.5.4 Mobility

The mobility of nanoparticles in soil is one of the major factors which may affect their availability to soil biota, their distribution through the soil profile, and effective persistence in soil (Lead and Wilkinson, 2006; Wiesner et al., 2006). Nanoparticle aggregation and deposition are the main processes which control the mobility of particles in a porous medium (Wiesner et al., 2006). These two processes may be affected by physicochemical properties of the manufactured nanoparticles such as size, surface charge and aggregation rate (Darlington et al., 2009) as well as the characteristics of the soils such as pH, clay content and type, amount and type of soil organic matter, ionic strength and composition of the soil solution, and flow velocity (Schrick et al., 2004).

Deposition on immobile surfaces has been identified as the main process in retarding mobility of nanoparticles through porous media (Lecoanet et al., 2004). Although it is commonly assumed that nanoparticles are highly mobile due to their small size, the large diffusivity of these particles in porous media that produces more frequent contacts may reduce their mobility (Hydutsky et al., 2007; Schrick et al., 2004; Wiesner et al., 2006). However, the rate of deposition may change over time. Migration of nanoparticles (TiO_2, SiO_2, ferroxan, alumoxane, fulleron and single-wall C nanotubes) in a packed bed of silicate beads was assessed by Lecoanet et al. (2004). The authors found that although different nanoparticles exhibited widely different transport behaviour, the rate of deposition decreased over time (Figure 13). The authors suggested this may be due to saturation or blocking of deposition sites within the porous medium. Opposite surface charges of nanoparticles and soil matrix can enhance deposition of nanoparticles due to electrostatic attractions (Darlington et al., 2009). Darlington et al. (2009) reported presence of Al nanoparticles at the point of application in the soil columns due to strong electrostatic attractions of surfaces with opposite charge. Binding of nanoparticles to soil matrix, further decreased the pore size of soil and increasingly restricted the transport of particles in soil columns.

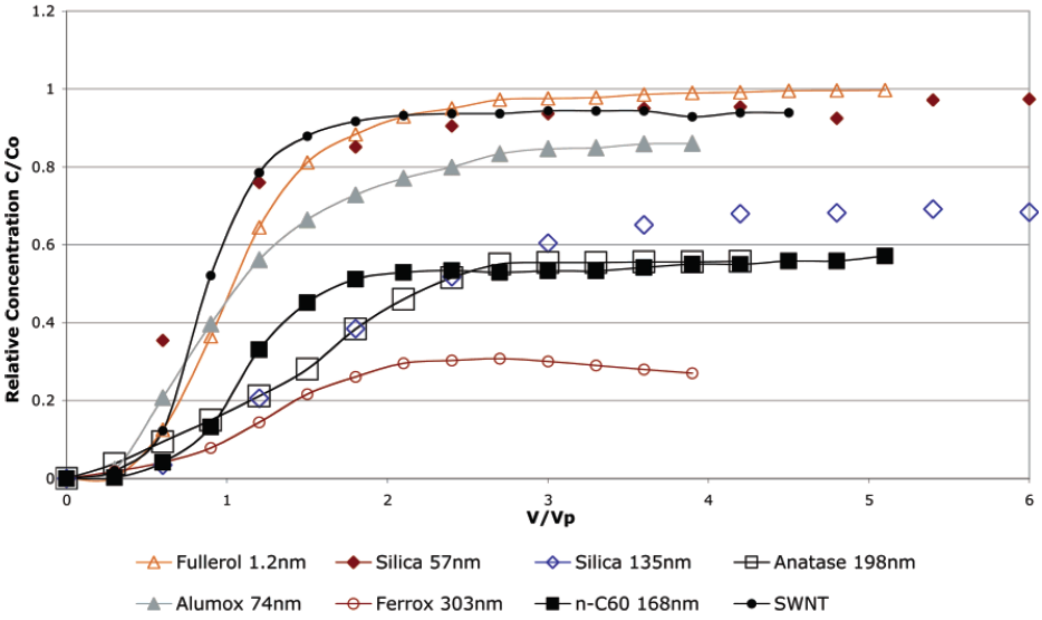

Figure 13- Breakthrough curve of nanoparticles, illustrating normalized concentration of nanoparticle in the effluent to the initial concentration (C/C_0) as a function of volume normalized to pore volume (V/V_p) (Lecoanet et al., 2004).

Aggregation of nanoparticles may also prevent their movement through soil (Darlington et al., 2009). This process may lead to efficient filtration of nanoparticles by an interception mechanism (Lin et al., 2010; Schrick et al., 2004). Hydutsky et al. (2007) investigated the transport of Fe nanoparticles through sand columns and suggested that aggregate straining rather than filtration is an important mechanism in particle capture and therefore large aggregates of nanoparticles may clog soil pores. Therefore, properties of soil systems accelerating aggregation process may be the same characteristics preventing mobility of nanoparticles in soils. Surface coatings of nanoparticles, which have been designed for specific applications of nanoparticles (e.g. Fe nanoparticles used in groundwater remediation), may alter their aggregation tendency. Three classes of coatings are used: polymers; polyelectrolytes; and surfactants and all can alter the particles' surface charge and stabilize them against aggregation and deposition (Wiesner et al., 2006). The effect of polyelectrolytes (Hydutsky et al., 2007) and hydrophilic C material coatings (Schrick et al., 2004) of nano-Fe particles on inhibition of aggregation and attachment efficiency are reported in column studies. Darlington et al. (2009) studied the effect of surface functionalization and solution conditions which mimic surface water conditions on aggregation and transport of Al nanoparticles in soil columns. The results indicated that application of surface modifiers increased long term stability of nanoparticles and enhanced their transport in soil columns whereas high ionic strengths and pH near PZC of Al nanoparticles decreased electrostatic repulsion between nanoparticles and allowed rapid aggregation process which in turn limited mobility by deposition.

1.6 Effect of soil properties on the fate of nanoparticles

A number of studies have investigated the effect of percolating solution properties on the transport and fate of a wide range of nanoparticles through porous media such as clean quartz sand and glass beads (Guzman et al., 2006; Hydutsky et al., 2007; Lecoanet and Wiesner, 2004; Lecoanet et al., 2004; Schrick et al., 2004). Although these investigations inferred possible influences of different soil characteristics on the mobility and fate of nanoparticles, recent investigations showed that the behaviour of nanoparticles in the soil environment (containing organic matter and clay minerals) can be different to that in non-reactive porous media previously studied (Wang et al., 2010). Soil factors such as pH, ionic strength of the soil solution, the presence of organic matter and soil clay content can dominate the fate and behaviour of nanoparticles in soil (Cornelis et al., 2011; Peralta-Videa et al., 2011).

1.6.1 pH

The mobility and aggregation of nanoparticles in soils are strongly affected by the surface charge of the nanoparticles, which is pH-dependent. As the pH reaches the PZC of the manufactured nanoparticles, the greatest self-aggregation (homo-coagulation) occurs (Illes and Tombacz, 2006) which may be followed by deposition. Guzman et al. (2006) investigated the effect of pH on the aggregation and transport of TiO_2 nanoparticles in microchannels of a porous medium fabricated from Pyrex wafers having a diameter of 700 µm with spacing between wafers of 110 µm, resulting in a porosity of 0.414. The authors showed that where the pH of the percolating solution was within one unit of the pH_{zpc} of the nanoparticles, transport was limited due to aggregation and deposition (Guzman et al., 2006). Thus, if a particular nanoparticles product is to remain mobile in the soil environment, it will be important to alter the surface properties of the particle so that the PZC is well outside normal soil pH values. Similarly, a nanoparticle with a high PZC is unlikely to be mobile in soils with net negative charge versa it would be mobile in positively charged soils of tropical regions.

1.6.2 Ionic strength of the soil solution

Concentration of salts in the soil solution may strongly affect the aggregation as well as deposition of nanoparticles. As the ionic strength of the soil solution increases, electrostatic repulsion between particles reduces and van der Waals attractions outweigh the repulsion (Hotze et al., 2010). Therefore, homo-aggregation (nanoparticles-nanoparticle attachment in soil solution) and hetero-aggregation (dissimilar particles such as nanoparticles-clay) in soil solution or attachment efficiency between nanoparticle surfaces and the porous medium in soil may increase (Espinasse et al., 2007; Hotze et al., 2010). The repulsive energy barriers for attachment can be completely screened at a certain ionic concentration for each specific particle which is known as critical coagulation concentration (CCC). At the CCC and above that concentration, the attachment efficiency remains constant (Chen and Elimelech, 2007). In addition to size, shape and type of nanoparticles, surface modifiers and valence of counter ions have principal importance in the CCC value of nanoparticles (Hotze et al., 2010).

Aggregation and deposition of manufactured nanoparticles may be influenced by the ionic composition of the soil solution as was already observed for natural nanoparticles (Perdrial et al., 2010). Divalent ions such as Ca^{2+} or Mg^{2+} have been shown to be more effective in speeding up kinetics of particle deposition than monovalent ions like Na^{+}, and hence particle deposition occurs at much lower concentrations of Ca^{2+} than Na^{+} (Chen and Elimelech, 2007). The same phenomenon may be expected to occur for manufactured nanoparticles in soil solution. Saleh et al. (2008) reported enhanced deposition of Fe nanoparticles in sand columns with an increase in the salt concentration. Moreover, it was observed that at the same ionic strength, Ca^{2+} was more effective than monovalent Na^{+} in reducing the mobility of Fe nanoparticles due to increased attachment of nanoparticles to the sand grains (Figure 14).

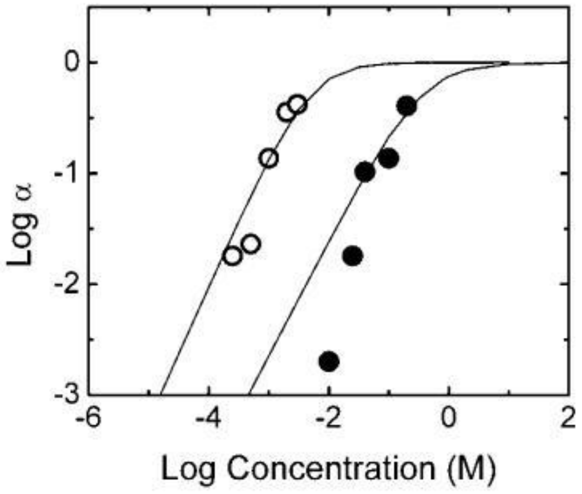

Figure 14- Attachment coefficient of Fe nanoparticles as a function of ionic strength in NaCl (black circles) and $CaCl_2$ (open circles) determined in sand column (Saleh et al., 2008).

From the effect of ionic strength and composition on aggregation and deposition, it can be speculated that saline soils have a higher potential to aggregate and retain manufactured nanoparticles. Moreover, it can be expected that calcareous soils would have a greater tendency to retain nanoparticles. In the same way, sodic soils which are not saline might allow dispersion and higher mobility of nanoparticles.

1.6.3 Organic matter

Organic macromolecules are ubiquitous in the soil environment and their effect on the surface reactions and mobility of nanoparticles in the soil may be the same as their effect on nanoparticles in the aquatic environment. The physicochemical natures of organic matter can dominate the effect of organic matter in stability of nanoparticles (Ghosh et al., 2008). Soil organic matter exists as solid particles (humin) or as dissolved organic matter in soil solution (fulvic and humic acids). Solid state organic matter in soil has variable charge due to H^+ dissociation from carboxylic (-COOH) and to a lesser extent phenolic (-OH) functional groups (Swift, 1996). The resultant negative charge at the surface can effectively adsorb charged nanoparticles by electrostatic attraction (Peralta-Videa et al., 2011). This process can remove nanoparticles to solid phases and it is likely to decrease their bioavailability (Navarro et al., 2008). In contrast, dissolved organic matter in soil can be sorbed onto nanoparticle surfaces and affect their surface speciation and charge through steric or charge alteration (PZC reduction) and increased hydrophilicity of the surfaces (Espinasse et al., 2007; Lecoanet et al., 2004). Therefore, aggregation of nanoparticles can be inhibited which may result in increased mobility and bioavailability of nanoparticles in terrestrial environments (Peralta-Videa et al., 2011). Yang et al. (2009) reported adsorption of humic acids at the surface of TiO_2, Al_2O_3, and ZnO nanoparticles. In their experiments, coating of metal oxide nanoparticles with humic acids decreased their zeta potential indicating enhanced electrostatic repulsion between nanoparticles and increased stability in the environment (Yang et al., 2009). However, dissociation of natural organic matter adsorbed to the surface of nanoparticles at environmental pH values less than the pK_a of the functional groups in organic matter may produce polar groups (e.g. –OH) at the surface of adsorbed organic matter and subsequently bridge coated nanoparticles through hydrogen bonds (Lin et al., 2010). It has also been established that polysaccharides will affect the stability and mobility of nanoparticles (Ghosh et al., 2008). They can bridge between nanoparticles (especially in the presence of Ca^{2+}) and promote aggregation and deposition (Espinasse et al., 2007). Gosh et al. (2008) also reported that long-chain weakly charged fractions of organic matter can entrap Al_2O_3 nanoparticles in a gel-like network and therefore limit their transport and mobility.

1.6.4 Soil texture

Interactions of nanoparticles with soil minerals can affect their mobility, bioavailability, reactivity and persistence in soils. The complexity of soil minerals makes it difficult to study the mechanisms involving in the reactions of soil minerals with nanoparticles. Nevertheless, a few recent investigations have addressed the effect of clay minerals on the mobility and retention of nanoparticles in soil suspensions or soil columns (Cornelis et al., 2010; Cornelis et al., 2011; Fang et al., 2009; Schrick et al., 2004; Wang et al., 2010). Cornelis et al. (2011) related retention of CeO_2 nanoparticles as single particles or aggregates less than 0.45 µm in soil suspensions with physicochemical properties of sixteen soils. The authors reported a positive correlation between clay content of the soils and retention of CeO_2 nanoparticles due to electrostatic attraction of positively charged clay minerals (at the pH values measured in the soil suspensions) and the negatively charged nanoparticles (Cornelis et al., 2011). Heterocoagulation of nanoparticles with mobile natural colloids such as clays can decrease collisions of nanoparticles with the soil and facilitate transport of nanoparticles in soil (Hotze et al., 2010), probably by the same mechanism as observed for colloid-meditated transport of soil contaminants. Fang et al. (2009) studied the retention of TiO_2 nanoparticles in twelve soils and also found a positive correlation of clay content and retention of the nanoparticles. Schrick et al. (2004) also reported that in clay-rich soils, clay platelets can act as surface modifiers and stabilise Fe nanoparticles and therefore facilitate transport of Fe nanoparticles through soil.

1.7 Manufactured nanoparticles and soil organisms

Environmental exposure models have predicted that soils are the main environmental compartment receiving nanomaterials, particularly through application of sewage sludge (Gottschalk et al., 2009). On the other hand, successful application of nanoparticles in different industrial sectors has generated interest in the application of nanoparticles in agriculture for targeted release of agrochemicals or efficient nutrient utilization which may enhance plant growth and health (DeRosa et al., 2010; Nair et al., 2010). Therefore, enhanced abundance of nanoparticles in the environment has primed researchers to be concerned about the possible negative or positive effects of manufactured nanoparticles on terrestrial organisms. Moreover, biological uptake mechanisms, translocation, transformation and degradation of nanoparticles via soil organisms and plants can be important pathways in the life cycle of manufactured nanoparticles which influences their fate in soil systems (Lin et al., 2010).

Despite the urgent need to address biological impact of nanoparticles, some technical and analytical problems have challenged the environmental relevance of these studies. To date, experiments on the biological impact of nanoparticles are primarily conducted in laboratory-controlled media due to analytical problems in separation of nanoparticles from naturally occurring nanoparticles in soil. In terrestrial environments, nanoparticles may undergo surface modification (aggregation, complexation with natural organic/inorganic components of soil) which highly affects their bioavailability (Bernhardt et al., 2010). Therefore, experiments conducted in controlled environments such as culture media or hydroponic systems might not represent well the influence of nanoparticles on soil biota. Moreover, transformation of added nanoparticles makes it difficult to detect nanoparticles in terrestrial media except at exceedingly high concentrations, which are well above their predicted abundance in the environment (Bernhardt et al., 2010). Although high rates of application can provide important information on the toxicity mechanisms, it cannot predict the dose-response relationship at environmentally relevant concentrations. Bernhardt et al. (2010) illustrated hypothetical dose-response scenarios for low addition rates of nanoparticles extrapolated from high spike rate observations (Figure 15). One scenario represents linear increase of toxicity as a function of concentration (scenario A). Whereas, low concentration of some nanoparticles (e.g. nanoparticles containing trace elements such as ZnO) may promote growth and toxic effects may only appear at high concentrations above the threshold (Scenario B). Scenario C shows high toxicity of nanoparticles at low concentrations. At higher doses, large aggregates may have developed which can theoretically reduce bioavailability and toxicity of nanoparticles. Another shortcoming in biological impact studies is differentiating the direct effect of nanoparticles from indirect influence caused by dissolved components released from nanoparticles (Klaine et al., 2008).

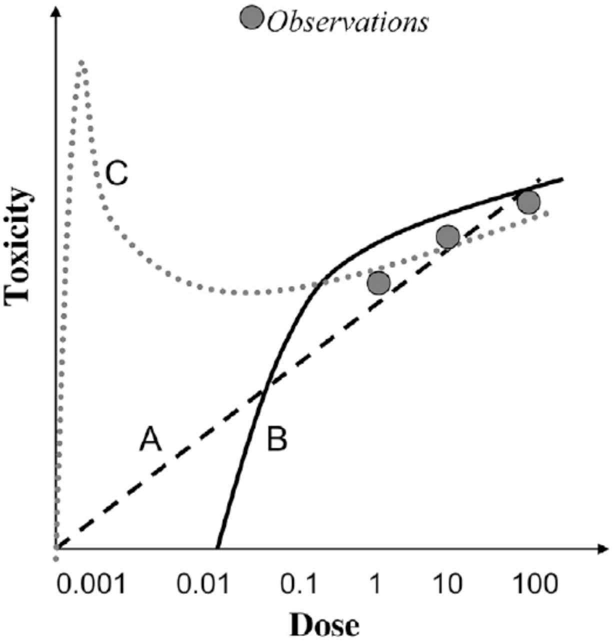

Figure 15 – Biological response as a function of introduced dose of manufactured nanoparticles. Gray dots show actual observations at high addition rates and three lines represent possible dose-response fits to the observations (Bernhardt et al., 2010).

1.7.1 Uptake by, and toxicity to, soil organisms

Given studies on aquatic organisms have shown antimicrobial and toxic effect of metal (oxide) nanoparticle (Sondi and Salopek-Sondi, 2004; Yoon et al., 2007; Zhang et al., 2007), it can be expected that nanoparticles might adversely affect the activity of nitrogen-fixing bacteria, symbiotic relationships involving fungi, bacteria, and plants or the role of fungi in protecting host plants against phytopathogens or oxidative stress (Navarro et al., 2008). On the other hand, a recent experiment has reported slight increase in basal soil respiration after 38 days following incubation of soils with low rates of ZnO nanoparticles (Ge et al., 2011). Therefore, it can be assumed that metal oxide nanoparticles may provide essential nutrients such as trace elements for microbial and plant growth in micronutrient-deficient soils and therefore promote biomass production and nutrient cycling. Therefore, investigations on the influence of manufactured nanoparticles on soil biota are of importance for evaluating the ecosystem services provided by soil organisms.

The uptake and behaviour of nanoparticulate metals by soil organisms after contact with nanoparticles are strongly dependent on physiology of cell membrane, physical and chemical properties of the nanoparticles (Brayner et al., 2006) as well as soil properties (Shoults-Wilson et al., 2011). It is believed that the cell wall acts as the primary site for interaction of nanoparticles with organisms and also as a barrier for entrance of NPs into the cell (Navarro et al., 2008). Transmission electron microscopy images have confirmed that adsorption of SiO_2 nanoparticles at the surface of green alga *Pseudokirchneriella subcapitata* is the primary reason for their toxicity (Figure 16) (Hoecke et al., 2008). Moreover, the rigidity of the cell wall can be disrupted as a result of oxidative stress caused by nanoparticles which will subsequently affect permeability of the membrane and can enhance the entrance of nanoparticles into the cell (Wiesner et al., 2006). Brayner et al. (2006) reported the disruption of cell membranes of Gram-negative *E. Coli* bacteria due to exposure to ZnO nanoparticles. Higher toxicity of ZnO nanoparticles to crustaceans (*Heterocyipris incongruens*) than soluble form of zinc was suggested to be due to adhesion of ZnO nanoparticle to the exoskeleton of crustaceans (Manzo et al., 2011). Raghupathi et al. (2011) reported the size-dependant toxicity of ZnO nanoparticles on both Gram-negative and Gram-positive microorganisms. The antibacterial activity of ZnO nanoparticles was suggested to be due to production of reactive oxygen species (ROS) and the accumulation of nanoparticles on the outer membrane or their entrance to the cytoplasm (Raghupathi et al., 2011). Once metal oxide nanoparticles enter the cell from damaged cells, they may dissolve and release damaging concentrations of metal ions in the cell (Kasemets et al., 2009). The physical and chemical properties of nanoparticles also can play a key role in their effect on soil organisms. The toxicity of ZnO nanoparticle to microorganisms was increased as the particle size of ZnO nanoparticles decreased (Raghupathi et al., 2011). The chemistry of the growth medium also reported to influence the toxicity of ZnO nanoparticles in *E. Coli* bacteria (Li et al., 2011).

The effect of nanoparticles on soil organisms can be governed by the soil properties and associated influence on nanoparticle behaviour. The accumulation of Ag in earthworm *Eisenia fetida* from silver nanoparticulate was compared in two different soils (Shoults-Wilson et al., 2011). The results showed higher accumulation of Ag in earthworms grown in soil with lower CEC and pH (higher Ag bioavailability). Aggregation of ZnO nanoparticles and their following deposition in soil matrix lowered the toxicity of ZnO nanoparticles compared to ionic Zn ($ZnCl_2$) in a soil matrix for *Eisnia veneta* earthworms (Hooper et al., 2011). Dissolution of metal oxide nanoparticles in soil also can greatly influence their effect on soil organisms. Synchrotron based X-ray absorption techniques have confirmed that free ions released from nanoparticles are responsible for the effect of Ag (Shoults-Wilson et al., 2011) and Cu nanoparticles (Unrine et al., 2010) to *Eisenia fetida* earthworms.

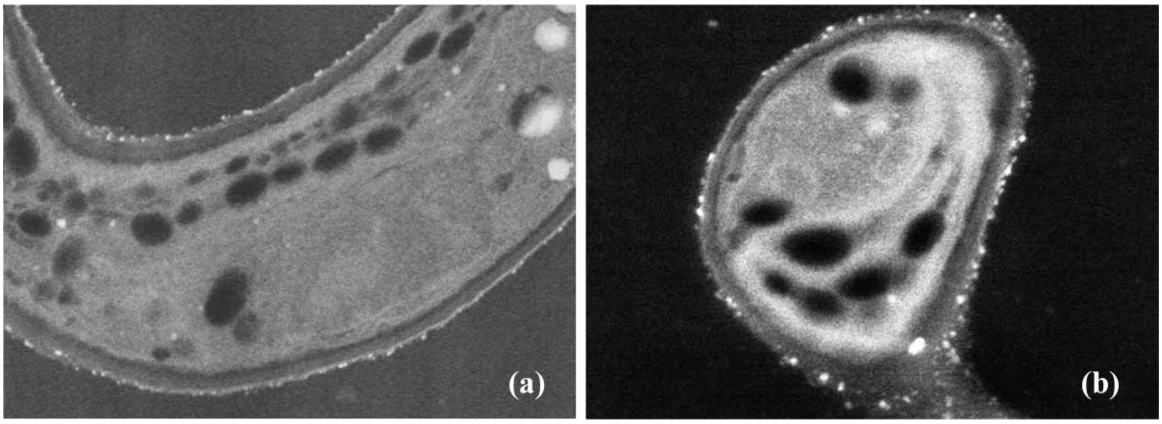

Figure 16- Green algae (*Pseudokirchneriella subcapitata*) exposed to100 mg L^{-1} of (a) 12.5 nm and (b) 27 nm SiO_2 nanoparticles. The low angel backscattered electron images of green algae illustrates adsorption of nanoparticles on the outer cell surface as bright dots (Hoecke et al., 2008).

1.7.2 Uptake and interactions in plants

The initial step in determining the effect of nanoparticles on plant growth and possible benefits of applying nanoparticles in agriculture would be to understand the uptake mechanisms, translocation and transformation of nanoparticles following application to soils. Although the majority of studies on plant uptake and negative or positive effects of nanoparticles have been conducted on seed germination and root elongation in culture media (Peralta-Videa et al., 2011), they provide evidence that plant uptake of nanoparticles and response to the exposed nanoparticles are primarily dependant on the physicochemical properties of nanoparticles (e.g. composition, shape and size) and plant type (Ma et al., 2010).

In plant uptake processes, solutes translocated by diffusion or mass flow to the external surface of plant roots, are taken up by movement across the cell wall and water-filled intercellular spaces of the root cortex (Marschner, 1995). The main barrier against passive solute movement in the apoplast is the Casparian strip in the endodermis, the innermost layer of cells of the cortex (Figure 18). To date, studies on plant uptake of nanoparticles from soil have suggested that the possible interactions of nanoparticles with higher plants are adsorption onto the root surfaces, incorporation into the cell walls and uptake into the cells (Ma et al., 2010; Nair et al., 2010; Nowack and Bucheli, 2007). Dissolution of metal (oxide) nanoparticles in soils and uptake of dissolved ions is also a critical pathway which may affect plant growth.

Given that the pore diameter of cell walls of plants are generally in the range of 3.5 – 3.8 nm for root hairs, only nanoparticles or aggregates with diameters less than the cell wall pore diameter can enter the cell wall of undamaged cells (Dietz and Herth, 2011). Formation of new and large size pores which allows internalization of nanoparticles through cell walls has also been reported (Ma et al., 2010; Navarro et al., 2008). Further internalization is possible by endocytosis which provides a cavity structure around the nanoparticles by the plasma membrane (Nair et al., 2010). Uptake of nanoparticles can be achieved at the point of lateral root formation zone where nanoparticles can enter xylem via cortex and apoplastic bypass (Dietz and Herth, 2011). Wounding of root system due to activities of herbivores and mechanical injuries are also speculated to be entry routes for nanoparticles through holes or damaged cells (Dietz and Herth, 2011; Nowack and Bucheli, 2007).

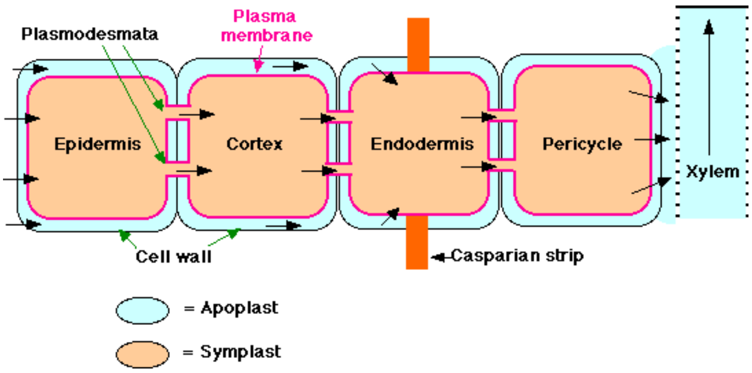

Figure 17 – Root structure in higher plants.

Adsorption of nanoparticles at the external surface root cells can affect plant uptake mechanisms by clogging the nanosized pores in the root cell wall. Asli et al. (2009) suggested that the average particle exclusion diameter of cell wall pores of maize (*Zea mays* L.) seedlings in a hydroponic medium to be from 3.0 to 6.6 nm and reported a decrease in transpiration and leaf growth as a result of exposure to a suspension of TiO_2 nanoparticles (mean diameter of 30 nm). The inhibited apoplastic flow of water evidenced by a decrease in hydraulic conductivity of roots suggested that reduced leaf transpiration and growth was due to a physical barrier created by adsorption of TiO_2 nanoparticles at the root surface rather than a chemically-induced toxicity (Asli and Neumann, 2009). However, shoot production was not affected and a possible adaptation mechanism and inhibited translocation of nanoparticles to shoots was suggested. Adsorption of TiO_2 nanoparticles at the root surface of wheat (*Triticum* *aestivum* L.) has recently been illustrated by transmission electron microscopy images of root tips of wheat plants grown in a loamy clay soil (Du et al., 2011). In this experiment, treatment with TiO_2 nanoparticles did not affect the Ti concentration of plant tissue. Therefore, it can be assumed that the adverse effect of TiO_2 nanoparticles on biomass production was mainly due to aggregation of nanoparticles in soil and their subsequent attachment to the wall of epidermal cells.

Experiments on cell internalization and upward translocation of ZnO nanoparticles by ryegrass (*Lolium perenne* L.) have also been conducted in hydroponic culture (Lin and Xing, 2008). Electron microscopy images confirmed that ZnO nanoparticles concentrated in the rhizosphere, adhered to the root surface, damaged the epidermal and cortical cells upon intake and increased Zn concentration in roots 3.6 times more than soluble Zn source when 1000 mg L^{-1} Zn were added to the hydroponic solution (Figure 18). However, translocation of Zn from roots to shoots for ZnO nanoparticles remained very low, much lower than that for Zn^{2+} (Lin and Xing, 2008). The reported phytotoxicity may be due to high rates of Zn (1000 mg L^{-1}) applied to the solution culture. Moreover, it can be assumed that the observed toxic effect would be less in soil systems due to partitioning to the soil solid phase. Development of breaks in the root endodermis is a common process due to development of roots in the solution culture which may facilitate the uptake of nanoparticles to the root system (Bell et al., 2003). Obviously, this effect would be limited in soil systems. In a recent experiment conducted by Kim et al. (2011), application of 2000 mg kg^{-1} Zn NPs and ZnO NPs compared to soluble Zn source in a natural soil did not affect biomass production and Zn concentration in cucumber (*Cucumis sativum)* plant tissue. However, Zn concentration in soils treated with nanoparticles were significantly higher than control plants and soil treated with soluble Zn. This may indicate that retention of nanoparticles in natural soil can effectively reduce plant toxicity of nanoparticles.

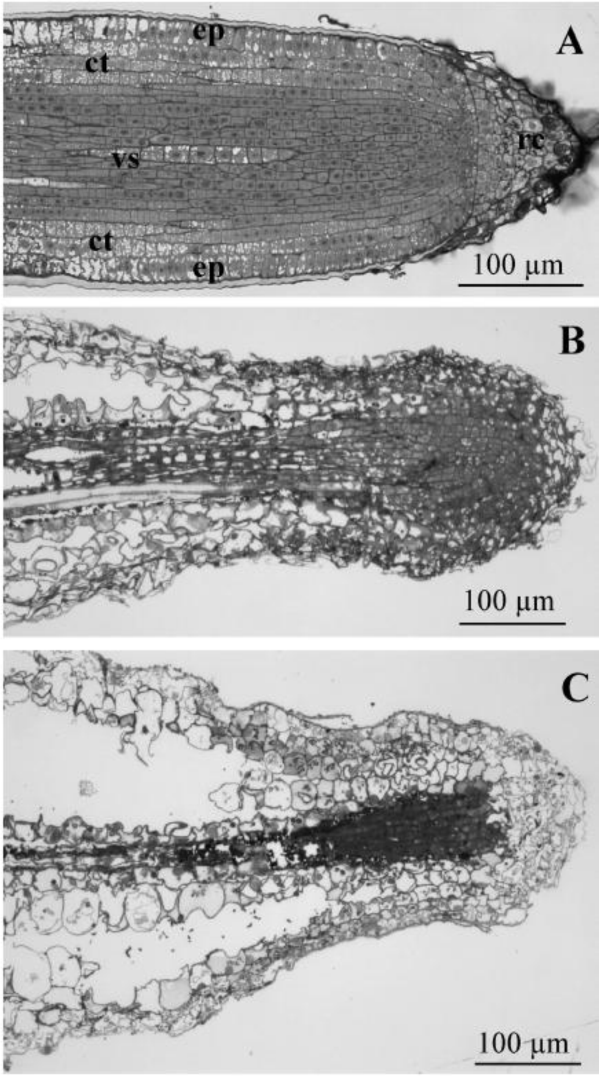

Figure 18- TEM images of ryegrass primary root treated as (A) control, (B) 1000 mg L^{-1} ZnO and (C) 1000 mg L^{-1} Zn^{2+} in hydroponic culture solution (Lin and Xing, 2008).

Potential dissolution of metal-based nanoparticles in soil or dissolution of nanoparticles within plant root cells may also affect plant growth by production of dissolved species. In the study carried out in a clay loamy soil, Du et al. (2011) investigated the effect of applying ZnO nanoparticles on growth of wheat plants. Although Zn concentration of wheat tissue increased as a result of application of ZnO nanoparticles to soil, no ZnO nanoparticles were observed in primary roots of wheat plants grown in soil. Therefore, uptake of ZnO nanoparticles may not be responsible for Zn accumulation in the plants and more likely it was dissolution of ZnO nanoparticles during the 2 month incubation period which increased soil Zn availability (Du et al., 2011). In another experiment, Zn speciation in soybean (*Glycine max*) roots germinated in a Petri dish system with 0, 500, 1000, 2000 and 4000 mg Zn L^{-1} as ZnO nanoparticles showed that the nanoparticles were not present in the root. Synchrotron X-ray absorption spectroscopy results showed that at the 4000 mg L^{-1} spike rate, Zn coordinated in the same manner as Zn nitrate or Zn acetate and no ZnO was present in the root (López-Moreno et al., 2010). Nevertheless, application of ZnO nanoparticles slightly increased seed germination of soybean plants up to 1000 mg Zn L^{-1} in the solution culture. However, the uptake of Zn was reduced by increasing the spike rate of ZnO nanoparticles above 1000 mg L^{-1} which might be due to aggregation of ZnO nanoparticles at the higher rates and inhibition of dissolution in the experimental media.

The uptake and distribution efficiency of metal oxide nanoparticles in plants depend on nanoparticle type and the surface properties (e.g. surface modifications) of nanoparticles. Yang and Watts (2005) investigated the effect of Al oxide nanoparticles on root elongation of corn (*Zea mays*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), cabbage (*Brassica oleracea*) and carrot (*Daucus carota*) and reported that root elongation can be inhibited in the presence of uncoated Al oxide. This effect on root elongation was reduced effectively by coating the Al oxide nanoparticles with phenanthrene which indicates relevance of surface modifications in reduction of phytotoxicity (Yang and Watts, 2005). Given the effect of soluble Al^{3+} as a powerful root toxicant was not considered in this study, high solubility of Al_2O_3 nanoparticles and enhanced concentration of Al species could have inhibited the root growth (Murashov, 2006).

Different plants may also behave differently to addition of the same nanoparticles (Nair et al., 2010) and also their response may be dependent on the growth stage. The influence of plant type on nanoparticle (multi-walled C nanotube, Al,

Al_2O_3, Zn, and ZnO) phytotoxicity (seed germination and root growth) was examined by Lin and Xing (2007) using radish (*Raphanus sativum*), rape (*Brassica napus*), ryegrass (*Lolium perenne*), lettuce (*Lactuca sativa*), corn (*Zea mays*), and cucumber (*Cucumis sativum*). The authors found only ZnO nanoparticles at high rates (2000 mg L^{-1}) significantly inhibited seed germination and root growth of corn plants. Dissolution of Zn and ZnO nanoparticles was not responsible for the phytotoxicity observed; however, the authors did not measure the dissolution of Zn and ZnO nanoparticles right on the root surface (Lin and Xing, 2007). Doshi et al. (2008) also investigated the effect of Al nanoparticles addition to soil on Al uptake by Californian kidney beans (*Phaseolus vulgaris*) and ryegrass (*Lolium perenne*). Amendments up to 10,000 mg kg^{-1} did not significantly affect the Al concentration in red kidney beans whereas the Al concentration in ryegrass almost doubled following treatment with the same amount of Al nanoparticles (Doshi et al., 2008). Comparison of inhibitory concentrations of ZnO nanoparticles on ryegrass at different growth stages showed that newly germinated ryegrass seedlings were more sensitive to ZnO nanoparticles than older seedlings (Lin and Xing, 2007; Lin and Xing, 2008).

As outlined above, the majority of studies investigating the effect of nanoparticles on plants have been conducted *in vitro* – in Petri dishes or hydroponic culture media. Interactions of NPs with soil surfaces, effects of soil on NP dissolution and the mode of uptake of elements by roots in soil will all markedly affect the outcomes from NP dosing experiments. There is therefore a need to study the uptake, translocation and biotransformation of NPs in natural soil environments.

1.8. Zinc: vital element in human health and plant nutrition

Zinc is an essential element for human health and plant growth and development. This trace element plays vital functions in structural molecules such as

DNA and activates different metabolic and regulatory enzymes. It has been reported that nearly 925 proteins in humans and over 500 proteins in plants contain Zn (Graham, 2008). As a result, insufficient supply of Zn would adversely affect its important physiological functions which may cause a wide variety of health problems in humans, including impairment of immune system function and brain function, DNA damage and cancer development (Cakmak, 2008). Plants also may develop symptoms such as interveinal chlorosis, bronzing of leaves, abnormally shaped leaves, stunting or rosetting which can effectively reduce crop production and health (Alloway, 2004). According to the World Health Organization, the average prevalence of Zn deficiency in the world population is 31% which may range from 4% to 73% in different countries (Caulfield and Black, 2004).

Agricultural systems are the main pathway from which nutrients including Zn enter the human food chain. Therefore, Zn malnutrition must be directly dependant on the inability of cropping systems to deliver enough Zn to the food crops (Welch, 2008). Global occurrence of Zn-deficient diets is particularly correlated with the distribution of Zn deficiency areas (Graham, 2008). Intensification of crop production in the last 70 years, which includes increased use of macronutrient fertilizers, growing higher yielding cultivars with higher Zn requirements, and increased use of agrochemicals for controlling pests and diseases, has amplified the risk of Zn deficiency in crops (Alloway, 2008). Hence, there is increasing interest in fortifying crops with Zn. Zinc enrichment of plants not only enhances Zn input to the food chain and subsequent improvements in human health but it can also contribute to increasing crop production which is of critical importance in satisfying the rising global demand for food.

Different agricultural strategies have been used to provide a solution for Zn deficiency problems in agricultural crops. Plant breeding strategies (genetic biofortification) have been introduced as a sustainable and cost effective approach to increase Zn content of crops by developing new genotypes with a higher ability to take up Zn (Cakmak, 2008). Although genetic biofortification can increase Zn uptake capacity of plants, Zn uptake by newly developed cultivars cannot be improved when plants are grown in Zn-deficient soils. Since the uptake of Zn from soil is directly related to the available pool of Zn in soil, special attention must be given to improving the Zn status of soils and developing agronomic methods to enhance the availability of soil Zn to plants.

1.8.1 Zinc deficiency in soils and its correction

Zinc deficiency is one of the most ubiquitous micronutrient deficiency problems in the world and has been reported on most major soils (Alloway, 2004; Takkar and Walker, 1993). A global study estimated that 49% of important agricultural soils are Zn deficient (Sillanpaa, 1982; Sillanpaa, 1990). A summary of possible factors causing Zn deficiency in crops is listed in Figure 19. Apart from limited genetic capacity of some crops for Zn uptake, Zn deficiency in crops is mainly associated with physical and chemical properties of soils such as total Zn content, soil pH, organic matter and calcite ($CaCO_3$) content, soil moisture, microbial activity in the rhizosphere, concentration of dissolved salts in soil solution and phosphate content of soils (Alloway, 2004; Cakmak, 2008; Takkar and Walker, 1993).

Zinc deficiency in crops due to low content of Zn in soil is considered as a “primary deficiency” which is mainly found in sandy soils or strongly leached tropical soils developed on highly weathered parent material (Alloway, 2009). Soil physical and chemical properties which may restrict bioavailability of soil Zn are known as “secondary deficiencies”. Among these properties, soil pH has the most marked influence on availability of Zn to plants. The activity of free Zn^{2+} ions, which is the most plant available form of Zn in soil, is inversely proportional to the square of proton activity in soil solution (Alloway, 2009). In the pH range between 5.5 and 7.0, a 30 to 45 times decrease in the equilibrium concentration of Zn in soil solution was reported for each unit increase in soil pH (Marschner, 1993). Increase in soil pH enhances the adsorption of Zn to soil solid phases (clay minerals, Fe/Al oxides) through increased pH-dependant negative charge at the surface of soil solids and reduces the desorption of adsorbed Zn (Cakmak, 2008). The main factors responsible for low Zn solubility in high pH soils are considered to be high $CaCO_3$ content of soils (from pedogenic origin or heavy liming of soil), high salt content of soils and reducing soil conditions (Alloway, 2009). Consequently, severe Zn deficiency symptoms are prone to be observed in crops grown in calcareous, saline or sodic soils which have high pH values or in waterlogged and flooded soils.

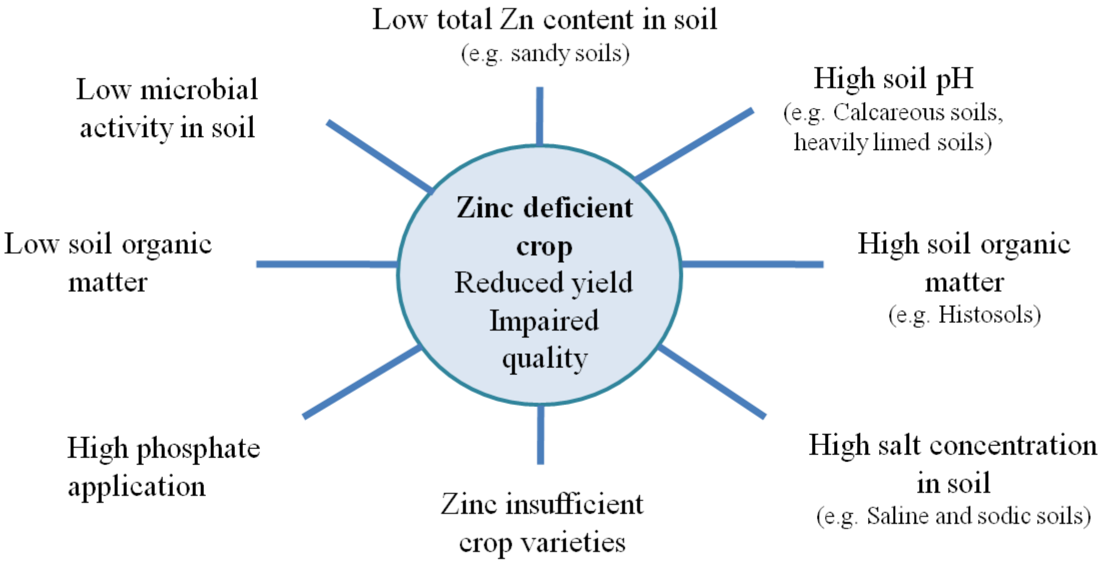

Figure 19- A summary of most important factors reducing Zn uptake in crops.

Soil organic matter has a critical role in the solubility and transport of Zn to plant roots (Cakmak, 2008). Presence of low molecular-weight organic anion exudates in the rhizosphere could enhance desorption of Zn^{2+} and supply rate of Zn to plant roots. Adsorption of Zn onto soil organic matter may also increase diffusion of zinc in soil (Marschner, 1993). Nevertheless, soils with extremely high amounts of organic matter (e.g. Histosols) can sorb Zn strongly and reduce availability of Zn for plant uptake (Fertilizer Industry Federation of Australia, 2006).

Increased microbial activity of soil can also increase Zn availability to plants. Biologically produced chelators may enhance mobilization of insoluble forms of Zn to readily available forms in the soil solution (Marschner, 1993). Moreover, vesicular-arbuscular mycorrhizae (VAM) effectively extend the root system and can increase plant acquisition of soil Zn (Marschner, 1993).

Soil moisture is another key factor controlling the transport of Zn to root surfaces for plant uptake. Zinc diffusion to the root surface is the major route for supplying plants with Zn demands (Marschner, 1993). Following Zn uptake, a depletion zone can be produced around the root hair which promotes diffusion of Zn towards the root surfaces (Marschner, 1993). Therefore, in water stressed conditions; Zn nutrition of soil can be adversely affected due to limited Zn diffusion in soil solution. Investigations have confirmed more severe Zn deficiency symptoms in crops grown in rainfed systems compared to irrigated conditions (Cakmak, 2008).

High phosphate fertilization of soils is also considered to produce Zn deficiency symptoms in crops (Alloway, 2009). The possible mechanisms responsible in the antagonistic relationship of Zn and P are discussed by Loneragan and Webb (1993). In soils with marginal Zn and P concentrations, P fertilization may enhance plant growth and consequently dilute Zn in plant tissues. Moreover, high levels of P in soil may reduce Zn uptake by plant roots through their effect on suppressing mcorrhizal (VAM) symbiosis, promoting partitioning of Zn to solid phases in soil or precipitating of Zn phosphates with varying solubility (Loneragan and Webb, 1993). Plants grown on soils with a high amount of available Zn may also express Zn deficiency symptoms, especially when high rates of P fertilizer are applied due presumably to immobilization of Zn in plant as Zn phytate (Loneragan and Webb, 1993). This may explain the situation when P fertilization caused Zn deficiency symptoms but total Zn content of Zn in plant remained unaffected.

One factor or combinations of factors recognized to produce Zn deficiency symptoms in crops can be found on many soils in different climatic zones of the world. However, most severe Zn deficiency problem is associated with calcareous soils, sandy soils, saline or sodic soils (Alloway, 2004). These soils are widespread in major agricultural area especially in India, Pakistan, China, Iran, Turkey and Australia. Therefore, Strategies for Zn enrichment of crops grown in Zn deficient areas is of great importance in improving human Zn nutrition in these regions. Increased concentration of Zn in edible parts of crops, which is known as biofortification, can be achieved by plant breeding or fertilization (White and Broadley, 2005). Breeding new cultivars with higher genetic capacity for Zn uptake is a sustainable approach in fortifying crops (Cakmak, 2008). However, it is a long-term procedure which requires extensive screening, crossing and adaptation to a large range of crop and soil management practices in different regions (Cakmak et al., 2010). Moreover, the genetic potential of newly developed varieties in Zn uptake cannot be expressed in a soil with low bioavailable Zn. Hence, application of Zn fertilizers is a short-term solution to the Zn deficiency problem which also ensures success of breeding strategy in a long term.

1.8.2 Zinc fertilizers

Various Zn compounds which vary considerably in Zn content, chemical state, effectiveness for crops and associated cost have been used as Zn fertilizers. Four main sources for Zn fertilizers include inorganic compounds, synthetic chelates, natural organic complexes and inorganic complexes (Mortvedt and Gilkkes, 1993). A list of commonly used compounds used as a Zn fertilizers is provided in Table 1. When selecting a Zn source for use as Zn fertilizer, effectiveness of the Zn compound in increasing crop yield as well as economic effectiveness must be considered. Although chelated Zn sources are more agronomically effective (more response per unit of applied micronutrient), inorganic sources of Zn are more economical to apply and mainly are preferred to chelated ones in large scale applications (Takkar and Walker, 1993). Inorganic sources of Zn such as zinc oxides (ZnO) and zinc sulphates ($ZnSO_4{\cdot}H_2O$ or $ZnSO_4{\cdot}7H_2O$) are the most commonly used Zn fertilizers to correct Zn deficiency (Mortvedt, 1992).

Soil application is the most common method in providing plants with required Zn (Mortvedt and Gilkes, 1993). Given the recommended application rate of Zn is usually less than 10 kg ha^{-1} (Mortvedt and Gilkkes, 1993), one of the main challenges in Zn fertilization is even and cost effective broadcasting of a very small amount of required Zn over the Zn deficiency affected area (Graham, 2008). Addition of Zn sources to macronutrient fertilizers is a common method which can reduce the cost of distribution and result in more uniform application of Zn in the field. Zinc source can be bulk blended with or incorporated to the macronutrient fertilizer in the manufacturing process of these fertilizers. Although these methods can deliver recommended levels of Zn, they are not ideal. Bulk blending of Zn source with macronutrient fertilizer might result in segregation of Zn source during handling and transport due to different particle sizes and/or hardness of the macro- and micronutrient components (Graham, 2008; Mortvedt and Gilkkes, 1993). Incorporation of Zn source with macronutrient fertilizer during the granulation process can help overcome segregation problem. However, chemical reactions of Zn source with components in the macronutrient fertilizers, particularly P, reduce fertilizer grade and plant availability of Zn (Graham, 2008). A more recent approach is coating of finely powdered Zn sources (ground to less than 0.25 µm in diameter) onto the surface of macronutrient fertilizers with a binding agent such as light oils, waxes, water and even ammonium polyphosphate solution (Mortvedt, 1991).

Table 1 – Commonly used Zn compounds in Zn fertilizers (Mortvedt and Gilkes, 1993).

Compound	Formula	Zn content (%)
Inorganic compounds		
Zinc sulphate monohydrate	$ZnSO_4.H_2O$	36 - 37
Zinc sulphate heptahydrate	$ZnSO_4.7H2O$	22 - 23
Zinc oxysulphate	$xZnSO_4.xZnO$	20 - 50
Basic Zn sulphate	$ZnSO_4.4Zn(OH)_2$	55
Zinc oxide	ZnO	50 - 80
Zinc carbonate	$ZnCO_3$	50 - 56
Zinc chloride	$ZnCl_2$	50
Zinc nitrate	$Zn(NO_3)_2.3H_2O$	23
Synthetic chelates		
Disodium zinc EDTA	Na_2-ZnEDTA	8 - 14
Sodium zinc HEDTA	Na_2-ZnHEDTA	6 - 10
Sodium zinc EDTA	NaZnEDTA	9 - 13
Natural organic complexes		
Zinc polyflavonoid	-	5 - 10
Zinc lignosulphonate	-	5 - 8
Inorganic complexes		
Ammoniated zinc sulphate solution	$Zn(NH_3)_4SO_4$	10

Information on the relative effectiveness of different methods of addition of Zn source to the macronutrient fertilizers is very limited in the literature. Mortvedt and Gilkkes (1993) cited the result of the experiment by Ellis et al. (1965) in which different methods of Zn application (bulk blending, incorporation and coating) were compared for their effect on yield and Zn concentration in pea beans (*Phaseoulus vulgaris* L.). The results showed that Zn concentrations in plant tissue were not affected by incorporation or coating of Zn source at the surface of macronutrient fertilizer (Table 2). However, greater plant availability of Zn and higher yield production were observed when Zn source bulk blended with the macronutrient fertilizer (Ellis et al., 1965). Although bulk blended treatment in this experiment revealed more promising results, this method would not be very practical in large scale due to the segregation problem and uneven broadcast of Zn in the field. Richard et al. (1969) compared incorporation and coating methods in agronomic effectiveness of Zn fertilizer. When the Zn source was coated onto the macronutrient fertilizer, it was more effective than Zn incorporated fertilizers (Richards, 1969). The benefit of coating method was claimed to be as a result of providing less favourable condition for chemical reaction between the Zn source and macronutrient fertilizer.

Table 2 – Grain yield and Zn concentration of pea bean plants as affected by addition method of ZnSO4 to the macronutrient fertilizer (Ellis et al., 1965) cited in (Mortvedt and Gilkkes, 1993).

Method of application	Yield ($kg\ ha^{-1}$)	Leaf Zn concentration ($mg\ kg^{-1}$)
No application	1,230	20
Blended	1,660	40
Incorporated	1,640	31
Coated	1,670	34
LSD (0.05)	*170*	*3*

The effectiveness of Zn fertilizers for providing plants with required Zn in Zn-deficient area mainly depends on the solubility of the Zn source in soil (Amrani et al., 1999; Mortvedt, 1968). Mortvedt and Giordano (1969) found a significant correlation between water-soluble fractions of Zn from several macronutrient fertilizers with zinc oxide or zinc sulfate incorporated and Zn availability to crops (Mortvedt and Giordano, 1969). Further investigations have confirmed that water-soluble Zn, not total Zn concentration, is the major parameter controlling the effectiveness of Zn enriched fertilizers for plant growth and development (Amrani et al., 1999; Gangloff et al., 2002; Shaver et al., 2007; Westfall et al., 2005). Dissolution of Zn fertilizer in soil can lead to diffusion of Zn into the surrounding soil. More soluble Zn fertilizers may affect larger volume of soil and increase root interception with the Zn-enriched soil and consequently increase plant uptake of Zn. As an example, it has been suggested that the Zn fertilizers must contain nearly 50% water-soluble Zn to supply sufficient Zn level for corn crops (Amrani et al., 1999; Mortvedt, 1992).

In addition to the effect of water solubility of Zn source in effectiveness of Zn fertilizer, the ability of the Zn source to maintain adequate Zn in the soil solution for plant uptake is another important factor determining the suitability of the Zn fertilizer (Mortvedt, 1985). Although $ZnSO_4$ is more soluble than ZnO, the experiment conducted by Giordano and Mortvedt (1973) showed that ZnO was more effective in providing rice plants with adequate Zn and resulted higher dry matter production than $ZnSO_4$ (Giordano and Mortvedt, 1973). Immediate dissolution of $ZnSO_4$ after application in soil may result in a sharp increase in the Zn concentration of soil solution followed by a rapid decline. However, ZnO dissolves more slowly and retains sufficient level of Zn in the soil solution for longer period of time (Mortvedt, 1985).

Particle size of the Zn source in the Zn fertilizer may also affect the effectiveness of Zn fertilizer (Mortvedt, 1992). When smaller Zn particles are used, the number of Zn particles per unit of applied Zn to soil would increase. Moreover, smaller particles have higher specific surface area which subsequently enhances dissolution rate of Zn source, especially in Zn sources with lower solubility such as ZnO. Mortvedt (1992) cited the result of an experiment carried out by Allen and Terman (1966) on the effect of three particle sizes of $ZnSO_4$ on dry matter and Zn uptake by corn plants. The results revealed that fine $ZnSO_4$ particles (<0.15mm in diameter) were more effective than larger $ZnSO_4$ particles (1.4 – 2.0 mm and 0.8 – 1.2 mm in diameter) (Allen and Terman, 1966).

1.8.3 Zinc oxide nanoparticles as potential Zn fertilizers

Given that the solubility and particle size of Zn source in the Zn fertilizer are among the main parameters determining the effectiveness of the Zn fertilizer, application of nanoparticles in fertilizer formulation may improve the performance of Zn fertilizer. Nanoparticles have smaller particle sizes, higher specific surface area and an increased proportion of reactive surface atoms compared to bulk particles (Wigginton et al., 2007). Since the dissolution kinetics of particles depends on surface area, it is speculated that rate and extent of dissolution is greater for nanoparticles compared to bulk materials (Borm et al., 2006). Therefore, application of nanoparticles in Zn fertilizers may promote Zn dissolution in soils, increase the volume of Zn-enriched soil and Zn uptake by plants.

Zinc oxide nanoparticle is the most common Zn nanoparticle which is being used as UV protector (e.g. in personal care products, coatings and paints), biosensors, electronics, and rubber manufacture (Brayner et al., 2010; Kool et al., 2011).The wide range of industrial applications for ZnO nanoparticle can predict future increase the production volume of these nanoparticles by developing economical synthesis methods and reducing the manufacture costs. Hence, economical application of ZnO nanoparticles as Zn fertilizers can be practical in large scale in the world. Moreover, nanotechnology may assist fertilizer industry by designing Zn fertilizers which could release Zn on demand and therefore preventing the interactions of Zn in soil with soil compartments, water and microorganisms which reduce availability of Zn for crops (DeRosa et al., 2010). However, it is important to consider that different properties of soils (pH, ionic strength, organic matter, solid phases etc.) may strongly affect the fate of nanoparticles in the soil. Therefore, the behaviour of nanoparticles can deviate from the ones theoretically expected. Given research on the transformation of ZnO nanoparticles in soils is very limited, it is critical to develop understanding on the fate and behaviour of ZnO nanoparticles in soils and their possible influence on the Zn uptake by plants in Zn deficient area.

1.9 Research gaps

Food security is one of the major challenges that the global community has been faced with this century. Practical solutions for this problem require deployment of existing and emerging technologies, such as nanotechnology, to introduce novel agrochemicals for sustainable agriculture. Smarter use of fertilizers in crop production could be achievable through using nanoparticles with high reactivity in soils. However, a thorough understanding of the fate and reactions of nanoparticles in soils and interactions of nanoparticles with plants is required prior to general recommendation of these novel materials. As discussed so far in this literature review, the study of the reactions and bioavailability of nanoparticles in environmental systems are mainly restricted to aquatic environments or a number of investigations using clean bed porous media or culture media. The fate of nanoparticles in soils is scarcely investigated and there is very limited information on the plant uptake and availability of nanoparticles added to soil. Although investigations reviewed in this chapter can provide invaluable insights into the possible reactions of nanoparticles in soils, the behaviour of nanoparticles is likely to be strongly affected by soil properties. Difficulties in identifying and separating naturally occurring nanoparticles from manufactured nanoparticles in soil, or distinguishing the effect of nanoparticles from the dissolved components derived from nanoparticles (especially in the case on metal oxide nanoparticles) have limited investigations of the fate and plant uptake of nanoparticulate metals. In this thesis, I aimed to adapt advanced techniques for imaging and tracing nanoparticles to provide a better understanding of the reactions and bioavailability of manufactured ZnO nanoparticles in soil, and of the factors which can affect this behaviour. Such information will ensure safer and more sustainable application of manufactured nanoparticulate metals as an efficient new source of Zn fertilizer for plants, and better management of their potential risks.

1.10 Specific objectives of this study

The aim of this thesis was to investigate the reactions and bioavailability of manufactured ZnO nanoparticles in soils for potential use as Zn fertilizers in Zn deficient soils. The specific objectives of this thesis were to:

- Develop an understanding of the solubility and dissolution kinetics of ZnO nanoparticles, both individually introduced or associated with macronutrient fertilizers to porous media;
- Investigate diffusion, transformation and changes in solid phase speciation of ZnO nanoparticles introduced into Zn-deficient soil;
- Study the partitioning of ZnO nanoparticles in a range of Australian soils and to evaluate the influence of different soil properties in partitioning behaviour of ZnO nanoparticles in soils; and
- Evaluate the potential bioavailability and response of plants to ZnO nanoparticles added to different soils and the effect of Zn spike rate and aging on bioavailability of Zn derived from ZnO nanoparticles.

1.11 Literature cited

Abbas, Z., C. Labbez, S. Nordholm, and E. Ahlberg. 2008. Size-dependent surface charging of nanoparticles. J. Phys. Chem. C 112:5715-5723.

Adams, L.K., D.Y. Lyon, and P.J.J. Alvarez. 2006. Comparative eco-toxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions. Water Res. 40:3527-3532.

Aitken, R.J., M.Q. Chaudhry, A.B.A. Boxall, and M. Hull. 2006. Manufacture and use of nanomaterials: Current status in the UK and global trends. Occup. Med.-Oxf. 56:300-306.

Allen, S.E., and G.L. Terman. 1966. Response of maize and sudangrass to zinc in granular micronutrients. International Soil Science Society, Aberdeen, Scotland.

Alloway, B.J. 2004. Zinc in soils and crop nutrition. International Zinc Association, Brussels.

Alloway, B.J. 2008. Micronutrients and crop production: An introduction, p. 1, *In* B. J. Alloway, ed. Micronutrient Deficiencies in Global Crop Production. Springer Science

Alloway, B.J. 2009. Soil factors associated with zinc deficiency in crops and humans. Environ. Geochem. Health 31:537-548.

Amrani, M., D.G. Westfall, and G.A. Peterson. 1999. Influence of water solubility of granular zinc fertilizers on plant uptake and growth. J. Plant Nutr. 22:1815-1827.

Asli, S., and P.M. Neumann. 2009. Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. Plant, Cell Environ. 32:577-584.

Baalousha, M., A. Manciulea, S. Cumberland, K. Kendall, and J.R. Lead. 2008. Aggregation and surface properties of iron oxide nanoparticles: Influence of pH and natural organic matter. Environ. Toxicol. Chem. 27:1875-1882.

Banfield, J., and H. Zhang. 2001. Nanoparticles in the Environment, p. 1-58, *In* J. Banfield and A. Navrotsky, eds. Nanoparticles and the Environment, Vol. 44. Mineralogical Society of America, Washington, DC.

Baruah, S., and J. Dutta. 2009. Nanotechnology applications in pollution sensing and degradation in agriculture: a review. Environ. Chem. Lett. 7:191-204.

Batley, G.E., and M.J. McLaughlin. 2010. Fate of Manufactured Nanomaterials in the Australian Environment. CSIRO, Adelaide.

Becheri, A., M. Durr, P. Lo Nostro, and P. Baglioni. 2008. Synthesis and characterization of zinc oxide nanoparticles: application to textiles as UV-absorbers. J. Nanopart. Res. 10:679-689.

Beddington, J. 2010. Food security: contributions from science to a new and greener revolution. Philos. Trans. R. Soc. B-Biol. Sci. 365:61-71.

Bell, P.F., M.J. McLaughlin, G. Cozens, D.P. Stevens, G. Owens, and H. South. 2003. Plant uptake of C-14-EDTA, C-14-Citrate, and C-14-Histidine from chelator-buffered and conventional hydroponic solutions. Plant Soil 253:311-319.

Bernhardt, E., B. Colman, M. Hochella, B. Cardinale, R. Nisbet, and C. Richardson. 2010. An ecological perspective on nanomaterial impacts in the environment. J. Environ. Qual. 39:1954-1965.

Bian, S.W., I.A. Mudunkotuwa, T. Rupasinghe, and V.H. Grassian. 2011. Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: Influence of pH, ionic strength, size, and adsorption of humic acid. Langmuir 27:6059-6068.

Biswas, P., and C.Y. Wu. 2005. Nanoparticles and the environment. J. Air Waste Manage. Assoc. 55:708-746.

Borm, P., F.C. Klaessig, T.D. Landry, B. Moudgil, J. Pauluhn, K. Thomas, R. Trottier, and S. Wood. 2006. Research strategies for safety evaluation of nanomaterials, Part V: Role of dissolution in biological fate and effects of nanoscale particles. Toxicol. Sci. 90:23-32.

Brant, J., H. Lecoanet, and M.R. Wiesner. 2005. Aggregation and deposition characteristics of fullerene nanoparticles in aqueous systems. J. Nanopart. Res. 7:545-553.

Brayner, R., R. Ferrari-Iliou, N. Brivois, S. Djediat, M.F. Benedetti, and F. Fievet. 2006. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett. (USA) 6:5 -5.

Brayner, R., S.A. Dahoumane, C. Yepremian, C. Djediat, M. Meyer, A. Coute, and F. Fievet. 2010. ZnO nanoparticles: Synthesis, characterization, and ecotoxicological studies. Langmuir 26:6522-6528.

Buffle, J. 2006. The key role of environmental colloids/nanoparticles for the sustainability of life. Environ. Chem. 3:155-158.

Buffle, J., K.J. Wilkinson, S. Stoll, M. Filella, and J. Zhang. 1998. A generalized description of aquatic colloidal interactions: The three-colloidal component approach. Environ. Sci. Technol. 32:2887-2899.

Burleson, D.J., M.D. Driessen, and R.L. Penn. 2004. On the characterization of environmental nanoparticles. J. Environ. Sci. Health 39:2707-2753.

Cakmak, I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant Soil 302:1-17.

Cakmak, I., W.H. Pfeiffer, and B. McClafferty. 2010. Biofortification of durum wheat with zinc and iron. Cereal Chem. 87:10-20.

Cakmak, I., A. Yilmaz, M. Kalayci, H. Ekiz, B. Torun, B. Erenoglu, and H.J. Braun. 1996. Zinc deficiency as a critical problem in wheat production in central Anatolia. Plant Soil 180:165-172.

Caulfield, L.E., and R.E. Black. 2004. Zinc Deficiency, *In* M. Ezzati, et al., eds. Comparative Quantification of Health Risks : Global and Regional Burden of Disease Attributable to Selected Major Risk Factors. World Health Organization, Geneva.

Chen, K.L., and M. Elimelech. 2007. Influence of humic acid on the aggregation kinetics of fullerene (C60) nanoparticles in monovalent and divalent electrolyte solutions. J. Colloid Interface Sci. 309:126-134.

Colvin, V.L. 2003. The potential environmental impact of engineered nanomaterials. Nat. Biotechnol. 21:1166-1170.

Cornelis, G., J.K. Kirby, D. Beak, D. Chittleborough, and M.J. McLaughlin. 2010. A method for determination of retention of silver and cerium oxide manufactured nanoparticles in soils. Environ. Chem. 7:298-308.

Cornelis, G., B. Ryan, M.J. McLaughlin, J.K. Kirby, D. Beak, and D. Chittleborough. 2011. Solubility and batch retention of CeO2 nanoparticles in soils. Environ. Sci. Technol. 45:2777-2782.

Darlington, T.K., A.M. Neigh, M.T. Spencer, O.T. Nguyen, and S.J. Oldenburg. 2009. Nanoparticle characteristics affecting environmental fate and transport through soil. Environ. Toxicol. Chem. 28:1191-1199.

Degen, A., and M. Kosec. 2000. Effect of pH and impurities on the surface charge of zinc oxide in aqueous solution. J. European Ceram. Soc. 20:667-673.

Derjaguin, B., and L. Landau. 1941. Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. Acta Physico Chemica URSS 14:633.

DeRosa, M.C., C. Monreal, M. Schnitzer, R. Walsh, and Y. Sultan. 2010. Nanotechnology in fertilizers. Nat. Nanotechnol. 5:91-91.

Dewick, P., K. Green, and M. Miozzo. 2004. Technological change, industry structure and the environment. Futures 36:267-293.

Dietz, K.-J., and S. Herth. 2011. Plant nanotoxicology. Trends Plant Sci. 16:582-589.

Domingos, R.F., C. Peyrot, and K.J. Wilkinson. 2009. Aggregation of titanium dioxide nanoparticles: role of calcium and phosphate. Environ. Chem. 7:61-66.

Doshi, R., W. Braida, C. Christodoulatos, M. Wazne, and G. O'Connor. 2008. Nano-aluminum: Transport through sand columns and environmental effects on plants and soil communities. Environ. Res. 106:296-303.

Du, W.C., Y.Y. Sun, R. Ji, J.G. Zhu, J.C. Wu, and H.Y. Guo. 2011. TiO(2) and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. J. Environ. Monit. 13:822-828.

Elimelech, M., and C.R. Omelia. 1990. Effect of particle-size on collision efficiency in the deposition of brownian particles with electrostatic energy barriers. Langmuir 6:1153-1163.

Ellis, B.G., J.F. Davis, and W.H. Judy. 1965. Effect of method of incorporation of znc in fertilizer on zinc uptake and yield of pea beans (*Phaseolus vulgaris L.*). Soil Sci. Soc. Am. Proc. 29:635-636.

Espinasse, B., E.M. Hotze, and M.R. Wiesner. 2007. Transport and retention of colloidal aggregates of C-60 in porous media: Effects of organic macromolecules, ionic composition, and preparation method. Environ. Sci. Technol. 41:7396-7402.

Fang, J., X.Q. Shan, B. Wen, J.M. Lin, and G. Owens. 2009. Stability of titania nanoparticles in soil suspensions and transport in saturated homogeneous soil columns. Environ. Pollut. 157:1101-1109.

Fertilizer Industry Federation of Australia. 2006. Australian Soil Fertility Manual 3rd ed. CSIRO Publishing, Collingwood, Vic.

Franklin, N.M., N.J. Rogers, S.C. Apte, G.E. Batley, G.E. Gadd, and P.S. Casey. 2007. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga (*Pseudokirchneriella subcapitata*): The importance of particle solubility. Environ. Sci. Technol. 41:8484-8490.

Freundlich, H. 1926. Capillary and Colloid Chemistry Methuen and Co., Ltd., London.

Gangloff, W.J., D.G. Westfall, G.A. Peterson, and J.J. Mortvedt. 2002. Relative availability coefficients of organic and inorganic Zn fertilizers. J. Plant Nutr. 25:259-273.

Ge, Y.G., J.P. Schimel, and P.A. Holden. 2011. Evidence for negative effects of TiO(2) and ZnO nanoparticles on soil bacterial communities. Environ. Sci. Technol. 45:1659-1664.

Gericke, M., and A. Pinches. 2006. Biological synthesis of metal nanoparticles. Hydrometallurgy 83:132-140.

Ghosh, S., H. Mashayekhi, B. Pan, P. Bhowmik, and B.S. Xing. 2008. Colloidal behavior of aluminum oxide nanoparticles as affected by pH and natural organic matter. Langmuir 24:12385-12391.

Gimbert, L.J., R.E. Hamon, P.S. Casey, and P.J. Worsfold. 2007. Partitioning and stability of engineered ZnO nanoparticles in soil suspensions using flow field-flow fractionation. Environ. Chem. 4:8-10.

Giordano, P.M., and J.J. Mortvedt. 1973. Zinc source and method of application for rice Agron. J. 65:51-53.

Gottschalk, F., T. Sonderer, R.W. Scholz, and B. Nowack. 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. Environ. Sci. Technol. 43:9216–9222.

Graham, R.D. 2008. Micronutrient Deficiencies in Crops and Their Global Significance, p. 41-61, *In* B. J. Alloway, ed. Micronutrient Deficiencies in Global Crop Production. Springer Netherlands.

Guzman, K.A.D., M.P. Finnegan, and J.F. Banfield. 2006. Influence of surface potential on aggregation and transport of titania nanoparticles. Environ. Sci. Technol. 40:7688-7693.

Hartley, W., R. Edwards, and N.W. Lepp. 2004. Arsenic and heavy metal mobility in iron oxide-amended contaminated soils as evaluated by short- and long-term leaching tests. Environ. Pollut. 131:495-504.

He, Y., J. Wan, and T. Tokunaga. 2008. Kinetic stability of hematite nanoparticles: the effect of particle sizes. J. Nanopart. Res. 10:321-332.

Hoecke, K.v., K.A.C.d. Schamphelaere, P.v.d. Meeren, S. Lucas, and C.R. Janssen. 2008. Ecotoxicity of silica nanoparticles to the green alga Pseudokirchneriella subcapitata: importance of surface area. Environ. Toxicol. Chem. 27:1948-1957.

Hooper, H.L., K. Jurkschat, A.J. Morgan, J. Bailey, A.J. Lawlor, D.J. Spurgeon, and C. Svendsen. 2011. Comparative chronic toxicity of nanoparticulate and ionic zinc to the earthworm Eisenia veneta in a soil matrix. Environ. Int. 37:1111-1117.

Hotze, E.M., T. Phenrat, and G.V. Lowry. 2010. Nanoparticle aggregation: Challenges to understanding transport and reactivity in the environment. J. Environ. Qual. 39:1909-1924.

Huynh, K.A., and K.L. Chen. 2011. Aggregation kinetics of citrate and polyvinylpyrrolidone coated silver nanoparticles in monovalent and divalent electrolyte solutions. Environ. Sci. Technol. 45:5564-5571.

Hydutsky, B.W., E.J. Mack, B.B. Beckerman, J.M. Skluzacek, and T.E. Mallouk. 2007. Optimization of nano- and microiron transport through sand columns using polyelectrolyte mixtures. Environ. Sci. Technol. 41:6418-6424.

Illes, E., and E. Tombacz. 2006. The effect of humic acid adsorption on pH-dependent surface charging and aggregation of magnetite nanoparticles. J. Colloid Interface Sci. 295:115-123.

Jiang, X.J., M.P. Tong, H.Y. Li, and K. Yang. 2010. Deposition kinetics of zinc oxide nanoparticles on natural organic matter coated silica surfaces. J. Colloid Interface Sci. 350:427-434.

Kanel, S.R., and S.R. Al-Abed. 2011. Influence of pH on the transport of nanoscale zinc oxide in saturated porous media. J. Nanopart. Res. 13:4035-4047.

Kasemets, K., A. Ivask, H.-C. Dubourguier, and A. Kahru. 2009. Toxicity of nanoparticles of ZnO, CuO and TiO_2 to yeast Saccharomyces cerevisiae. Toxicol. in Vitro 23:1116-1122.

Kim, S., J. Kim, and I. Lee. 2011. Effects of Zn and ZnO nanoparticles and Zn^{2+} on soil enzyme activity and bioaccumulation of Zn in Cucumis sativus. Chem. Ecol. 27:49-55.

Klabunde, K.J. 2001. Introduction to Nanotechnology, p. 1-13, *In* K. J. Klabunde, ed. Nanoscale Materials in Chemistry. John Wiely & sons, Inc., Hoboken, NJ.

Klaine, S.J., P.J.J. Alvarez, G.E. Batley, T.F. Fernandes, R.D. Handy, D.Y. Lyon, S. Mahendra, M.J. McLaughlin, and J.R. Lead. 2008. Nanomaterials in the environment: Behaviour, fate, bioavailability and effects. Environ. Toxicol. Chem. 27:1825–1851.

Kool, P.L., M.D. Ortiz, and C.A.M. van Gestel. 2011. Chronic toxicity of ZnO nanoparticles, non-nano ZnO and $ZnCl_2$ to Folsomia candida (Collembola) in relation to bioavailability in soil. Environ. Pollut. 159:2713-2719.

Lead, J.R., and K.J. Wilkinson. 2006. Aquatic colloids and nanoparticles: Current knowledge and future trends. Environ. Chem. 3:159-171.

Lecoanet, H.F., and M.R. Wiesner. 2004. Velocity effects on fullerene and oxide nanoparticle deposition in porous media. Environ. Sci. Technol. 38:4377-4382.

Lecoanet, H.F., J.Y. Bottero, and M.R. Wiesner. 2004. Laboratory assessment of the mobility of nanomaterials in porous media. Environ. Sci. Technol. 38:5164-5169.

Li, M., L.Z. Zhu, and D.H. Lin. 2011. Toxicity of ZnO nanoparticles to *Escherichia coil*: Mechanism and the influence of medium components. Environ. Sci. Technol. 45:1977-1983.

Lin, D., and B. Xing. 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environ. Pollut. 150:243-250.

Lin, D., and B. Xing. 2008. Root uptake and phytotoxicity of ZnO nanoparticles. Environ. Sci. Technol. 42:5580-5585.

Lin, D.H., X.L. Tian, F.C. Wu, and B.S. Xing. 2010. Fate and transport of engineered nanomaterials in the environment. J. Environ. Qual. 39:1896-1908.

Lin, X.M., and A.C.S. Samia. 2006. Synthesis, assembly and physical properties of magnetic nanoparticles. J. Magn. Magn. Mater. 305:100-109.

Liu, W.T. 2006. Nanoparticles and their biological and environmental applications. J. Biosci. Bioeng. 102:1-7.

Liu, X.Y., M. Wazne, T.M. Chou, R. Xiao, and S.Y. Xu. 2011. Influence of Ca^{2+} and Suwannee River Humic Acid on aggregation of silicon nanoparticles in aqueous media. Water Res. 45:105-112.

Logan, B.E. 1999. Environmental Transport Processes J. Wiley, New York.

Loneragan, J.F., and M.J. Webb. 1993. Interactions Between Zinc and Other Nutrients Affecting the Growth of Plants, *In* A. D. Robson, ed. Zinc in Soils and Plants. Kluwer Academic Publishers, Dordrecht ; Boston.

López-Moreno, M.L., G. de la Rosa, J.A. Hernández-Viezcas, H. Castillo-Michel, C.E. Botez, J.R. Peralta-Videa, and J.L. Gardea-Torresdey. 2010. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (Glycine max) plants. Environ. Sci. Technol. 44:7315–7320.

Luther, W. 2004. Industrial Application of Nanomaterials - Chances and Risks VDI Technologiezentrum GmbH, Dusseldorf, Germany.

Ma, H., N.J. Kabengi, P.M. Bertsch, J.M. Unrine, T.C. Glenn, and P.L. Williams. 2011. Comparative phototoxicity of nanoparticulate and bulk ZnO to a free-living nematode *Caenorhabditis elegans*: The importance of illumination mode and primary particle size. Environ. Pollut. 159:1473-1480.

Ma, X., J. Geiser-Lee, Y. Deng, and A. Kolmakov. 2010. Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. Sci. Total Environ. 408:3053-3061.

Ma, Y.B., and N.C. Uren. 2006. Effect of aging on the availability of zinc added to a calcareous clay soil. Nutr. Cycling Agroecosyst. 76:11-18.

Manceau, A., K.L. Nagy, M.A. Marcus, M. Lanson, N. Geoffroy, T. Jacquet, and T. Kirpichtchikova. 2008. Formation of metallic copper nanoparticles at the soil-root interface. Environ. Sci. Technol. 42:1766–1772.

Mandal, D., M.E. Bolander, D. Mukhopadhyay, G. Sarkar, and P. Mukherjee. 2006. The use of microorganisms for the formation of metal nanoparticles and their application. Appl. Microbiol. Biotechnol. 69:485-492.

Manzo, S., A. Rocco, R. Carotenuto, F.D. Picione, M.L. Miglietta, G. Rametta, and G. Di Francia. 2011. Investigation of ZnO nanoparticles' ecotoxicological effects towards different soil organisms. Environ. Sci. Pollut. Res. 18:756-763.

Marschner, H. 1993. Zinc Uptake from Soils, *In* A. D. Robson, ed. Zinc in Soils and Plants. Kluwer Academic Publishers, Dordrecht ; Boston.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Second edition ed. Academic Press Limited, London.

Mohanpuria, P., N. Rana, and S. Yadav. 2008. Biosynthesis of nanoparticles: technological concepts and future applications. J. Nanopart. Res. 10:507-517.

Mortvedt, J.J. 1968. Crop response to applied zinc in ammoniated phosphate fertilizers. J. Agric. Food Chem. 16:241-245.

Mortvedt, J.J. 1985. Micronutrient fertilizers and fertilization practices. Nutr. Cycling Agroecosyst. 7:221-235.

Mortvedt, J.J. 1991. Micronutrient Fertilizer Technology, p. 523-549, *In* J. J. Mortvedt, et al., eds. Micronutrient in Agriculture. SSSA Inc., Madison, USA.

Mortvedt, J.J. 1992. Crop response to level of water -soluble zinc in granular zinc fertilizers. Fert. Res. 33:249-255.

Mortvedt, J.J., and P.M. Giordano. 1969. Extractability of zinc granulated with macronutrient fertilizers in relation to its agronomic effectivemess. J. Agric. Food Chem. 17:1272-1275.

Mortvedt, J.J., and R.J. Gilkkes. 1993. Zinc Fertilizers, *In* A. D. Robson, ed. Zinc in Soils and Plants. Kluwer Academic Publishers, Dordrecht ; Boston.

Murashov, V. 2006. Comments on "Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles" by Yang, L., Watts, D.J., Toxicology Letters, 2005, 158, 122-132. Toxicol. Lett. 164:185-187.

Nair, R., S.H. Varghese, B.G. Nair, T. Maekawa, Y. Yoshida, and D.S. Kumar. 2010. Nanoparticulate material delivery to plants. Plant Sci. 179:154-163.

Navarro, E., A. Baun, R. Behra, N.B. Hartmann, J. Filser, A.J. Miao, A. Quigg, P.H. Santschi, and L. Sigg. 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology 17:372-386.

Nel, A., T. Xia, L. Madler, and N. Li. 2006. Toxic potential of materials at the nanolevel. Science 311:622-627.

Nowack, B., and T.D. Bucheli. 2007. Occurrence, behavior and effects of nanoparticles in the environment. Environ. Pollut. 150:5-22.

Oberdorster, G., E. Oberdorster, and J. Oberdorster. 2005. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 113:823-839.

Ostwald, W. 1900. On the assumed isomerism of red and yellow mercury oxide and the surface-tension of solid bodies. Z. Phys. Chem. Stoechiom. Verwandtschafts. 34:495-503.

Parker, J. 2001. Application of Nanocrystals, p. 279-285, *In* K. J. Klabunde, ed. Nanoscale Materials in Chemistry. John Wiely & sons, Inc, Hoboken, NJ.

Peralta-Videa, J.R., L.J. Zhao, M.L. Lopez-Moreno, G. de la Rosa, J. Hong, and J.L. Gardea-Torresdey. 2011. Nanomaterials and the environment: A review for the biennium 2008-2010. J. Hazard. Mater. 186:1-15.

Perdrial, N., J.N. Perdrial, J.E. Delphin, F. Elsass, and N. Liewig. 2010. Temporal and spatial monitoring of mobile nanoparticles in a vineyard soil: evidence of nanoaggregate formation. Eur. J. Soil Sci. 61:456-468.

Powers, K.W., S.C. Brown, V.B. Krishna, S.C. Wasdo, B.M. Moudgil, and S.M. Roberts. 2006. Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation. Toxicol. Sci. 90:296-303.

Qafoku, N.P. 2010. Terrestrial Nanoparticles and Their Controls on Soil/geo Processes and Reactions p. 33-91 Advances in Agronomy, Vol 107, Vol. 107. Elsevier Academic Press Inc, San Diego.

Raghupathi, K.R., R.T. Koodali, and A.C. Manna. 2011. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir 27:4020-4028.

Richards, G.E. 1969. Water solubility of zinc in a granular mixed fertilizer as affected by zine source and method of addition. Soil Sci. Soc. Am. J. 33:310-313.

Rickerby, D.G., and M. Morrison. 2007. Nanotechnology and the environment: A European perspective. Sci. Technol. Adv. Mater. 8:19-24.

The Royal Society & Royal Academy of Engineering. 2000. Nanoscience and Nanotechnologies [Online]. Available by http://www.nanotec.org.uk/finalReport.htm.

Saleh, N., H.J. Kim, T. Phenrat, K. Matyjaszewski, R.D. Tilton, and G.V. Lowry. 2008. Ionic strength and composition affect the mobility of surface-modified Fe-0 nanoparticles in water-saturated sand columns. Environ. Sci. Technol. 42:3349-3355.

Sasson, Y., G. Levy-Ruso, O. Toledano, and I. Ishaaya. 2007. Nanosuspensions: Emerging Novel Agrochemical Formulations Springer-Verlag Berlin.

Sastry, M., A. Ahmad, M.I. Khan, and R. Kumar. 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr. Sci. 85:162-170.

Schmid, G. 2001. Metals, p. 15-59, *In* K. J. Klabunde, ed. Nanoscale Materials in Chemistry. Wiley-Interscience, New York.

Schrick, B., B.W. Hydutsky, J.L. Blough, and T.E. Mallouk. 2004. Delivery vehicles for zerovalent metal nanoparticles in soil and groundwater. Chem. Mater. 16:2187-2193.

Shaver, T.M., D.G. Westfall, and M. Ronaghi. 2007. Zinc fertilizer solubility and its effects on zinc bioailability over time. J. Plant Nutr. 30:123-133.

Shoults-Wilson, W.A., B.C. Reinsch, O.V. Tsyusko, P.M. Bertsch, G.V. Lowry, and J.M. Unrine. 2011. Role of particle size and soil type in toxicity of silver nanoparticles to earthworms. Soil Sci. Soc. Am. J. 75:365-377.

Sillanpaa, M. 1982. Micronutrients and the Nutrient Status of Soils: A Global Study. FAO, Rome.

Sillanpaa, M. 1990. Micronutrient Assessment at Country Level: An International Study. FAO, Rome.

Sondi, I., and B. Salopek-Sondi. 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J. Colloid Interface Sci. 275:177-182.

Stumm, W., (ed.) 1987. Aquatic Surface Chemistry: Chemical Processes at the Particle-Water Interface. Wiley, New York

Stumm, W., and J.J. Morgan. 1996. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. 3rd ed. Wiley, New Yok.

Sun, Y.P., X.Q. Li, W.X. Zhang, and H.P. Wang. 2007. A method for the preparation of stable dispersion of zero-valent iron nanoparticles. Colloid Surf. A-Physicochem. Eng. Asp. 308:60-66.

Swift, R.S. 1996. Organic Matter Characterization, *In* D. L. Sparks, et al., eds. Methods of Soil Analysis. Prat 3. Chemical Methods. Soil Science Society of America, Inc., Madison, USA.

Takkar, P.N., and C.D. Walker. 1993. The Distribution and Correction of Zinc Deficiency, *In* A. D. Robson, ed. Zinc in Soils and Plants, Vol. 55. Kluwer Academic Publishers, Dordrecht ; Boston

Tang, R., C.A. Orme, and G.H. Nancollas. 2004. Dissolution of crystallites: Surface energetic control and size effects. ChemPhysChem 5:688-696.

Tavakoli, A., M. Sohrabi, and A. Kargari. 2007. A review of methods for synthesis of nanostructured metals with emphasis on iron compounds. Chem. Pap. 61:151-170.

Thomas, C., S. George, A. Horst, Z. Ji, R. Miller, and J. Peralta-Videa. 2011. Nanomaterials in the environment: From materials to high-throughput screening to organisms. Acs Nano 5:13-20.

Tran, C., K. Donaldson, V. Stones, T. Fernandez, A. Ford, N. Christofi, J. Ayres, M. Steiner, J. Hurley, R. Aitken, and A. Seaton. 2005. A Scoping Study to Identify Hazard Data Needs for Addressing the Risks Presented by Nanoparticles and Nanotubes. Institute of Occupational Medicine, UK.

Tratnyek, P.G., and R.L. Johnson. 2006. Nanotechnologies for environmental cleanup. Nano Today 1:44-48.

Tso, C., C. Zhung, Y. Shih, Y. Tseng, S. Wu, and R. Doong. 2010. Stability of metal oxide nanoparticles in aqueous solutions. Water Sci. Technol. 61:127-133.

Tufenkji, N., and M. Elimelech. 2004. Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media. Environ. Sci. Technol. 38:529-536.

Unrine, J.M., S.E. Hunyadi, O.V. Tsyusko, W. Rao, W.A. Shoults-Wilson, and P.M. Bertsch. 2010. Evidence for bioavailability of Au nanoparticles from soil and biodistribution within earthworms (Eisenia fetida). Environ. Sci. Technol. 44:8308-8313.

USEPA. 2005. Nanotechnology white paper -external review draft [Online]. Available by U.S. Environmental Protection Agency http://www.epa.gov/osa/pdfs/EPA_nanotechnology_white_paper_external_review_draft_12-02-2005.pdf.

Vayssieres, L. 2005. On the thermodynamic stability of metal oxide nanoparticles in aqueous solutions. Int. J. Nanotechnol. 2:411 - 439.

Vayssieres, L. 2009. On the effect of nanoparticle size on water-oxide interfacial chemistry. J. Phys. Chem. C 113:4733-4736.

Verwey, E.J.W., and J.T.G. Overbeek. 1948. Theory of the Stability of Lyophobic Colloids Elsevier, New York.

Vogelsberger, W., J. Schmidt, and F. Roelofs. 2008. Dissolution kinetics of oxidic nanoparticles: The observation of an unusual behaviour. Colloid Surf. A-Physicochem. Eng. Asp. 324:51-57.

Wang, Y.G., Y.S. Li, H. Kim, S.L. Walker, L.M. Abriola, and K.D. Pennell. 2010. Transport and retention of fullerene nanoparticles in natural soils. J. Environ. Qual. 39:1925-1933.

Welch, R.M. 2008. Linkages Between Trace Elements in Food Crops and Human Health, p. 41-61, *In* B. J. Alloway, ed. Micronutrient Deficiencies in Global Crop Production. Springer Science.

Westfall, D.G., J.J. Mortvedt, G.A. Peterson, and W.J. Gangloff. 2005. Efficient and environmentally safe use of micronutrients in agriculture. Commun. Soil Sci. Plant Anal. 36:169-182.

White, P.J., and M.R. Broadley. 2005. Biofortifying crops with essential mineral elements. Trends Plant Sci. 10:586-593.

Wiesner, M.R., G.V. Lowry, P. Alvarez, D. Dionysiou, and P. Biswas. 2006. Assessing the risks of manufactured nanomaterials. Environ. Sci. Technol. 40:4336-4345.

Wigginton, N.S., K.L. Haus, and M.F.H. Jr. 2007. Aquatic environmental nanoparticles. J. Environ. Monit. 9:1306 -1316.

Xiang, H.F., H.A. Tang, and Q.H. Ying. 1995. Transformation and distribution of forms of zinc in acid, neutral and calcareous soils of China. Geoderma 66:121-135.

Yang, K., D.H. Lin, and B.S. Xing. 2009. Interactions of humic acid with nanosized inorganic oxides. Langmuir 25:3571-3576.

Yang, L., and D.J. Watts. 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicol. Lett. 158:122-132.

Yoon, K.-Y., J. Hoon Byeon, J.-H. Park, and J. Hwang. 2007. Susceptibility constants of Escherichia coli and Bacillus subtilis to silver and copper nanoparticles. Sci. Total Environ. 373:572-575.

Zhang, L.L., Y.H. Jiang, Y.L. Ding, M. Povey, and D. York. 2007. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). J. Nanopart. Res. 9:479-489.

Zhang, S.G. 2003. Fabrication of novel biomaterials through molecular self-assembly. Nat. Biotechnol. 21:1171-1178.

Zhang, Y., Y.S. Chen, P. Westerhoff, K. Hristovski, and J.C. Crittenden. 2008. Stability of commercial metal oxide nanoparticles in water. Water Res. 42:2204-2212.

2 Dissolution kinetics of macronutrient fertilizers coated with manufactured zinc oxide nanoparticles

NARGES MILANI[a],

MIKE J. MCLAUGHLIN[ab],

SAMUEL P. STACEY[a],

JASON K. KIRBY[b],

GANGA, M. HETTIARACHCHI[bc],

DOUGLAS G. BEAK[d],

GEERT CORNELIS [a],

[a] Soil Science, School of Agriculture, Food& Wine, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia

[b] CSIRO Land and Water, Nanosafety, Advanced Materials Transformational Capability Platform, Environmental Biogeochemistry Program, Waite Campus, Waite Road, Glen Osmond, SA 5064, Australia.

[c] Department of Agronomy, Trockmorton Plant Sciences Centre, Kansas State University, Manhattan, KS 66506, USA.

[d] U.S. Environmental Protection Agency, National Risk Management Research Laboratory, 919 Kerr Research Dr., Ada, OK, U.S.A.

Journal of Agricultural and Food Chemistry, 2012; 60:3991-3998

Supporting information of this manuscript is contained in Chapter 6, section 6.1.

STATEMENT OF AUTHORSHIP

DISSOLUTION KINETICS OF MACRONUTRIENT FERTILIZERS COATED WITH MANUFACTURED ZINC OXIDE NANOPARTICLES

MILANI, N. (Candidate)

Performed data collection and analysis, critically interpreted data, wrote manuscript and acted as corresponding author.
I hereby certify that the statement of contribution is accurate.

Signed Date

MCLAUGHLIN, MIKE J.

Supervised development of the work, helped in data interpretation, provided critical evaluation and revision of manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

STACEY, SAMUEL P.

Supervised development of the work, helped in data interpretation, reviewed and edited the manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

KIRBY, JASON K.

Supervised development of the work, helped in data interpretation, reviewed and edited the manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.
Signed Date

HETTIARACHCHI GANGA, M.

Helped in data interpretation and manuscript evaluation, reviewed and edited the manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.
Signed Date

BEAK, DOUGLAS G.

Helped in data interpretation and manuscript evaluation, reviewed and edited the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis

Signed Date

CORNELIS, GEERT.

Helped in data interpretation and manuscript evaluation, reviewed and edited the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

Milani, N., McLaughlin, M.J., Stacey, S.P., Kirby, J.K., Hettiarachchi, G.M., Beak, D.G. & Cornelis, G. (2012)
Dissolution kinetics of macronutrient fertilizers coated with manufactured zinc oxide nanoparticles.
Journal of Agricultural and Food Chemistry, v. 60, pp. 3991-3998

NOTE:
This publication is included on pages 86-112 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1021/jf205191y

3 Fate of nanoparticulate zinc oxide fertilizers in an alkaline calcareous soil

NARGES MILANI[a],

GANGA, M. HETTIARACHCHI[bc],

DOUGLAS G. BEAK[d],

MIKE J. MCLAUGHLIN[ab],

JASON K. KIRBY[b]

SAMUEL P. STACEY[a]

[a] Soil Science, School of Agriculture, Food& Wine, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia

[b]CSIROLand and Water, Nanosafety, Advanced Materials Transformational Capability Platform, Environmental Biogeochemistry Program, Waite Campus, Waite Road, Glen Osmond, SA 5064, Australia.

[c] Department of Agronomy, Trockmorton Plant Sciences Centre, Kansas State University, Manhattan, KS 66506, USA.

[d]U.S. Environmental Protection Agency, National Risk Management Research Laboratory, 919 Kerr Research Dr., Ada, OK, U.S.A.

Submitted to the Soil Science Society of America Journal

STATEMENT OF AUTHORSHIP

FATE OF NANOPARTICULATE ZINC OXIDE FERTILIZERS IN AN ALKALINE CALCAREOUS SOIL

MILANI, N. (Candidate)

Performed sample preparation and data analysis, critically interpreted data, wrote manuscript and acted as corresponding author.
I hereby certify that the statement of contribution is accurate.

Signed Date

HETTIARACHCHI GANGA, M.

Supervised development of the work and data collection at APS, helped in data interpretation, reviewed the manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

BEAK, DOUGLAS G.

Supervised the analysis of μ-XANES and μ-EXAFS spectra, helped in data interpretationand reviewedthe manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.
Signed Date

MCLAUGHLIN, MIKE J.

Supervised development of the work and data collection at APS,provided critical evaluation and revision of manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

KIRBY, JASON K.

Supervised manuscript evaluation, reviewed and edited manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

STACEY, SAMUEL P.

Supervised manuscript evaluation, reviewed and edited manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

Milani, N., Hettiarachchi, G.M., Beak, D.G., McLaughlin, M.J., Kirby, J.K. & Stacey, S.P. Fate of Nanoparticulate zinc oxide fertilizers in an alkaline calcareous soil.
Soil Science Society of America Journal, forthcoming.

NOTE:
This publication is included on pages 116-143 in the print copy
of the thesis held in the University of Adelaide Library.

4 Retention and lability of manufactured zinc oxide nanoparticles in natural soils

NARGES MILANI[a],

MIKE J. MCLAUGHLIN[ab],

JASON K. KIRBY[b],

GEERT CORNELIS [ac],

SAMUEL P. STACEY[a],

[a] Soil Science, School of Agriculture, Food& Wine, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia

[b] CSIRO Land and Water, Nanosafety, Advanced Materials Transformational Capability Platform, Environmental Biogeochemistry Program, Waite Campus, Waite Road, Glen Osmond, SA 5064, Australia.

[c] Chemistry department, University of Gothenburg, Kemivägen 10, 41296 Gothenburg, Sweden

Supporting information of this manuscript is contained in chapter 6, section 6.2.

STATEMENT OF AUTHORSHIP

RETENTION AND LABILITY OF MANUFACTURED ZINC OXIDE NANOPARTICLES IN NATURAL SOILS

.

MILANI, N. (Candidate)

Performed data collection and analysis, critically interpreted data, wrote manuscript and acted as corresponding author.
I hereby certify that the statement of contribution is accurate.

Signed Date

MCLAUGHLIN, MIKE J.

Supervised development of the work,helped in data interpretation, provided critical evaluation and revision of manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

KIRBY, JASON K.

Supervised development of the work, helped in data interpretation, reviewed and edited the manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.
Signed Date

CORNELIS, GEERT.

Supervised development of the work, helped in data interpretation, reviewed and edited the manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.
Signed Date

STACEY, SAMUEL P.

Supervised development of the work, helped in data interpretation, reviewed and edited the manuscript.
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.
Signed Date

ABSTRACT

Dissolution and solid- liquid partitioning of zinc oxide (ZnO) nanoparticles (NPs) are major factors governing bioavailability and fate of ZnO NPs in soils. The investigation of the retention and bioavailability of ZnO NPs in soils at environmentally relevant concentrations and distinguishing the specific ecotoxicological effect of ZnO NPs from the effect of dissolved Zn species released from ZnO NPs have been limited by lack of suitable methods. The retention, dissolution of ZnO NPs and partitioning of dissolved Zn species from ZnO NPs were investigated as a function of concentration using microfiltration and ultrafiltration in 5 soils having diverse chemical and physical properties. Zinc bioavailability from ZnO NPs, non-nano (bulk) ZnO particles and soluble Zn to durum wheat (*Triticum durum*) plants applied at 4 mg kg^{-1} and 100 mg kg^{-1} spike rates were also investigated in 2 soils using isotopic dilution technique. Strong retention of ZnO NPs were observed especially in alkaline soils due to great dissolution and/or high degree of aggregation and possible deposition of ZnO NPs. Rapid dissolution of nanoparticulate and bulk ZnO and release of Zn^{2+} to the labile pool over the plant growth period was evident in the isotopic dilution experiment, even in alkaline soils where ZnO NP retention and dissolved Zn partitioning was high. Application of ZnO NPs did not significantly improve shoot dry matter production, Zn uptake at agronomic rate of Zn application (4 mg kg^{-1}) and percent of Zn derived from Zn treatment in plants over bulk source of ZnO. Generally, rapid dissolution of ZnO NPs and bulk ZnO particles in soils which followed by strong retention in soil eliminated the effect of particle size on bioavailability.

KEYWORDS

Zinc, ZnO, Nanoparticle, solubility, partitioning, bioavailability

ABBREVIATIONS USED

DLS, dynamic light scattering; EDAX, energy dispersive X-ray analysis; ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma-optical emission spectroscopy; NPs, nanoparticles; SEM, scanning electron microscopy; TEM, transmission electron microscopy; XRD, X-ray diffraction; ZnO, Zinc oxide;

4.1 INTRODUCTION

Rapid development of nanotechnology has lead to manufacturing and application of nanoparticles (NPs) in various sectors. Zinc oxide nanoparticles (ZnO NPs) are receiving much attention due to their efficiency in blocking ultraviolet radiation and antibacterial properties (Becheri et al., 2008; Brayner et al., 2010). The specific characteristics of ZnO NPs such as small size and high specific surface area may provide new opportunities in the agricultural sector to develop new zinc (Zn) fertiliser products to increase crop production in Zn deficient soils (DeRosa et al., 2010)

Zinc deficiency is one of the most widely distributed micronutrient problems that adversely affect agricultural productivity, particularly in alkaline and calcareous soils (Takkar and Walker, 1993). Zinc oxide nanoparticles could potentially be applied to fertilizer formulations to supply Zn for plant growth in Zn-deficient soils and increase Zn contents of cereal grain for improved human and animal nutrition. Our previous work suggested that ZnO nanoparticles coated onto macronutrient fertilisers provided no advantage in terms of Zn dissolution and diffusion in a calcareous soil

(Milani et al., 2012a). However, ZnO can also be applied as a separate dressing or separately to macronutrients in bulk fertiliser blends to soils to avoid adverse pH conditions or chemical reactions that reduce Zn solubility when incorporated into nitrogen or phosphatic fertilisers.

The important parameters governing bioavailability and fate of ZnO NPs in soils are a combination of solubility of ZnO NPs in soils, retention of ZnO NPs and partitioning of dissolved Zn species derived from the ZnO NPs (i.e. adsorption and precipitation reactions). Theoretically, solubility and dissolution kinetics of particles are assumed to be correlated with surface area and thus particle size (Borm et al., 2006). Consequently, application of ZnO NPs rather than ZnO particles in micro- or millimetre size (bulk ZnO) as a source of Zn for plant nutrition may enhance Zn availability to plants through enhancements of the rate and extent of Zn dissolution. However, we previously found no significant effect of particle size on the rate or extent of dissolution of ZnO in sand columns (Milani et al., 2012b). One other possible advantage of nanoparticulate forms of ZnO could be the better distribution of ZnO throughout the soil, compared to bulk sources of ZnO. It has been shown that distribution of Zn in soil is a key factor controlling crop access to fertiliser Zn (Soper et al., 1989). Diffusion of ZnO NPs are expected to be much greater than bulk ZnO in the soil volume for the same rate of added Zn and therefore ZnO NPs can be transported in soil pores over considerable distances before they dissolve, thus possibly delivering Zn over a much larger soil volume than bulk ZnO. A decisive property in this respect is the retention of ZnO NPs and partitioning of dissolved Zn species from ZnO NPs between soil solids and soil solution, which has been scarcely investigated thus far.

Few studies have examined effects of ZnO NPs on plant species, as reviewed by Rico et al. (2011). The current literature has provided some indications that plant species (Lin and Xing, 2007) and surface properties of the suspension ZnO NPs (e.g. functional groups and stability) (Stampoulis et al., 2009) affect plant response to ZnO NPs, but the majority of the investigations to date have been short term or hydroponic experiments using high rates of ZnO NPs application (up to 4000 mg kg^{-1}) (Lin and Xing, 2007; Lin and Xing, 2008; López-Moreno et al., 2010; Stampoulis et al., 2009). Plant roots and vascular systems are not fully developed at initial growth stages so the reported results may not represent the effect of ZnO NPs on mature plants. The root system and particularly the Casparian strip that acts as a barrier for uptake of most particles, is more likely to be compromised in hydroponic systems than in actual soil due to mechanical stress (Handy et al., 2011). Moreover, results of most of these studies using high spike rates, may not predict the effect of low rate application of ZnO NPs which are likely to promote plant growth (Bernhardt et al., 2010). Kim et al (2011) compared longer term effects of a high rate of application (2000 mg kg^{-1}, unspecified whether based on Zn or ZnO concentration) of soluble Zn ($ZnCl_2$), Zn NPs (50 nm) and ZnO NPs (50 nm) on growth of cucumber (*Cucumis sativius*) in an acidic (pH 5.5) soil. Plant Zn concentrations indicated a much higher bioavailability and toxicity of Zn from soluble Zn (despite a lower measured dose in soil) compared to Zn added as NPs, which they attributed to NP aggregation and retention in the soil (Kim et al., 2011). Du et al. (2011) also reported the results of a field experiment where ZnO NPs (40 nm) were added at a rate equivalent to 45 mg ZnO kg^{-1} to an alkaline soil (pH 7.36) in lysimeters and incubated for 2 months prior to growth of wheat (*Triticum aestivum*). Wheat growth was reported to be slightly reduced and plant Zn concentrations slightly increased by addition of ZnO NPs (Du et al., 2011). However, soluble Zn or bulk ZnO treatments were not included in this study and in it is unclear if replicate treatments were compared. The magnitude of the Zn concentrations in plants (~30 mg kg^{-1} in leaf tissue) were in the normal range for agricultural crops and certainly well below those reported to cause phytotoxicity (Reuter and Robinson, 1997). Hence, the growth reduction was unrelated to addition of Zn in the form of NPs.

Results from toxicity experiments using soil invertebrates and proper soluble Zn and bulk ZnO controls suggest that any toxicity of ZnO NPs is due to dissolution of Zn from the NPs (Kool et al., 2011). In terrestrial environments, ZnO NPs may undergo dissolution or surface modifications (aggregation, complexation with natural organic/inorganic components of soil) which highly affect their bioavailability (Bernhardt et al., 2010). Solid- liquid partitioning of ZnO NPs or dissolved Zn^{2+} released from ZnO NPs would also affect their bioavailability through their retention in soil (Kool et al., 2011). Different soil properties such as pH, ionic strength, dissolved organic carbon, clay content or zeta potential of soil solids may affect aggregation or retention of NPs in soils (Cornelis et al., 2011; Peralta-Videa et al., 2011). There is little information available on the solid-solution partitioning of ZnO NPs in soil. Kool et al. (2011) showed that pore water Zn concentrations (and toxicity to soil invertebrates) were much lower in an acidic soil treated with ZnO NPs (nominally < 200 nm) compared to similar soluble Zn treatments, suggesting limited dissolution over the 4 wk incubation. This contrasts with the small differences in Zn dissolution between bulk and nanoparticulate forms of ZnO found by Franklin et al. (1997) by dialysis methods in algae culture media and by Milani et al. (2012b) in dissolution experiments in sand columns.

The present study therefore aimed to examine retention of ZnO NPs in a range of soils with different physical and chemical properties and to evaluate the bioavailability and bioaccumulation of Zn from ZnO NPs in relation to potential retention and solubility of NPs as affected by soil properties using isotopic dilution techniques.

4.2 MATERIALS AND METHODS

4.2.1 Nanoparticulate/ bulk ZnO characterisation

Two commercially available ZnO NPs powders namely NanoAmor ZnO NPs (nominal diameter 20 nm; Nanostructure & Amorphous Material Inc., Houston, USA) and NanoSun ZnO NPs (nominal diameter 30 nm; Micronisers Pty Ltd, Melbourne, Australia) were purchased for the study. Zinc oxide powder with nominal size < 250 µm was purchased from Umicore Zinc Chemicals (Eijsden, The Netherlands) which will be named bulk ZnO to be distinguishable from ZnO NPs. Size, structure and morphology of nanoparticulate and bulk ZnO samples was examined using transmission electron microscopy (TEM; Phillips CM200, Eindhoven, The Netherlands). Mineralogy of ZnO powders were identified using X-ray diffraction (XRD; PANalytical X'Pert Pro, Almelo, The Netherlands) from which the crystallite size was estimated using the Scherrer equation (Hammond, 2009). The specific surface area of particles was determined using the Brunauer-Emmett-Teller (BET) surface area equation (Brunauer et al., 1938) after liquid N_2 adsorption (Quanta Chrome, USA). A 400 mg Zn L^{-1} stock suspension of ZnO NPs was prepared in ultrapure deionised water (Milli-Q, Millipore, Billerica, MA) and sonicated for 3 min. at 1500 W L^{-1} using an ultrasonic probe (VirtisVirsonic, Gardiner, NY) followed by centrifugation at 4000 g for 15 min. The z-averaged hydrodynamic diameter and electrophoretic mobility of ZnO nanoparticles in the resulting suspension was determined using dynamic light scattering (DLS; Zetasizer Nano ZS, Malvern Instruments Ltd., U.K.) which allowed calculation of zeta potentials.

4.2.2 Soil sampling and characterization

Five soil samples with diverse physical and chemical properties were collected from the A horizon of selected soils in South Australia and Queensland, Australia (Table 1). Soil samples were air-dried at 25 °C and ground to pass through a 2 mm stainless steel sieve. Soil pH and EC of soils were measured in 1:5 m/v soil: deionised water following 1 h of end-over-end shaking. Total Zn concentrations of soils were determined using open vessel *aqua regia* extraction (1:3 HCl: HNO_3) at $140^{o}C$ followed by determination of total Zn concentrations in solutions by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Spectro, Kleve, Germany). Cation exchange capacity (CEC), total carbon, dissolved organic carbon (DOC) and calcium carbonate content of soils were measured according to standard methods (Rayment and Higginson, 1992). The percentages of clay, sand and silt in soils were measured using the pipette method described by Gee and Bauder (1986).

Table 1 - Selected properties of the soils.

	Port Kenny	Bute	Emerald Black	Mount Compass	Ingham
pH (1:5 water)	8.6	7.7	7.1	5.9	5.4
EC (1:5 water), dS m^{-1}	0.41	0.09	0.10	0.05	0.02
Total C, mg kg^{-1}	1507	672	169	103	520
DOC, mg kg^{-1}	1017	469	77	73	512
CEC, cmol kg^{-1}	16.6	3.6	65.7	2.0	6.8
Carbonate content as $CaCO_3$, %	24	<0.5	<0.5	0.2	<0.5
Clay, %	24	6	59	4	30
Sand, %	66	92	27	96	44
Texture	Sandy clay loam	Sand	Clay	Sand	Clay loam
Total Zn, mg kg^{-1}	19.59	16.02	2.44	1.24	19.43
Total Mn, mg kg^{-1}	119.99	129.9	18.9	4.21	401.2
Total Al, %	1.97	1.68	3.81	0.05	4.25
Total Fe, %	1.02	1.32	0.16	0.05	5.31

4.2.3 Adsorption isotherms

Adsorption isotherms of NanoSun and NanoAmor ZnO NPs, soluble Zn ($ZnSO_4$; AnalaR®, 99.5%) were obtained for 5 selected soils using a batch equilibrium technique (Cornelis et al., 2010; OECD, 2000). For soluble Zn, adsorption isotherms (n = 3) were determined in soil suspensions (2.5 ±0.01 g of each soil: 25 mL of 2 mmol L^{-1} KNO_3; Aldrich®, 99.999%) spiked to add 1.5, 5, 10, 25, 50 and 100 mg Zn kg^{-1} soil. The suspensions were equilibrated for 24 h in an end-over-end shaker, centrifuged for 15 min. at 2300 g and filtered through 0.45 µm pore size filters (Millipore, Ireland). All 0.45 µm filters were pre-conditioned using 0.1 mmol L^{-1} $Cu(NO_3)_2$ solution to occupy adsorption sites at the surface of filters with Cu and eliminate Zn adsorption because preliminary experiments showed Cu pre-conditioning improved Zn recovery from less than 25% to nearly 100% in solution containing less than 100 µg Zn L^{-1} (data not shown). Total Zn concentrations in filtrates were determined using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce, USA). The amount of adsorbed Zn ($[Zn_{sorb}]$; mg kg^{-1}) was calculated for all Zn forms using the equation:

$$[\mathrm{Zn}_{sorb}] = [\mathrm{Zn}_{add}] - ([\mathrm{Zn}_{MF}] - [\mathrm{Zn}_{geo}]) \times \frac{L}{S} \qquad (1)$$

where $[Zn_{add}]$ is the total amount of spiked Zn (mg kg^{-1}) , $[Zn_{MF}]$ is the equilibrium concentration of Zn in solution passed through 0.45 µm filters (mg L^{-1}), $[Zn_{geo}]$ is the geogenic concentration of Zn in solution from control (mg L^{-1}) and L/S is the liquid to solid ratio for the experiment. The plot of Zn_{sorb} (mg kg^{-1}) versus Zn_{MF} (mg L^{-1}) was used to fit experimental data to adsorption isotherm models in each soil. The empirical Freundlich equation was fitted to the experimental data using:

$$[\mathrm{Zn}_{sorb}] = k_{f(Zn^{2+})} \times [\mathrm{Zn}_{MF}]^{\frac{1}{n}(Zn\,2+)} \qquad (2)$$

where $K_{f\ (Zn2+)}$ (L kg^{-1}) is the Freundlich adsorption coefficient and $1/n_{(Zn2+)}$ is an empirical constant (dimension less) which is indicative of the shape of the adsorption-concentration relationship (Mohan and Singh, 2002). The non-linearity constant of the Freundlich equation (*1/n*) normally ranges between 0 and 1 for solutes having high solubility compounds (i.e. precipitation is not important) and this indicates adsorption to charged sites dominates sorption to soil, whereas values larger than 1 suggest precipitation of poorly soluble solid phases dominates sorption (Foo and Hameed, 2010).

Stock solutions for ZnO NPs were prepared for each ZnO NP treatment (see supporting information for optimisation procedure of stock preparations) and Zn concentrations in stock NP suspensions were measured using ICP-MS following digestion with concentrated HNO_3 (Cornelis et al., 2010). Adsorption isotherms (n = 5) for ZnO NPs were determined by equilibrating 2.5 ±0.01 g of each soil with 25 mL of 2 mmol L^{-1} KNO_3 (Aldrich®, 99.999%) containing stable suspensions of ZnO NPs to give additions of 1.5, 5, 10, 25, 50 and 100 mg Zn kg^{-1} soil. The samples were shaken end-over-end for 24 h and subsequently centrifuged at 2300 g for 15 min. The supernatant were filtered through Cu pre-conditioned 0.45 µm pore size filters to separate dissolved Zn species and ZnO nanoparticles (<450 nm) (Zn_{MF}) from larger colloidal and aggregated materials. In order to separate ZnO NPs from the "truly dissolved" Zn species, the <0.45 µm filtrates were passed through 1 kDa cut-off ultra-filtration centrifugal devices (Pall-Gellman, USA) at 4000 g for 15 min. Pre-conditioning of 1 kDa filters using Cu^{2+} was unnecessary as recoveries of soluble Zn from these filters was not significantly improved following Cu pre-conditioning of 100 µg Zn L^{-1} solution (data not shown). Total Zn concentrations in <0.45 µm and <1 kDa filtrates (Zn_{MF} and Zn_{UF}, respectively) were measured using ICP-MS following digestion with HNO_3. The < 1kDa filtrate was operationally defined as truly dissolved

Zn (Zn_{ionic}) , whereas the <0.45 um filtrate contains suspended individual ZnO NP and small aggregates (Zn_{NP}) as well as dissolved Zn adsorbed to natural colloids and truly dissolved Zn. To prepare adsorption isotherm of total ZnO NPs and associated dissolved Zn species, total adsorbed Zn was calculated using equation 1 and the plot of Zn_{sorb} (mg kg^{-1}) versus Zn_{MF} (mg L^{-1}) was fitted to the Freundlich equation which resulted in adsorption coefficients for ZnO NPs and dissolved species ($K_{f\,(ZnO\&Zn2+)}$ *and* $1/n_{(ZnO\&Zn2+)}$).

In order to distinguish the sorption of ZnO NPs from dissolved Zn species derived from ZnO NPs, the concentration of ionic Zn (Zn_{ionic}) and nanoparticulate ZnO (Zn_{NP}) in solution were calculated as:

$$[\mathrm{Zn}_{ionic}] = [\mathrm{Zn}_{UF}] - [\mathrm{Zn}_{geo}] \qquad (\mathrm{mg\ L^{-1}}) \qquad (4)$$

$$[\mathrm{Zn}_{NP}] = [\mathrm{Zn}_{MF}] - [\mathrm{Zn}_{UF}] \qquad (\mathrm{mg\ L^{-1}}) \qquad (5)$$

This assumes that there is negligible particulate Zn in the < 0.45 μm fraction from geogenic sources (i.e. in Zn_{geo}).

To calculate the concentration of ZnO NPs adsorbed to soil surfaces, the concentration of adsorbed ionic Zn derived from NPs ($Zn_{ionic/sorb}$) was estimated based on known Freundlich parameters ($K_{f\,(Zn2+)}$ and $1/n_{(Zn2+)}$) for soluble Zn^{2+} in the same soil and at similar concentrations of Zn^{2+} in the equilibration solution:

$$\left[\mathrm{Zn}_{ionic\,/sorb}\right] = k_{f\,(Zn^{2+})} \times [\mathrm{Zn}_{ionic}]^{\frac{1}{n}(Zn\,2+)} \qquad (\mathrm{mg\ kg^{-1}}) \qquad (6)$$

Therefore, the concentration of ZnO NPs sorbed to soil surfaces was calculated according to:

$$\left[Zn_{NP/sorb}\right] = [Zn_{sorb}] - [Zn_{ionic\,/sorb}] \qquad (\mathrm{mg\ kg^{-1}}) \qquad (7)$$

The plots of [$Zn_{NP/sorb}$] as a function of [Zn_{NP}] in different soils were fitted to the Freundlich adsorption model to evaluate the affinity and trend of adsorption of ZnO NPs in soils with diverse physical and chemical characteristics. The Freundlich coefficients for ZnO NPs ($K_{f\ (ZnO)}$ and $1/n_{\ (ZnO)}$) were determined for different soil studied.

4.2.4 Plant availability of nanoparticulate ZnO

Separation of the effect of ZnO NPs on plant uptake and production from background Zn in soil is problematic (Klaine et al., 2008). Isotopic dilution techniques offer one solution to improve our understanding of the biological fate of Zn in ZnO NPs in soils and to distinguish the role of added Zn from geogenic Zn. In this experiment, the specific activity of Zn (ratio of radioactive to unlabeled Zn) in plants grown in a ^{65}Zn-labeled soil were used to quantify the dissolution and plant uptake of Zn derived from ZnO NPs in comparison to soluble Zn^{2+} and bulk ZnO. The Port Kenny and Mount Compass soils were spiked with carrier-free ^{65}Zn at the rate of 1 MBq kg^{-1} soil and brought to 20% field capacity (FC, determined by 100 mm water suction or pF2) with deionised water and thoroughly mixed for 2 h. The spiked soils were covered and allowed to equilibrate for 2 wk at 60% of FC. The soils were air-dried at the end of equilibration period and subsamples (505 g pot^{-1}) were placed in closed-bottom pots before spiking with Zn treatments. Zinc treatments (NanoAmor ZnO NPs, Bulk ZnO and $ZnSO_4.7H_2O$) were added as dry powders to pots in order to standardise method of addition and to avoid discrepancies in spike rate due to instability of bulk and nanoparticulate ZnO suspensions. Final spike rates of 4 and 100 mg Zn kg^{-1} in soil were obtained by addition of appropriate amount of each Zn treatment. The mixture of ^{65}Zn radioisotope-labeled soil and Zn treatments were then agitated end-over-end for 3 h and subsequently incubated for 0 and 3 mo. in the dark at 22°C. Each treatment had 4 replications in a completely randomized design. Control pots were treated and incubated exactly the same way.

Following each incubation period, a basal nutrient solution was added to each pot to ensure that Zn was the only nutrient affecting plant growth. Each pot received nutrient solution containing 4.16 mg Mn as Mn sulphate ($MnSO_4.H_2O$), 6.29 mg Cu as Cu sulphate ($CuSO_4.5H_2O$), 114.11 mg K as K sulphate (K_2SO_4), 15.93 mg Fe as Fe sulphate ($FeSO_4.7H_2O$). Nitrogen was provided as urea ammonium nitrate in nutrient solution and as monoammonium phosphate granules (MAP) at a rate of 64 mg N per pot. Three granules (40 mg each) of MAP were placed at 40 mm depth from the surface of the pots to provide phosphorus (P) requirements of plants. The position of pots was randomized in a controlled environment growth chamber (12 h days at 20°C and 12 h dark at 15°C, 40% humidity).

Wheat seeds (*Triticum durum*) were pre-germinated for 48 h at 25°C before being sown in pots (three seeds per pot). The plants were grown for 6 wk and the water content of pots was maintained at 80% FC by daily watering to weight with deionised water. Shoots were harvested and dry weights were recorded after drying the plant material to constant weight at 60°C. Plant materials were then ground and digested in concentrated HNO_3 (Zarcinas et al., 1987). Wheat seeds were also digested to quantify the contribution of seed Zn towards total plant Zn uptake. Soil samples were collected from each pot, dried in the oven at 60°C and then digested using *aqua regia* (1:3, HCl: HNO_3). All soil and plant digest solutions were analysed for total Zn using ICP-OES. The activity of ^{65}Zn in digests was determined using γ spectroscopy (Wallac Wizard 1480, PerkinElmer Corp., Waltham, MA).

The isotopically available pool of Zn as determined by plant uptake (L-value) (Larsen, 1952) was calculated as follows:

$$L = \frac{\mathrm{Zn}_{shoot} - \mathrm{Zn}_{seed}}{\mathrm{r}_{shoot}} \times \mathrm{R} \qquad (\mathrm{mg\ kg}^{-1}) \qquad (8)$$

where $\mathrm{Zn}_{\mathrm{shoot}}$ represents the concentration of Zn in plant shoot (mg kg^{-1}), $\mathrm{Zn}_{\mathrm{seed}}$ is the concentration of Zn in seeds (mg kg^{-1}), $\mathrm{r}_{\mathrm{shoot}}$ indicates the activity of isotope in plant shoots (MBq kg^{-1}) and R is the total radioactivity introduced into the soil (MBq kg^{-1}). The percentages of Zn derived from individual Zn treatments ($\mathrm{Zn}_{\mathrm{dft}}$) were calculated according to (Bertrand et al., 2006) as follows:

$$\%\, Zn_{dft} = \left(1 - \frac{L_{control}}{L_{Zn}}\right) \times 100 \qquad (9)$$

Analysis of variance (ANOVA) was performed using Genstat software (13th edition, VSN International, Hemel Hempstead, UK). The experimental design was a factorial using a completely randomized block design. Data were analysed using ANOVA with factors including 4 (3 Zn sources and no Zn control) × 2 (Zn rate) × 2 (soil) × 2 (incubation) in 4 blocks (replicates). The least significant difference (LSD, $P \leq 0.05$) was used to compare differences between treatment means.

4.3 RESULTS AND DISSCUSION

4.3.1 Characterisations of ZnO NPs and bulk ZnO powders

The TEM image of ZnO NPs in water suspension illustrated formation of large aggregates of nearly spherical particles (Figures 1a and 1b). Aggregation of ZnO NPs may be due to sample preparation procedure for TEM (air-drying) and/or aggregation of ZnO NPs in suspensions. The Z-averaged hydrodynamic diameter of the aggregates of ZnO NPs in suspension was estimated to be 312 nm and 130 nm for NanoAmor and NanoSun ZnO NPs, respectively (Table 2). Size estimates based on crystallite size determined by XRD and BET-N_2 analysis suggested a primary particle diameter for

ZnO NPs consistent with the nominal particle sizes (Table 2), which in combination with DLS results suggests that both ZnO NPs were aggregated readily in water suspension. A high degree of aggregation is expected at pH values close to the pH value where net surface charge is zero (pH_{PZC}) (Guzman et al., 2006; Kanel and Al-Abed, 2011). The pH of ZnO NPs suspension prepared for characterization (pH = 8) was close to pH_{PZC} of ZnO NPs (pH_{PZC} ranges between 8 and 10.3 based on surface chemistry (Kosmulski, 2001; Tso et al., 2010)). The zeta potential of NanoAmor and NanoSun ZnO NPs at pH = 8 was +15.7 mV and +34.2 mV, respectively. The magnitude of zeta potential is an indicator of the repulsive forces between particles and therefore it can provide a good estimation of the suspension stability (Hunter, 2001). The larger zeta potential values for NanoSun NPs compared to NanoAmor ZnO NPs explains their lower degree of aggregation and smaller z-averaged hydrodynamic diameter.

The TEM image of aqueous suspensions of bulk ZnO (< 250 µm) illustrated large star shaped particles in the aqueous suspension (Figure 1c). The average crystallite size of bulk ZnO particles was >500 nm, and the specific surface area of 1 m^2 g^{-1} corresponds to an estimated diameter of 1.07 µm using the BET-N_2 method (Table 2). The XRD analysis of both ZnO NP samples and bulk ZnO powder indicated that all ZnO samples were primarily zincite (ZnO).

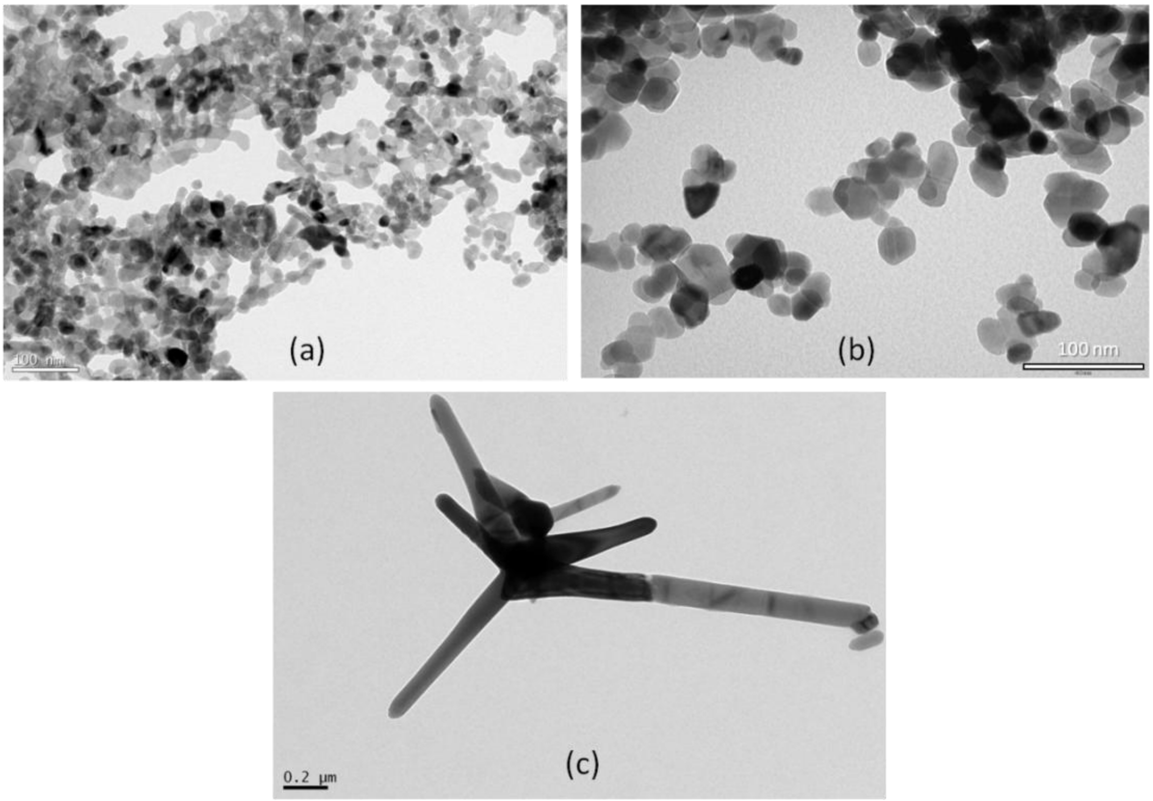

Figure 1- Transmission electron microscopy images of the ZnO powders applied in the experiment (a) NanoAmor ZnO (b) NanoSun and (c) bulk ZnO. The scale bar for ZnO NPs and bulk ZnO are 100 nm and 0.2 µm, respectively.

4.3.2 ZnO NP dissolution and retention in soil

The dissolution and retention of ZnO NPs in soil pore water was strongly dependent on the chemical and physical properties of soils (Figure 2). In the acidic soils (Mt Compass and Ingham), Zn was mainly found as soluble Zn species in equilibrated solutions due to rapid dissolution of ZnO NPs, likely by direct proton attack of the surface of ZnO NPs and formation of hydrolysis species in solution (Bian et al., 2011). A very small percentage (< 1.5%) of added ZnO NPs remained as nanoparticles (> 1 kDa) in the solution (< 0.45 µm) after equilibration for 24 h in the acidic soils (Figure 2). In the Ingham soil with lower pH and higher CEC values compared to the Mount Compass soil (Table 2), smaller concentration of Zn in equilibrated solution remained as ZnO NPs, indicating higher solubility and/or retention of ZnO NPs in this soil.

Table 2- Some of the characteristics of manufactured ZnO nanoparticles and bulk ZnO samples used in the experiment.

Property	NanoAmor ZnO	NanoSun ZnO	Bulk ZnO
Crystal structure	Zincite	Zincite	Zincite
Specific surface area (BET-N_2)	31 m^2g^{-1}	35 m^2g^{-1}	1 m^2g^{-1}
Zeta potential (mV)	+15.7	+34.2	NA
Nominal size	20 nm	30 nm	< 250µm
Z-averaged hydrodynamic diameter (DLS)	311 nm	130 nm	ND
Crystallite size (Scherrer equation estimate)	20 nm	35 nm	> 500 nm
Particle diameter (BET-N_2 estimate)	35 nm	31 nm	1.07 µm

In the neutral to alkaline soils (Emerald Black, Bute and Port Kenny), the concentrations of Zn found in < 0.45 µm solutions (Zn^{2+} and ZnO NPs) were one to two orders of magnitude less than the concentrations detected in acidic soils (Figure 2). Reduced solubility of ZnO NPs in the alkaline soils as well as expected high degree of aggregation in soil pH within one unit from literature pH_{PZC} value of ZnO NPs, likely decreased the Zn concentrations in < 0.45 µm solutions compared to the acidic soils. Furthermore, the high Ca concentrations in the highly calcareous Port Kenny soil could promote aggregation of ZnO NPs and their heterocoagulation with soil minerals. The ionic strength of Port Kenny soil was estimated from electrical conductivity of soil (Griffin and Jurinak, 1973) (ca. 4.9 mmol L^{-1}) was higher than the critical coagulation concentration of ZnO nanoparticles (0.125 mmol L^{-1} $CaCl_2$, (Shih et al., 2008)) which were conducive for nanoparticle aggregation in this soil (Hotze et al., 2010; Zhou and Keller, 2010). Port Kenny soil contained numerically higher concentrations of DOC than the Bute soil (Table 2). It is well established that DOC may adsorb onto the surface of NPs and dissociation of surface functional groups may produce polar sites (e.g. –OH) at the surface of these coatings and subsequently bridge nanoparticles through hydrogen bonding (Espinasse et al., 2007; Lin et al., 2010). Therefore, greater aggregation and retention of ZnO NPs could explain lower concentrations of Zn NPs in the Port Kenny soil compared to the Bute soil.

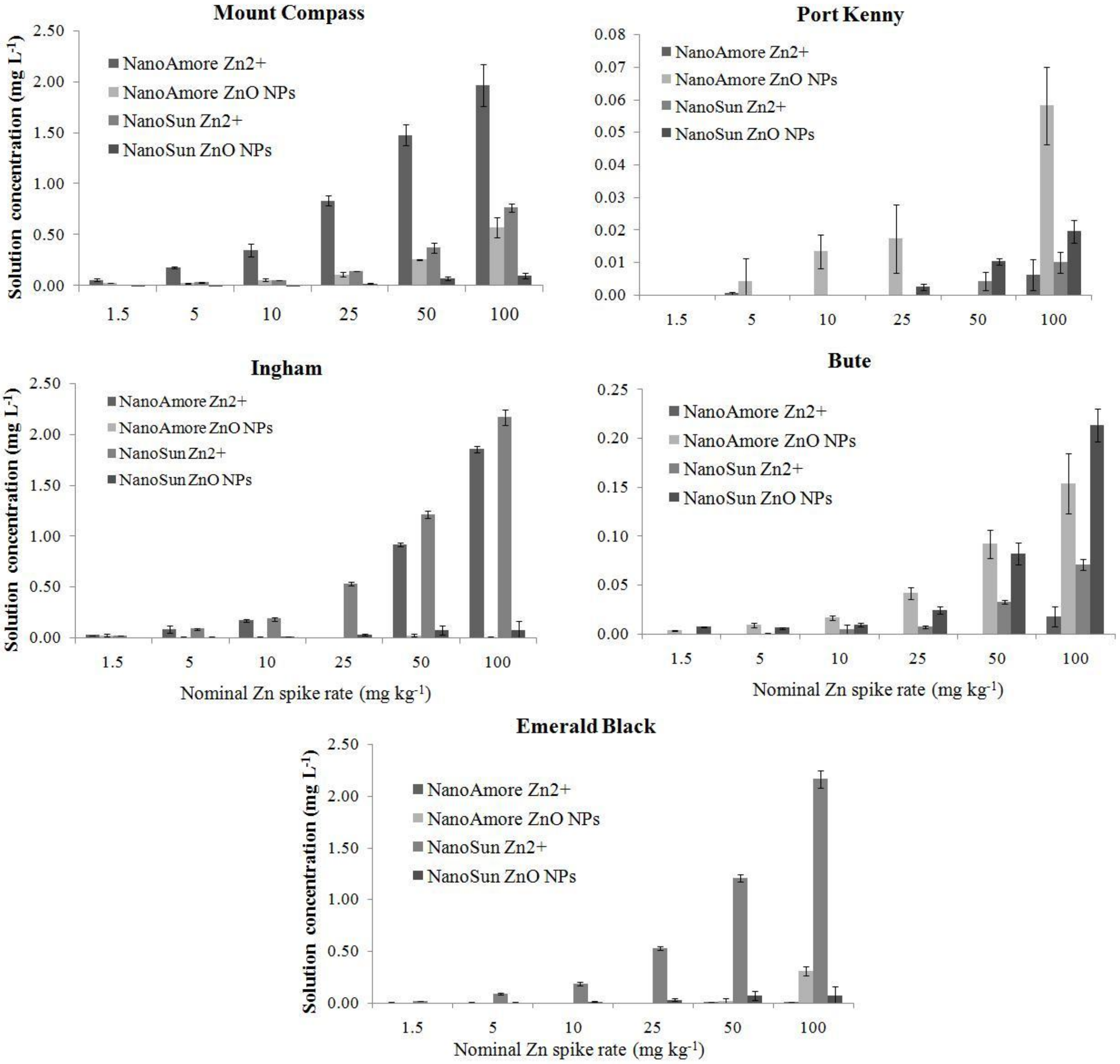

Figure 2- The concentrations of ZnO NPs (Zn concentration in < 0.45 µm filtrate minus the Zn concentration in < 1 kDa filtrate) and truly dissolved Zn species (Zn concentration in < 1 kDa filtrate) in solution following equilibrating soils with different rates of ZnO NPs.

Although the Emerald Black soil had neutral pH values (pH =7.1), the very high clay content and CEC of this soil may have effectively retained the ZnO NPs and any ionic Zn^{2+} dissolved from ZnO NPs due to electrostatic attraction of positively charged ZnO NPs and negatively charged clay surfaces at alkaline soil pH. These results indicate Zn NPs would undergo rapid dissolution and/or are highly sorbed and hence would have reduced mobility in high clay soils.

4.3.3 Adsorption isotherms

The adsorption affinity ($K_{f\ (ZnO\&Zn2+)}$) and adsorption intensity ($1/n_{(ZnO\&Zn2+)}$) of ZnO NPs and dissolved Zn^{2+} from ZnO NPs depended on the Zn source and the physico-chemical properties of the soils (Figure 3 and Table 3). The K_f parameter from the Freundlich equation can be used provide a relative measure for comparing adsorption tendencies of different soils (Singh et al., 2006). The K_f values of ZnO NPs and dissolved Zn from NPs (e.g. $K_{f\ (ZnO\&Zn2+)}$) ranged from 32 L kg^{-1} in the Mount Compass soil to 993 L kg^{-1} in the Port Kenny soil.

Soil pH showed a considerable influence on adsorption of both soluble and ZnO NPs in soils. The acidic soils (Mount Compass and Ingham) generally had smaller $K_{f(ZnO\&Zn2+)}$ values than alkaline soils indicating lower adsorption affinity of these soils (Table 3). The same low adsorption affinity was observed for soluble Zn added to acidic soils (Table 3). At alkaline soil pH, the negative surface charge of soil solid surfaces arises from fixed negative charge of layer silicates and, in combination with negatively charged functional groups due to deprotonation of pH-dependent surface groups, can increase the adsorption energy of soil surfaces for positively charged particles (Elzinga et al., 1999; Sparks, 1999). Given the pH of the soil suspensions were close to the pH_{PZC} of ZnO NPs, they are expected to have negligible or slightly positive surface charges which could enhance retention of ZnO NPs. Kanel and Al-Abed (2011) reported a strong influence of pH on mobility and retention of ZnO NPs in sand columns. Increases in the pH of the solution enhanced the deposition of ZnO NPs. At a solution pH equal to the pH_{PZC} of the ZnO NPs, strong aggregation and complete deposition of ZnO NPs were reported (Kanel and Al-Abed, 2011). However, they used silica sand as the porous medium and pH effects are likely to dominate where the particle size and surface properties of the solid phase are held relatively constant.

Porous media where organic matter content and clay content and mineralogy vary are likely to react quite differently with regard to NP retention as pH is varied.

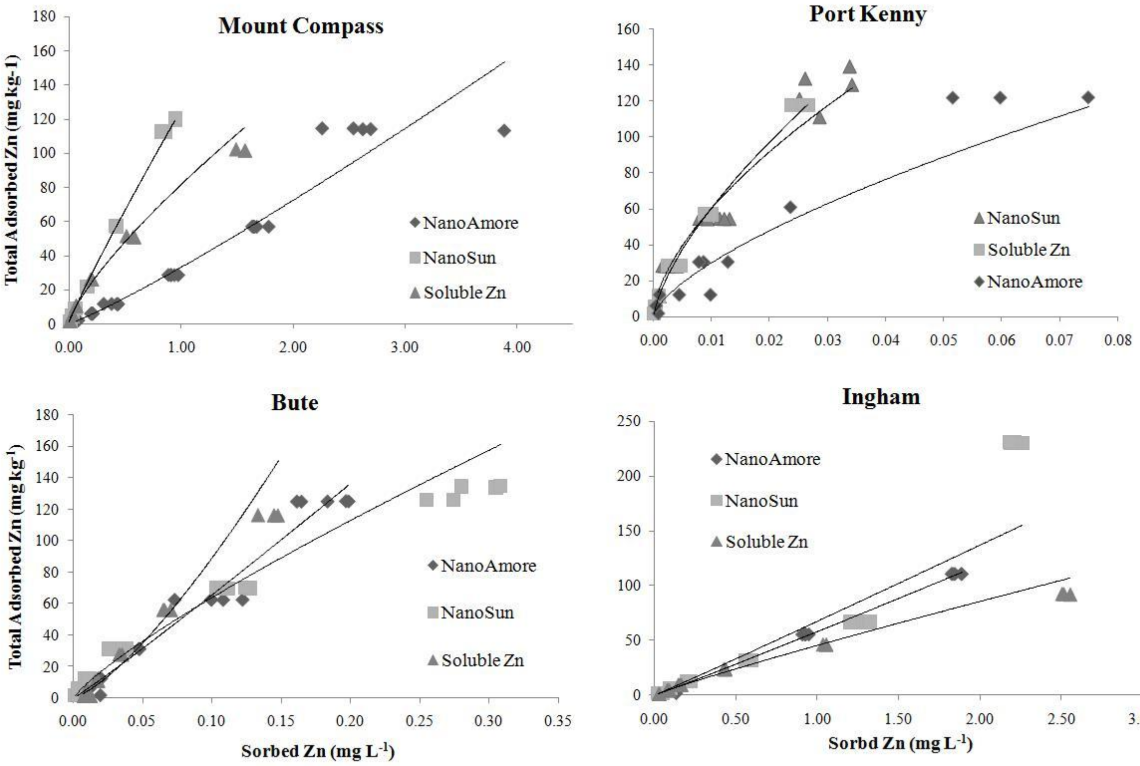

Figure 3- Sorption isotherm of ZnO NP$_S$ and dissolved Zn from ZnO NPs in 4 soils. Strong retention of Zn in the clay-rich Emerald Black soil at the ZnO spike rate investigated restricted plotting adsorption isotherm for this soil.

Effects of clay content on sorption are difficult to distinguish in with such a small set of soils where several soil properties are co-variant, but sorption was strong in the clayey Emerald Black soil where Zn concentrations in equilibrium solutions were less than the detection limit of the ICP-MS (0.1 µg L^{-1}) unless the spike rate exceeded 50 mg Zn kg^{-1} for NanoAmor ZnO NPs. The strong retention of Zn in this soil precluded fitting the Freundlich model to data. Cornelis et al. (2011) also reported a positive correlation between clay content of soils and retention of cerium oxide NPs due to hetero-coagulation of nanoparticles with clays. The adsorption is lower in Ingham soil, especially for NanoSun ZnO NPs, despite the high clay content. The clay content in this soil is predominantly composed of Fe-oxides as indicated by the relatively high oxalate extractable Fe-content (Table 2). At the pH of Ingham soils suspensions (pH 5.8 ± 0.1), these surfaces are positively charged, thus repelling the positively charged ZnO NPs.

Table 3– The Freundlich isotherm coefficent (K_F; L kg^{-1}), n (dimensionless) and goodness of fit (R^2) for the Freundlich equation fitted to the adsorption isotherms of total ZnO NPs and soluble Zn.

Soil	NanoAmor ZnO NP			NanoSun ZnO NP			Soluble Zn		
	$K_{f\,(ZnO\&Zn2+)}$	*1/n*	R^2	$K_{f\,(ZnO\&Zn2+)}$	*1/n*	R^2	$K_{f\,(Zn2+)}$	*1/n*	R^2
Mount Compass	32	1.15	0.99	125	0.92	0.99	81	0.77	0.99
Port Kenny	673	0.68	0.83	993	0.61	0.94	1381	0.68	0.99
Ingham	67	1.20	0.99	58	1.04	0.98	45	0.91	0.99
Emerald Black†	-	-	-	-	-	-	688	0.43	0.99
Bute	745	1.06	0.90	423	0.82	0.93	2018	1.35	0.91

† Strong retention of Zn in the clay-rich Emerald Black soil at the ZnO spike rate investigated restricted calculation of isotherm parameters.

The intensity of adsorption described by the *1/n* parameter of Freundlich model is revealed to be affected by the type of Zn source in the order of NanoAmor > NanoSun > soluble Zn except in the Bute soil. Different *1/n* values for ZnO NPs can be a reflection of the initial stability of the nanoparticles. The stability of NanoAmor ZnO NPs was less than NanoSun NPs based on zeta potential measurements (Table 2) which can explain greater aggregation and retention of NanoAmor NPs compared to NanoSun NPs. The *1/n* values for nanoparticles were close or higher than one indicating precipitation-like reactions (aggregation or hetero-coagulation) or cooperative adsorption (Foo and Hameed, 2010) of ZnO NPs and dissolved species derived from ZnO NPs in soils. To date, retention of ZnO NPs in different soils has not been documented. Kool et al. (2011) reported the *1/n* parameter for ZnO NPs (nominal size < 200 nm) in a loamy sand soil with low pH to be 1.5 compared to 0.42 for soluble Zn sources (Kool et al., 2011).

Retention of ZnO NPs that were not dissolved in soil solution was distinguished from partitioning of soluble Zn dissolved from ZnO NPs using ultrafiltration technique and calculated based of Freundlich parameters for soluble Zn source. Freundlich $K_{f(ZnO)}$ and $1/n_{(ZnO)}$ coefficients for ZnO NPs were generally greater than the ones which were calculated for NPs and soluble Zn species combined (Tables 3 and 4). This suggests that ZnO NPs are retained more strongly than soluble Zn in soils.

Table 4 – The Freundlich isotherm coefficent ($K_{f(ZnO)}$; L kg^{-1}), $1/n_{(ZnO)}$ (dimensionless) and goodness of fit (R^2) for the Freundlich equation fitted to the adsorption isotherms of ZnO NPs.

Soil	NanoAmor ZnO NP			NanoSun ZnO NP		
	$K_{f\,(ZnO)}$	$1/n_{(ZnO)}$	R^2	$K_{f\,(ZnO)}$	$1/n_{(ZnO)}$	R^2
Mount Compass	-	-	-	1672	1.6	0.95
Port Kenny	522075	3.1	0.93	5892	1.1	0.94
Ingham	1200	1.6	0.69	25	0.9	0.78
Emerald Black	-	-	-	-	-	-
Bute	434	0.8	0.83	134	0.4	0.98

4.3.4 Shoot dry matter production and zinc uptake

Shoot dry matter production of wheat plants was not significantly affected by the size of ZnO particles applied compared to wheat that received no Zn (Figure 4). Durum wheat is considered to be the most sensitive cereal plant to Zn deficiency

(Cakmak et al., 1997) and increases in plant yield have been reported following Zn application to Zn-deficient soils (Cakmak et al., 2001; Kalayci et al., 1999). Particularly, the Port Kenny soil is one which is generally noted to be Zn deficient under field conditions but optimum moisture and temperature in the controlled environment may have precluded this being expressed. Toxic effects of the high application rates of Zn on dry matter production were not observed (Figure 4) which is likely due to the strong partitioning of Zn in Port Kenny and Mount Compass soils. However, application of 100 mg kg^{-1} soluble Zn in both soils significantly reduced the plant shoot growth compared to agronomic spike rate of soluble Zn (Figure 4). Although a recent study by Du et al (2011) reported slight decrease in biomass of wheat shoots as a result of addition of 45 mg ZnO NPs kg^{-1} to a clay loam soil in lysimeters, they did not clarify if the growth reduction observed was significant and also soluble source of Zn and bulk ZnO were not included in their investigation.

Zinc uptake in shoot tissue was much higher at the high Zn spike rate compared to the low Zn spike rate (Table 5). As expected, Zn uptake was also significantly increased by application of Zn sources over control (data not shown). Even the low but agronomically relevant Zn addition rate (4 mg kg^{-1}) increased Zn uptake considerably and Zn concentrations in plants increased from ~20 mg kg^{-1} in the control treatments to ~30-50 mg kg^{-1} in the Zn treatments.

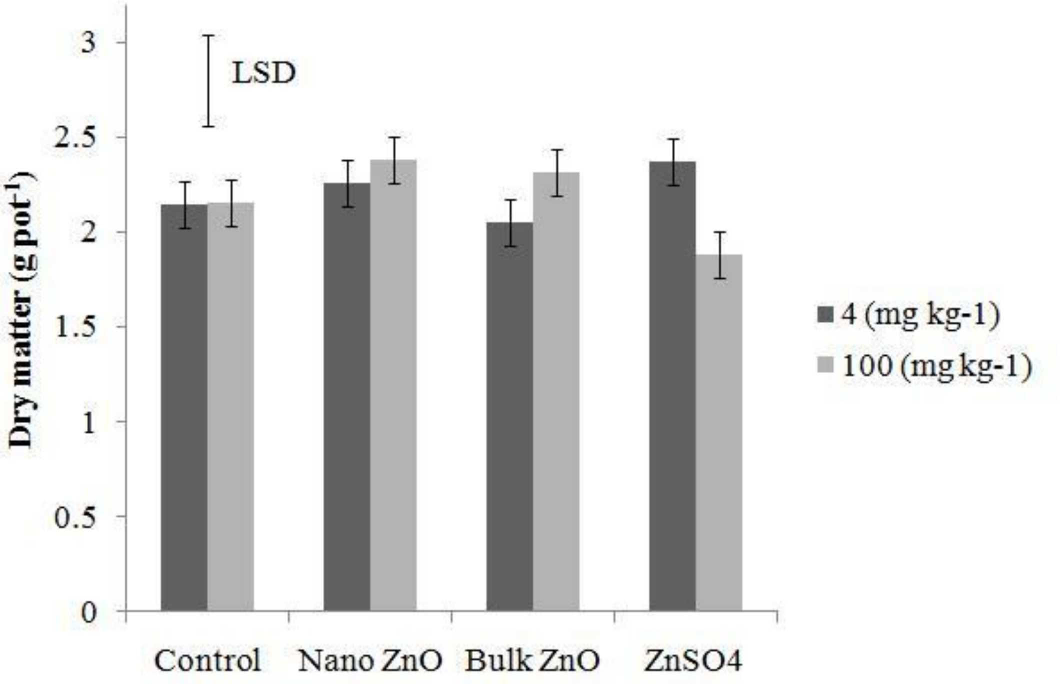

Figure 4 – Shoot dry matter production of durum wheat as a function of Zn source (ZnO NPs, bulk ZnO and soluble Zn ($ZnSO_4$)) and spike rate. The effect of Zn source was compared by pooling different Zn source treatments in 2 spike rates. Least significant difference of means (LSD; $P \leq 0.05$) shown was equal to 0.53.

At agronomic rate of Zn application, Zn uptake in plants treated with ZnO NPs and bulk ZnO were not significantly different and was similar to the Zn uptake from soluble Zn source. Whereas, at higher rate of Zn application Zn uptake was in the order of Zn^{2+} > ZnO NPs > bulk ZnO after grouping data (see supporting information). Plants grown on the acid soil (Mount Compass) were found to accumulate significantly higher Zn amounts compared to the alkaline calcareous soil (Port Kenny). Moreover, aging of the Zn sources in the acid soil significantly improved Zn uptake by plants. As it was reported earlier, acid soils had higher concentration of soluble Zn and ZnO NPs species in the soil solution compared to the alkaline soils (Figure 2). Dissolution of ZnO NPs in the acid soil over the 3-month incubation time likely led to the increased Zn uptake by plants in the 3-month incubation treatments.

Table 5 – Mean values for Zn uptake of wheat plants, labile Zn in soil (L value), percent of added Zn which remained labile (% added ZN labile) and percent of ZN derived from Zn treatment in the plant tissue (Zndft) with no Zn treatments or application of ZnO NPs, bulk ZnO and soluble Zn in 2 rates (4 mg kg^{-1} and 100 mg kg^{-1}) which is cultivated immediately (0 mo) or Zn source incubated for 3 mo in the soil. Least significant difference of means (LSD; $P < 0.05$) for Zn uptake, L value and Zndft and added labile Zn were 0.12, 0.11, 1.91 and 15.7 respectively. Within rows, values by the same letter are not significantly different ($P > 0.05$).

Soil	Incub. mo	Spike rate (mg kg^{-1})					
		4			100		
		Nano	Bulk	Zn^{2+}	Nano	Bulk	Zn^{2+}
		Zn uptake (mg Zn pot^{-1}) †					
Mt Compass	0	0.10 d	0.07 e	0.09 de	0.67 b	0.45 c	1.23 a
	3	0.14 c	0.11 d	0.16 c	0.80 b	0.91 b	1.43 a
Pt Kenny	0	0.07 c	0.07 c	0.05 d	0.48 a	0.36 b	0.45 ab
	3	0.06 c	0.08 c	0.08 c	0.57 a	0.42 b	0.45 b
		L value (mg kg^{-1}) †					
Mt Compass	0	3.68 b	3.29 bc	2.68 c	81.4 a	71.7 a	89.9 a
	3	2.67 b	3.02 b	2.71 b	77.8 a	76.6 a	81.2 a
Pt Kenny	0	6.05 c	5.52 c	3.57 d	65.6 a	54.3 ab	45.8 b
	3	4.16 d	4.16 cd	5.82 c	87.5 a	57.2 b	66.3 ab
		Zndft (%) †					
Mt Compass	0	83.5 b	82.3 b	79.7 c	99.0 a	99.0 a	99.1 a
	3	82.3 b	82.8 b	84.0 b	99.2 a	99.2 a	99.3 a
Pt Kenny	0	79.0 b	74.9 b	61.4 c	97.6 a	97.3 a	96.6 a
	3	53.2 d	66.6 c	71.3 b	97.2 a	96.6 a	97.1 a

† LSD reported for Zn uptake, L value data is related to the transformed values for data analysis

Generally, Zn uptake was higher in the acidic soils, with addition of soluble Zn and after incubation of the Zn treatments in soil, although the trend was not consistent, particularly in the Port Kenny soil with a high Zn retention capacity (see supporting information). These evidences suggest that Zn dissolution was an important mechanism supplying Zn for plant uptake. Du et al. (2011) also reported dissolution of ZnO NPs during 2 mo of incubation in a loamy clay soil was the possible reason for increased Zn concentrations in wheat tissue as no ZnO NPs was observed in wheat tissue following electron microscopy imaging (Du et al., 2011). Detailed X-ray absorption spectroscopy investigations also have confirmed that Zn can be mainly found as Zn coordinated as Zn-nitrates in plant roots exposed to ZnO NPs and no particulate ZnO has been identified in plant tissue (Hernandez-Viezcas et al., 2011; López-Moreno et al., 2010). Given the pore diameter of cell walls of plants are generally in the range of 3.5 – 3.8 nm for root hairs, only nanoparticles or aggregates with diameters less than the cell wall pore diameter can enter the cell wall of undamaged cells (Dietz and Herth, 2011). The crystallite sizes of ZnO NPs applied in this study were greater than diameter of ion channels in plant roots and therefore these channels cannot contribute in internalization of ZnO NPs. Alternative entry routs such as endocytosis through plasma membrane (Nair et al., 2010) or uptake at the point of lateral root formation zone (Dietz and Herth, 2011). These pathways were unlikely to have considerable contribution in ZnO NP uptake as rapid dissolution and retention of ZnO NPs in soils significantly limited the concentration of ZnO NPs in soil solution for plant uptake. Zinc concentration in plant shoots grown on soils with the high spike rate of Zn varied between 180 to 760 mg kg^{-1} which was less than the 2000 mg kg^{-1} toxicity threshold of Zn reported for wheat plants (Wheeler et al., 1993). This explains the lack of any adverse effects of ZnO NPs even at the high Zn spike rate on shoot growth.

4.3.5 Lability of Zn sources

The labile pool of Zn (L value) in soils was significantly higher in Zn treatments than in control soils ($P < 0.001$). Application of ZnO NPs did not enhance concentrations of labile Zn in soils compared to the bulk ZnO treatment (Table 5). The L value data can be used to assess the dissolution of ZnO *in situ* in the soils. It is evident from these data (Table 5) that significant dissolution (~ 50 - 100%) of the ZnO nanoparticles occurred in the soils, even with no incubation period prior to plant growth. Enhanced concentrations of labile Zn at high rates of Zn application did not adversely affect shoot growth. This contrasts with results from *in vitro* studies using nutrient solutions where ZnO NPs were found to adversely affect plant growth (Asli and Neumann, 2009; Lin and Xing, 2008). However, these studies used much higher doses of ZnO NPs and the exposure pathway did not account for interaction of soil with NPs or Zn^{2+} derived from NP dissolution – processes which significantly mitigate toxicity due to retention to the solid phase (see section 4.3.3).

The data for the high soluble Zn^{2+} dose (where by definition all Zn was 100% labile at time zero) indicate that significant amounts of labile Zn moved into non-labile pools in the Port Kenny soil, likely through precipitation of Zn^{2+} as Zn-carbonate or silicate species, as observed previously for fertiliser Zn (Hettiarachchi et al., 2008). Thus for the high Zn dose, it is likely that the lower L values in the Port Kenny soil compared to the Mount Compass soil represent either reduced dissolution of ZnO NPs, or more likely complete dissolution of the NPs and subsequent fixation of the soluble Zn^{2+} released from the NPs. Bulk ZnO has been previously found to dissolve quite rapidly in acidic to neutral soils over time (Smolders and Degryse, 2002). This would occur for ZnO NPs, even in alkaline calcareous soils based on the results observed. The calculated percentages of added Zn source which is labile in soils revealed a large percent of Zn (~ 60 – 99 %) remained labile in soil even following incubation for 3 mo in soils (see supporting information). The percentages of added Zn labile from ZnO NPs and bulk ZnO were high and similar to soluble Zn, indicating significant dissolution of ZnO treatments.

The percentages of Zn derived from Zn treatment (%Zndft) were not affected by the sources of Zn in soils which is likely due to the same rapid and similar dissolution of bulk and nanoparticulate sources of Zn with soluble Zn. (Table 5). Generally, there was no significant advantage in using nano-sized ZnO over bulk ZnO as a source of fertilizer Zn, due to the rapid dissolution of both these Zn forms in soil.

4.4 CONCLUSIONS

Strong retention of ZnO NPs and/or dissolved Zn species from ZnO NPs was found in all soils especially in alkaline and calcareous soils. The adsorption affinity of ZnO NPs was generally greater than that of soluble Zn, which suggested ZnO NPs were retained more strongly than soluble Zn in soils. Soil pH and clay content of soil appeared to be the most important soil properties affecting retention, although the number of soils used was too small to draw firm conclusions as soil parameters co-varied. Rapid and significant dissolution of both bulk ZnO and ZnO NPs was evident in the isotopic dilution experiment using durum wheat (*Triticum durum*) when the ZnO sources were uniformly mixed throughout the soils. A large percentage of the ZnO NPs dissolved and released Zn^{2+} to the labile pool over the plant growth period, confirming that there is low persistence of ZnO NPs, even in alkaline soils. Application of ZnO NPs did not significantly improved shoot dry matter production, Zn uptake at agronomic rate of Zn application and percent of Zn derived from Zn treatment in plants. There appears to be no significant advantage in using nano-sized ZnO over bulk ZnO as a source of fertilizer Zn at agronomic rates of addition to soil, due to the rapid dissolution and strong retention of both these Zn forms in soil. This also may suggest that the risks associated with the presence of ZnO NPs in soils would be the same as soluble sources of Zn.

ACKNOWLEDGEMENT

The authors would like to thank several CSIRO colleagues for their technical assistance namely Caroline Johnston, Gillian Cozens, Catherine Fiebiger, Margaret Yam, Lester Smith, Maria Manjarrez Martinez and Claire Wright. A part of this project has been conducted with the financial support from the Nanosafety Theme, Advanced Materials Transformational Capability Platform, CSIRO Land and Water.

4.5 LITERATURE CITED

Asli, S., and P.M. Neumann. 2009. Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. Plant, Cell Environ. 32:577-584.

Becheri, A., M. Durr, P. Lo Nostro, and P. Baglioni. 2008. Synthesis and characterization of zinc oxide nanoparticles: application to textiles as UV-absorbers. J. Nanopart. Res. 10:679-689.

Bernhardt, E., B. Colman, M. Hochella, B. Cardinale, R. Nisbet, and C. Richardson. 2010. An ecological perspective on nanomaterial impacts in the environment. J. Environ. Qual. 39:1954-1965.

Bertrand, I., M. McLaughlin, R. Holloway, R. Armstrong, and T. McBeath. 2006. Changes in P bioavailability induced by the application of liquid and powder sources of P, N and Zn fertilizers in alkaline soils. Nutr. Cycling Agroecosyst. 74:27-40.

Bian, S.W., I.A. Mudunkotuwa, T. Rupasinghe, and V.H. Grassian. 2011. Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: Influence of pH, ionic strength, size, and adsorption of humic acid. Langmuir 27:6059-6068.

Borm, P., F.C. Klaessig, T.D. Landry, B. Moudgil, J. Pauluhn, K. Thomas, R. Trottier, and S. Wood. 2006. Research strategies for safety evaluation of nanomaterials, Part V: Role of dissolution in biological fate and effects of nanoscale particles. Toxicol. Sci. 90:23-32.

Brayner, R., S.A. Dahoumane, C. Yepremian, C. Djediat, M. Meyer, A. Coute, and F. Fievet. 2010. ZnO nanoparticles: Synthesis, characterization, and ecotoxicological studies. Langmuir 26:6522-6528.

Brunauer, S., P.H. Emmett, and E. Teller. 1938. Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. 60:309-319.

Cakmak, I., H. Ekiz, A. Yilmaz, B. Torun, N. Koleli, I. Gultekin, A. Alkan, and S. Eker. 1997. Differential response of rye, triticale, bread and durum wheats to zinc deficiency in calcareous soils. Plant Soil 188:1-10.

Cakmak, O., L. Ozturk, S. Karanlik, H. Ozkan, Z. Kaya, and I. Cakmak. 2001. Tolerance of 65 durum wheat genotypes to zinc deficiency in a calcareous soil. J. Plant Nutr. 24:1831-1847.

Cornelis, G., J.K. Kirby, D. Beak, D. Chittleborough, and M.J. McLaughlin. 2010. A method for determination of retention of silver and cerium oxide manufactured nanoparticles in soils. Environ. Chem. 7:298-308.

Cornelis, G., B. Ryan, M.J. McLaughlin, J.K. Kirby, D. Beak, and D. Chittleborough. 2011. Solubility and batch retention of CeO2 nanoparticles in soils. Environ. Sci. Technol. 45:2777-2782.

DeRosa, M.C., C. Monreal, M. Schnitzer, R. Walsh, and Y. Sultan. 2010. Nanotechnology in fertilizers. Nat. Nanotechnol. 5:91-91.

Dietz, K.-J., and S. Herth. 2011. Plant nanotoxicology. Trends Plant Sci. 16:582-589.

Du, W.C., Y.Y. Sun, R. Ji, J.G. Zhu, J.C. Wu, and H.Y. Guo. 2011. TiO(2) and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. J. Environ. Monit. 13:822-828.

Elzinga, E.J., J.J.M. Van Grinsven, and F.A. Swartjes. 1999. General purpose Freundlich isotherms for cadmium, copper and zinc in soils. Eur. J. Soil Sci. 50:139-149.

Espinasse, B., E.M. Hotze, and M.R. Wiesner. 2007. Transport and retention of colloidal aggregates of C-60 in porous media: Effects of organic macromolecules, ionic composition, and preparation method. Environ. Sci. Technol. 41:7396-7402.

Foo, K.Y., and B.H. Hameed. 2010. Insights into the modeling of adsorption isotherm systems. Chem. Eng. J. 156:2-10.

Franklin, N.M., N.J. Rogers, S.C. Apte, G.E. Batley, G.E. Gadd, and P.S. Casey. 2007. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga (*Pseudokirchneriella subcapitata*): The importance of particle solubility. Environ. Sci. Technol. 41:8484-8490.

Gee, G.W., and J.W. Bauder. 1986. Particle size analysis, p. 337-382, *In* A. Klute, ed. Methods of Soil Analysis, Part 1-Physical and Mineralogical Methods, 2nd ed. ASA and SSSA, Madison, WI.

Griffin, R.A., and J.J. Jurinak. 1973. Estimation of activity-coefficients from electrical conductivity of natural aquatic systems and soil extracts Soil Sci. 116:26-30.

Guzman, K.A.D., M.P. Finnegan, and J.F. Banfield. 2006. Influence of surface potential on aggregation and transport of titania nanoparticles. Environ. Sci. Technol. 40:7688-7693.

Hammond, C. 2009. The Basics of Crystallography and Diffraction. 3rd ed. Oxford University Press, Oxford.

Handy, R.D., G. Cornelis, T. Fenandes, O. Tsyusko, A. Decho, T. Sabo-Attwood, C. Metcalfe, J.A. Steevens, S.J. Klaine, A.A. Koelmans, and N. Horne. 2011. Ecotoxicity test methods for engineered nanomaterials: Practical experiences and recommendations from the bench. Environ. Toxicol. Chem.:n/a-n/a.

Hernandez-Viezcas, J.A., H. Castillo-Michel, A.D. Servin, J.R. Peralta-Videa, and J.L. Gardea-Torresdey. 2011. Spectroscopic verification of zinc absorption and distribution in the desert plant Prosopis juliflora-velutina (velvet mesquite) treated with ZnO nanoparticles. Chem. Eng. J. 170:346-352.

Hettiarachchi, G.M., M.J. McLaughlin, K.G. Scheckel, D.J. Chittleborough, M. Newville, S. Sutton, and E. Lombi. 2008. Evidence for different reaction pathways for liquid and granular micronutrients in a calcareous soil. Soil Sci. Soc. Am. J. 72:98-110.

Hotze, E.M., T. Phenrat, and G.V. Lowry. 2010. Nanoparticle aggregation: Challenges to understanding transport and reactivity in the environment. J. Environ. Qual. 39:1909-1924.

Hunter, R.J. 2001. Foundations of Colloid Science 2nd ed. Oxford University Press, New York.

Kalayci, M., B. Torun, S. Eker, M. Aydin, L. Ozturk, and I. Cakmak. 1999. Grain yield, zinc efficiency and zinc concentration of wheat cultivars grown in a zinc-deficient calcareous soil in field and greenhouse. Field Crop. Res. 63:87-98.

Kanel, S.R., and S.R. Al-Abed. 2011. Influence of pH on the transport of nanoscale zinc oxide in saturated porous media. J. Nanopart. Res. 13:4035-4047.

Kim, S., J. Kim, and I. Lee. 2011. Effects of Zn and ZnO nanoparticles and Zn^{2+} on soil enzyme activity and bioaccumulation of Zn in Cucumis sativus. Chem. Ecol. 27:49-55.

Klaine, S.J., P.J.J. Alvarez, G.E. Batley, T.F. Fernandes, R.D. Handy, D.Y. Lyon, S. Mahendra, M.J. McLaughlin, and J.R. Lead. 2008. Nanomaterials in the environment: Behaviour, fate, bioavailability and effects. Environ. Toxicol. Chem. 27:1825–1851.

Kool, P.L., M.D. Ortiz, and C.A.M. van Gestel. 2011. Chronic toxicity of ZnO nanoparticles, non-nano ZnO and $ZnCl_2$ to Folsomia candida (Collembola) in relation to bioavailability in soil. Environ. Pollut. 159:2713-2719.

Kosmulski, M. 2001. Chemical Properties of Material Surfaces Marcel Dekker, New York

Larsen, S. 1952. The use of P^{32} in studies on the uptake of phosphorus by plants. Plant Soil 4:1-10.

Lin, D., and B. Xing. 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environ. Pollut. 150:243-250.

Lin, D., and B. Xing. 2008. Root uptake and phytotoxicity of ZnO nanoparticles. Environ. Sci. Technol. 42:5580-5585.

Lin, D.H., X.L. Tian, F.C. Wu, and B.S. Xing. 2010. Fate and transport of engineered nanomaterials in the environment. J. Environ. Qual. 39:1896-1908.

López-Moreno, M.L., G. de la Rosa, J.A. Hernández-Viezcas, H. Castillo-Michel, C.E. Botez, J.R. Peralta-Videa, and J.L. Gardea-Torresdey. 2010. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (Glycine max) plants. Environ. Sci. Technol. 44:7315–7320.

Milani, N., G.M. Hettiarachchi, D.G. Beak, M.J. McLaughlin, J.K. Kirby, and S.P. Stacey. 2012a. Fate of nanoparticulate zinc oxide fertilizers in an alkaline calcareous soil. Soil Sci. Soc. Am. J. Submitted.

Milani, N., M.J. McLaughlin, S.P. Stacey, J. Kirby, G.M. Hettiarachchi, D. Beak, and G. Cornelis. 2012b. Dissolution kinetics of macronutrient fertilizers coated with manufactured zinc oxide nanoparticles. J. Agric. Food Chem. 60:3991-3998.

Mohan, D., and K.P. Singh. 2002. Single- and multi-component adsorption of cadmium and zinc using activated carbon derived from bagasse - an agricultural waste. Water Res. 36:2304-2318.

Nair, R., S.H. Varghese, B.G. Nair, T. Maekawa, Y. Yoshida, and D.S. Kumar. 2010. Nanoparticulate material delivery to plants. Plant Sci. 179:154-163.

OECD. 2000 Guideline for the Testing of Chemicals Organisation for Economic Co-operation and Development, Paris.

Peralta-Videa, J.R., L.J. Zhao, M.L. Lopez-Moreno, G. de la Rosa, J. Hong, and J.L. Gardea-Torresdey. 2011. Nanomaterials and the environment: A review for the biennium 2008-2010. J. Hazard. Mater. 186:1-15.

Rayment, G.E., and F.R. Higginson. 1992. Australian Laboratory Handbook of Soil and Water Chemical Methods Inkarta Press, Melbourne.

Reuter, D.J., and J.B. Robinson. 1997. Plant Analysis : An Interpretation Manual. 2nd Edition ed. CSIRO Publishing, Melbourne, Victoria, Australia.

Rico, C.M., S. Majumdar, M. Duarte-Gardea, J.R. Peralta-Videa, and J.L. Gardea-Torresdey. 2011. Interaction of nanoparticles with edible plants and their possible implications in the food chain. J. Agric. Food Chem. 59:3485-3498.

Shih, Y.-h., S.C. Wu, and R.A. Doong. 2008. The fate and transformation of nanoparticles in water environmental media [Online]. Available by Taiwan EPA http://ehs.epa.gov.tw/Projects/EN_F_ResearchPrjs_Detail/1014.

Singh, D., R.G. McLaren, and K.C. Cameron. 2006. Zinc sorption-desorption by soils: Effect of concentration and length of contact period. Geoderma 137:117-125.

Smolders, E., and F. Degryse. 2002. Fate and effect of zinc from tire debris in soil. Environ. Sci. Technol. 36:3706-3710.

Soper, R.J., G.W. Morden, and M.W. Hedayat. 1989. The effect of zinc rate and placement on yeild and zinc utilization by blackbean (*Phaseolus-vulgaris* var Black Turtle) Canadian Journal of Soil Science 69:367-372.

Sparks, D.L. 1999. Soil physical chemistry 2nd ed. CRC Press, Boca Raton, Florida.

Stampoulis, D., S.K. Sinha, and J.C. White. 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environ. Sci. Technol.

Takkar, P.N., and C.D. Walker. 1993. The Distribution and Correction of Zinc Deficiency, *In* A. D. Robson, ed. Zinc in Soils and Plants, Vol. 55. Kluwer Academic Publishers, Dordrecht ; Boston

Tso, C., C. Zhung, Y. Shih, Y. Tseng, S. Wu, and R. Doong. 2010. Stability of metal oxide nanoparticles in aqueous solutions. Water Sci. Technol. 61:127-133.

Wheeler, D.M., I.L. Power, and D.C. Edmeades. 1993. Effect of various metal ions on growth of two wheat lines known to differ in aluminium tolerance. Plant Soil 155-156:489-492.

Zarcinas, B.A., B. Cartwright, and L.R. Spouncer. 1987. Nitric acid digestion and multi-element analysis of plant material by inductively coupled lasma spectroscopy Commun. Soil Sci. Plant Anal. 18:131-146.

Zhou, D., and A.A. Keller. 2010. Role of morphology in the aggregation kinetics of ZnO nanoparticles. Water Res. 44:2948-2956.

5 Summary and conclusions

Zinc oxide nanoparticles are theoretically more soluble and reactive compared to bulk ZnO particles due to their smaller size and higher specific surface area (Borm et al., 2006). Therefore, applications of ZnO NPs in Zn fertilizer formulations could theoretically improve bioavailability of ZnO for plant nutrition in Zn-deficient soils. However, the fate and bioavailability of ZnO NPs and dissolved Zn released from ZnO NPs in soils are predominantly governed by physical and chemical characteristics of soils and the interactions of ZnO NPs with soil components (Darlington et al., 2009; Lin et al., 2010, Bernhardt et al., 2010; Peralta-Videa et al., 2011). A review of the literature revealed very limited information on the fate and bioavailability of ZnO NPs in natural soils and interactions of ZnO NPs with plants at environmentally relevant concentrations in natural soils. Hence, the experimental components presented in this thesis were conducted to gain a better understanding of the dissolution, diffusion, transformation, partitioning and availability of manufactured ZnO NPs in natural soils.

5.1 Implications of the research findings

5.1.1 Fate of ZnO nanoparticles in soils

The dissolution of metal oxide NPs (including ZnO NPs) and subsequent solid-liquid partitioning of dissolved Zn^{2+} and/or retention of ZnO NPs in natural soils can greatly reduce the mobility of ZnO NPs and their availability for soil biota and plants. Dissolution and retention of ZnO NPs and Zn from ZnO NP powders (nominal sizes of 20 and 35 nm) were evaluated in five soils with diverse physical and chemical properties using six point adsorption isotherms. Following 24 h of equilibration with soils, ZnO NPs were predominantly removed from solution (less than 1.5% of added Zn remained as ZnO NPs in soil solutions). Rapid dissolution of ZnO NPs and inner sphere sorption of dissolved Zn (Scheckel et al., 2010) and/or retention of ZnO NPs are the reasons for the strong solid phase partitioning. The adsorption affinity of ZnO NPs was generally greater than the one for soluble Zn species indicating ZnO NPs were retained more strongly than soluble Zn in soils.

In general, alkaline soils showed greater adsorption affinity for ZnO NPs and dissolved Zn compared to acid soils due to the presence of negatively charged solid surfaces at alkaline pH which enhanced adsorption of positively charged soluble Zn species or ZnO NPs. Moreover, the pH values of the alkaline soils were close to the pH_{PZC} of ZnO NPs (pH_{PZC} = 8 – 10.3 (Kosmulski, 2001)), where electrostatic repulsion between individual NPs are negligible and strong aggregation is expected (Kanel and Al-Abed, 2011). The ionic strength of the suspensions alkaline calcareous soil was likely higher than the critical coagulation concentration of ZnO NPs and the high Ca concentration of the calcareous soil could also have led to aggregation of ZnO NPs (Hotze et al., 2010; Zhou and Keller, 2010). Therefore, deposition of aggregated ZnO NPs would reduce mobility in alkaline and calcareous soils. The clay content of soils was also found to be important in the partitioning of ZnO NPs in soils with greater retention of ZnO NPs and dissolved Zn species derived from nanoparticles in clay-rich soils. The positive correlation of soil clay content and retention of NPs in natural soils could be due to electrostatic attraction of clay minerals and NPs with opposite surface charges (Cornelis et al., 2011; Fang et al., 2009).

Rapid and significant dissolution of both bulk ZnO and ZnO NPs (nominal size of 20 nm) was evident in the isotopic dilution experiment using durum wheat (*Triticum durum*) when the ZnO sources were applied alone and uniformly mixed throughout the soils. This indicates that ZnO should not be regarded as an insoluble Zn source in soils, even in alkaline soils, and that results from solubility tests in water do not give an adequate indication of dissolution in soils. The significant dissolution in soil is due to the particles being uniformly mixed throughout the soil, so that any pH increases as a result of dissolution (consumption of protons) are small and can be buffered by the soil's solid phase. Also, rapid sorption of Zn^{2+} released from the ZnO particles drives the process towards completion (Le Chatelier principle).

On the basis of the results from the dissolution and sorption experiments in Chapters 2 and 4, ZnO NPs will not be persistent in soil systems and hence their mobility in soil would be limited. This will be particularly evident in alkaline calcareous soils, soils with high clay contents and CEC values, and soils with high ionic strengths in the soil solution (i.e. saline soils).

5.1.3 Use of ZnO NPs as micronutrient fertilisers

Solid Zn fertilizers are typically applied in combination with macronutrient fertilizers to maintain a more uniform and cost effective distribution of the small amounts of Zn required in Zn-deficient soils. Therefore, in this thesis nanoparticulate ZnO and bulk ZnO powders were coated onto two macronutrient fertilizers, urea or MAP. The Zn solid phase speciation at the surface of the coated MAP fertilizer granules revealed that Zn was mainly precipitated as zinc ammonium phosphate ($ZnNH_4PO_4$), whereas Zn speciation did not change following coating onto urea granules and remained as ZnO.

Solubility and dissolution kinetics of Zn from nanoparticulate and bulk ZnO coated granules were measured as water solubility is an important factor determining effectiveness of Zn-enriched macronutrient fertilizers (Amrani et al., 1999; Mortvedt and Giordano, 1969; Shaver et al., 2007; Westfall et al., 2005). Particle size of ZnO was found not to affect the solubility and dissolution kinetics of Zn from both coated fertilizer granules. Dissolution of Zn from coated MAP granules was controlled by $Zn(NH_4)PO_4$ precipitation on the surface of MAP granules coated with either particle size of ZnO. Although ZnO particles remained as zincite (ZnO) in the coating of urea granules, high pH and ionic strength in soil solutions from hydrolysis of urea would have promoted aggregation of any ZnO NPs released from the granules and masked the effect of particle size on solubility and mobility of ZnO particles. Moreover, size-independent release rates of Zn from coated urea granules or ZnO powders could be explained based on the solubility of the same compounds formed irrespective of the size of the original ZnO particles (Chapter 2). When coated granules were incubated in soil, again no significant advantage of nanoparticulate ZnO over bulk ZnO could be determined (Chapter 3). Hence there is no advantage from using nanoparticulate sources of ZnO for coating of macronutrient fertilizers. Given the rapid reaction of ZnO NPs to form Zn-phosphate species when coated onto MAP (Chapter 3), it is highly likely the same reactions would occur if the NPs were incorporated into the granule. Hence issue of limited diffusion of Zn away from fertiliser granules remains as a problem to be resolved.

Zinc oxides are also used as fertilisers alone, or in bulk blends with macronutrient fertilisers, where the interaction with macronutrients is either absent or minimised. While this method of Zn delivery is less effective than co-granulation of Zn within the macronutrient fertiliser granule due to poor Zn distribution in soil, it is common practice in some developing countries. The experiments in Chapter 4 revealed that when applied alone, nanoparticulate forms of ZnO again performed no better than bulk ZnO, likely due to rapid dissolution in soil. The surprising results was the high amount of dissolution of ZnO (bulk or NP) in the soils, especially in the alkaline soil, and indicates that ZnO can be regarded as an effective source of Zn when uniformly mixed throughout the soil.

In conclusion, application of ZnO NPs with macronutrient fertilizers and/or application alone appear to have no significant advantages over bulk ZnO as a source of fertilizer Zn.

5.1.2 Environmental implications of addition of ZnO NPs to soil

The release of ZnO NPs into environmental compartments following increased production and commercial use has become a focus of environmental risk assessment studies (Peralta-Videa et al., 2011). Soils are the main sinks for manufactured ZnO NPs in the environment (Gottschalk et al., 2009). This would emphasise the importance of risk assessment for ZnO NPs in soils. As yet, there has been little study of the fate of ZnO NPs in natural soils and most research has been undertaken in hydroponic systems or using artificial media.

The rapid dissolution of ZnO NPs and their strong retention by soils (Chapter 4) suggest that even if ZnO NPs are added to soils it is unlikely they would be mobile. Even if they remain as NPs in soils, their mobility would be significantly reduced by aggregation and adsorption mechanisms. These findings indicate ZnO NPs are unlikely to be persistent in soil and risk assessments in the soil environment would be similar to that for soluble sources of Zn.

To date, the majority of studies on the effect of ZnO NPs on food crops have been short-term experiments or have used hydroponic systems and evaluated the effect of high (not environmentally relevant) concentration of ZnO NPs (Kim et al., 2011; Lin and Xing, 2008; López-Moreno et al., 2010; Stampoulis et al., 2009). The toxicological findings for immature plant root and vascular systems at initial stages of plant growth in solution culture may not represent effects in soil systems with a great affinity to sorb ZnO NPs, promote heterocoagulation and dissolution. Although a recent experiment reported the effect of application of ZnO NPs at a low rate (Du et al., 2011), comparisons were not performed with bulk ZnO and soluble sources of Zn. Our findings revealed bulk and nanoparticulate sources of ZnO provided similar amounts of bioavailable Zn (L values) as soluble Zn. Generally, Zn utilized from different source of Zn was not influenced by the form of Zn exposed to durum wheat plants. Therefore, from an ecotoxicological point of view the risks associated with the presence of ZnO NPs in soils would be the same as soluble sources of Zn.

5.2 Suggestions for future studies

Further research is required to fully understand the behaviour of NPs in soils and to successfully apply them as Zn fertilizers. A few suggestions based on results presented in this thesis are summarized below:

- Commercially available ZnO NPs without surface modifiers were used in the experiments. Designing ZnO NPs with appropriate surface capping agents may improve stability of ZnO NPs and consequently availability of Zn in soils. Meanwhile, careful considerations have to be taken to use non-toxic surface modifiers for soil biota (such as fulvic and humic acids) which are naturally present in soils or can be degraded readily in soils.

- The size dependant features of ZnO NPs are likely to be more prominent when NPs are in very small size range (less than 10 nm) (Bian et al., 2011). Designing sources of ZnO NPs with a mixture different particle sizes and surface modifiers could be investigated for controlled release of Zn from ZnO NPs to improve the efficiency of ZnO NPs as fertilizers.

- Retention and mobility of ZnO NPs, surface-modified by different stabilisation agents, needs to be performed and a wider range of soils are required to be used to determine key soil properties governing transport of ZnO NPs through soils.

- Further investigations are needed on a bigger set of soils to model influence of different soil characteristics on solubility, retention and bioavailability of poorly-soluble ZnO nanoparticles in the soil. Moreover, findings of diffusion and solid phase speciation of ZnO in this thesis is limited to a single soil (an alkaline calcareous soil). A wide range of soils needs to be investigated to provide a better understanding of the diffusion and speciation of ZnO NPs in different soils.

- Although previous studies mainly have shown that ZnO NPs are not taken up by plants (Hernandez-Viezcas et al ., 2011; López-Moreno et al., 2010), it is worthwhile to further investigate the potential interactions of surface-modified ZnO NPs with the root cells (e.g. aggregation, deposition and uptake) of different plants using µ-X-ray absorption techniques or electron microscopy.

5.3 LITERATURE CITED

Amrani, M., D.G. Westfall, and G.A. Peterson. 1999. Influence of water solubility of granular zinc fertilizers on plant uptake and growth. J. Plant Nutr. 22:1815-1827.

Bian, S.W., I.A. Mudunkotuwa, T. Rupasinghe, and V.H. Grassian. 2011. Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: Influence of pH, ionic strength, size, and adsorption of humic acid. Langmuir 27:6059-6068.

Borm, P., F.C. Klaessig, T.D. Landry, B. Moudgil, J. Pauluhn, K. Thomas, R. Trottier, and S. Wood. 2006. Research strategies for safety evaluation of nanomaterials, Part V: Role of dissolution in biological fate and effects of nanoscale particles. Toxicol. Sci. 90:23-32.

Cornelis, G., B. Ryan, M.J. McLaughlin, J.K. Kirby, D. Beak, and D. Chittleborough. 2011. Solubility and batch retention of CeO2 nanoparticles in soils. Environ. Sci. Technol. 45:2777-2782.

Darlington, T.K., A.M. Neigh, M.T. Spencer, O.T. Nguyen, and S.J. Oldenburg. 2009. Nanoparticle characteristics affecting environmental fate and transport through soil. Environ. Toxicol. Chem. 28:1191-1199.

Du, W.C., Y.Y. Sun, R. Ji, J.G. Zhu, J.C. Wu, and H.Y. Guo. 2011. TiO(2) and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. J. Environ. Monit. 13:822-828.

Fang, J., X.Q. Shan, B. Wen, J.M. Lin, and G. Owens. 2009. Stability of titania nanoparticles in soil suspensions and transport in saturated homogeneous soil columns. Environ. Pollut. 157:1101-1109.

Gottschalk, F., T. Sonderer, R.W. Scholz, and B. Nowack. 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. Environ. Sci. Technol. 43:9216–9222.

Hernandez-Viezcas, J.A., H. Castillo-Michel, A.D. Servin, J.R. Peralta-Videa, and J.L. Gardea-Torresdey. 2011. Spectroscopic verification of zinc absorption and distribution in the desert plant Prosopis juliflora-velutina (velvet mesquite) treated with ZnO nanoparticles. Chem. Eng. J. 170:346-352.

Hotze, E.M., T. Phenrat, and G.V. Lowry. 2010. Nanoparticle aggregation: Challenges to understanding transport and reactivity in the environment. J. Environ. Qual. 39:1909-1924.

Kanel, S.R., and S.R. Al-Abed. 2011. Influence of pH on the transport of nanoscale zinc oxide in saturated porous media. J. Nanopart. Res. 13:4035-4047.

Kim, S., J. Kim, and I. Lee. 2011. Effects of Zn and ZnO nanoparticles and Zn^{2+} on soil enzyme activity and bioaccumulation of Zn in Cucumis sativus. Chem. Ecol. 27:49-55.

Kosmulski, M. 2001. Chemical Properties of Material Surfaces Marcel Dekker, New York

Lin, D., and B. Xing. 2008. Root uptake and phytotoxicity of ZnO nanoparticles. Environ. Sci. Technol. 42:5580-5585.

Lin, D.H., X.L. Tian, F.C. Wu, and B.S. Xing. 2010. Fate and transport of engineered nanomaterials in the environment. J. Environ. Qual. 39:1896-1908.

López-Moreno, M.L., G. de la Rosa, J.A. Hernández-Viezcas, H. Castillo-Michel, C.E. Botez, J.R. Peralta-Videa, and J.L. Gardea-Torresdey. 2010. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (Glycine max) plants. Environ. Sci. Technol. 44:7315–7320.

Mortvedt, J.J., and P.M. Giordano. 1969. Extractability of zinc granulated with macronutrient fertilizers in relation to its agronomic effectivemess. J. Agric. Food Chem. 17:1272-1275.

Peralta-Videa, J.R., L.J. Zhao, M.L. Lopez-Moreno, G. de la Rosa, J. Hong, and J.L. Gardea-Torresdey. 2011. Nanomaterials and the environment: A review for the biennium 2008-2010. J. Hazard. Mater. 186:1-15.

Scheckel, K.G., T.P. Luxton, A.M. El Badawy, C.A. Impellitteri, and T.M. Tolaymat. 2010. Synchrotron speciation of silver and zinc oxide nanoparticles aged in a kaolin suspension. Environ. Sci. Technol. 44:1307-1312.

Shaver, T.M., D.G. Westfall, and M. Ronaghi. 2007. Zinc fertilizer solubility and its effects on zinc bioailability over time. J. Plant Nutr. 30:123-133.

Stampoulis, D., S.K. Sinha, and J.C. White. 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environ. Sci. Technol. 43: 9473-9479

Westfall, D.G., J.J. Mortvedt, G.A. Peterson, and W.J. Gangloff. 2005. Efficient and environmentally safe use of micronutrients in agriculture. Commun. Soil Sci. Plant Anal. 36:169-182.

Zhou, D., and A.A. Keller. 2010. Role of morphology in the aggregation kinetics of ZnO nanoparticles. Water Res. 44:2948-2956.

6 Supporting Information

6.1 Supporting information of Chapter 2: Dissolution kinetics of macronutrient fertilizers coated with manufactured zinc oxide nanoparticles

6.1.1 XRD patterns of ZnO NPs, bulk ZnO and coatings of fertilizer granules

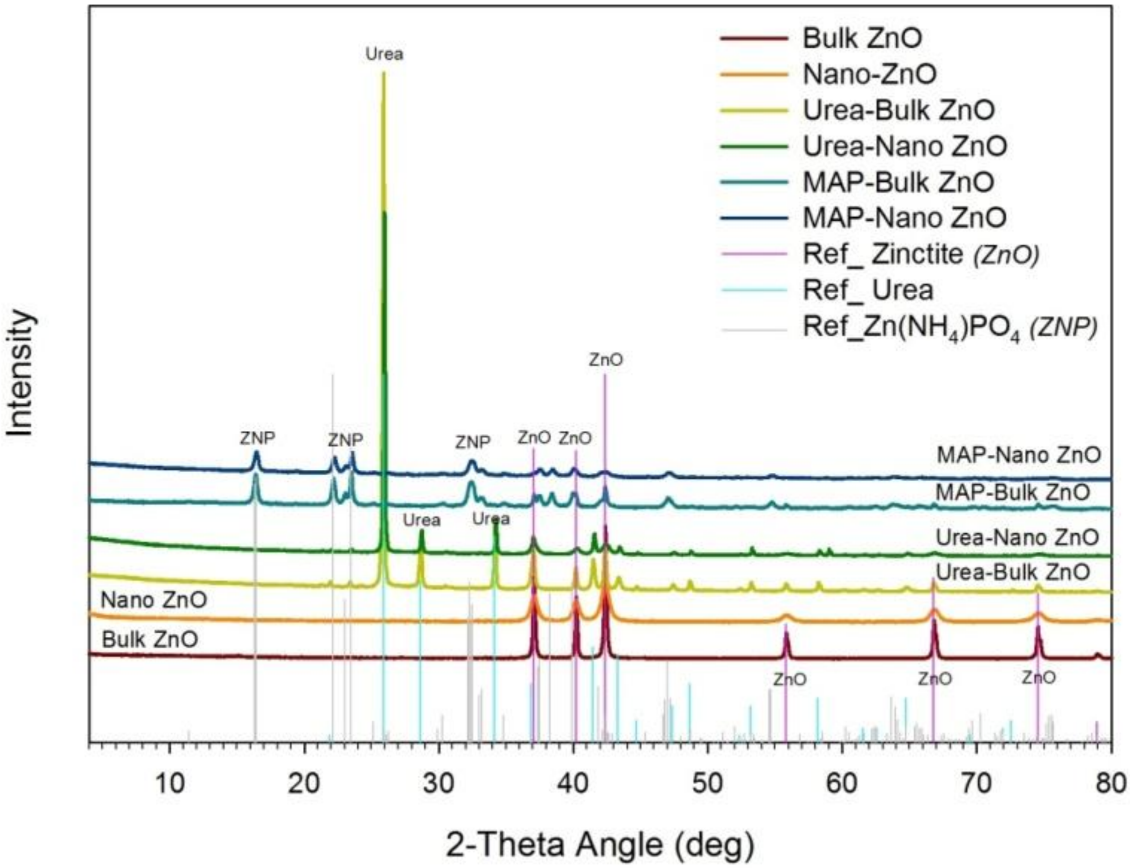

Figure 6.1- XRD patterns of ZnO NPs, bulk ZnO and coatings of fertilizer granules.

6.1.2 Scanning electron microscopy (SEM) images of coated MAP and urea granules with ZnO NPs and bulk ZnO

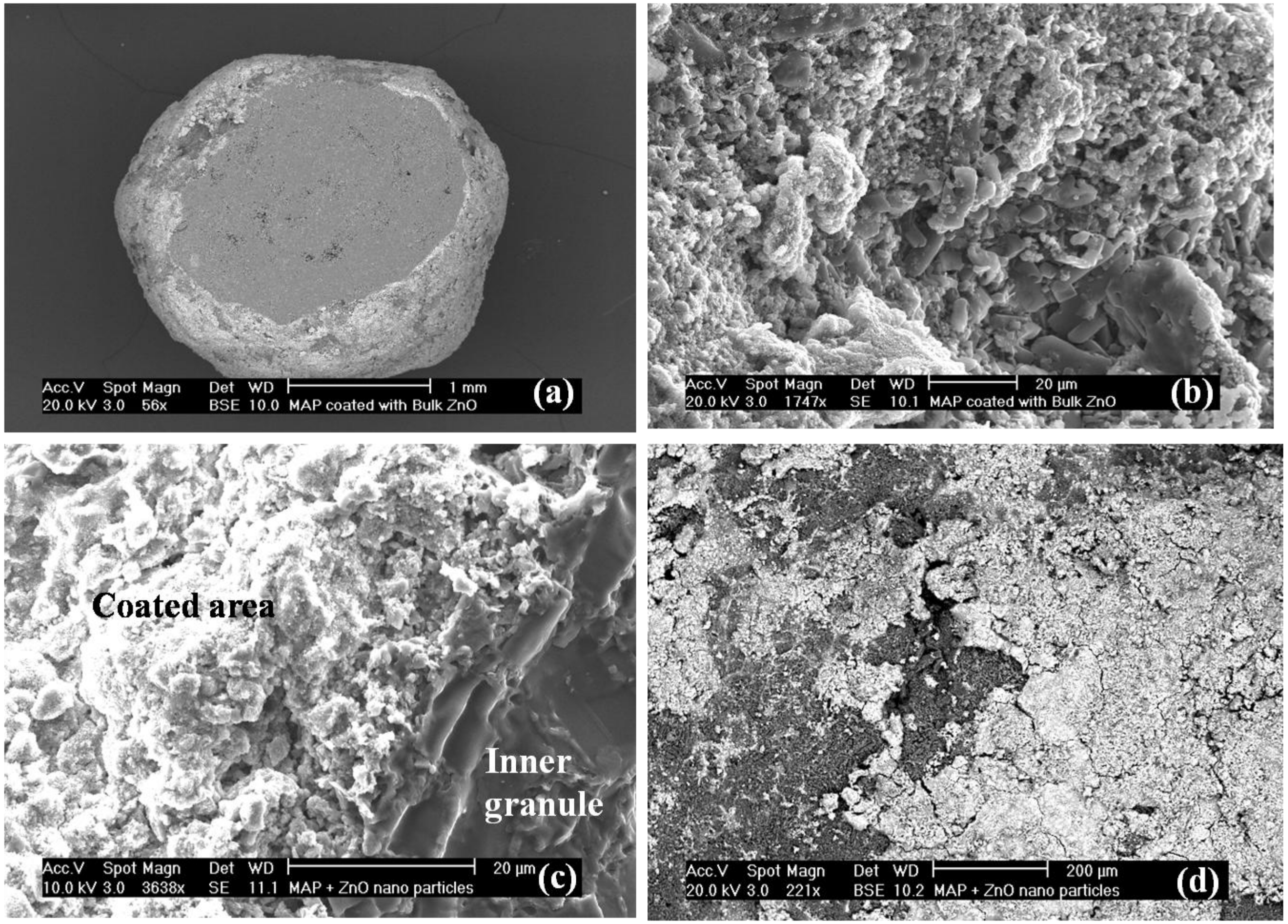

Figure 6.2- Scanning electron microscopy (SEM) images of (a) cross-sectioned MAP granule coated with bulk ZnO in a backscatter mode, (b) surface of MAP granule coated with bulk ZnO, (c) cross sectioned MAP granule with ZnO NPs showing inner MAP granule and coated surface and (d) backscattered image of the surface of MAP granule coated with ZnO NPs.

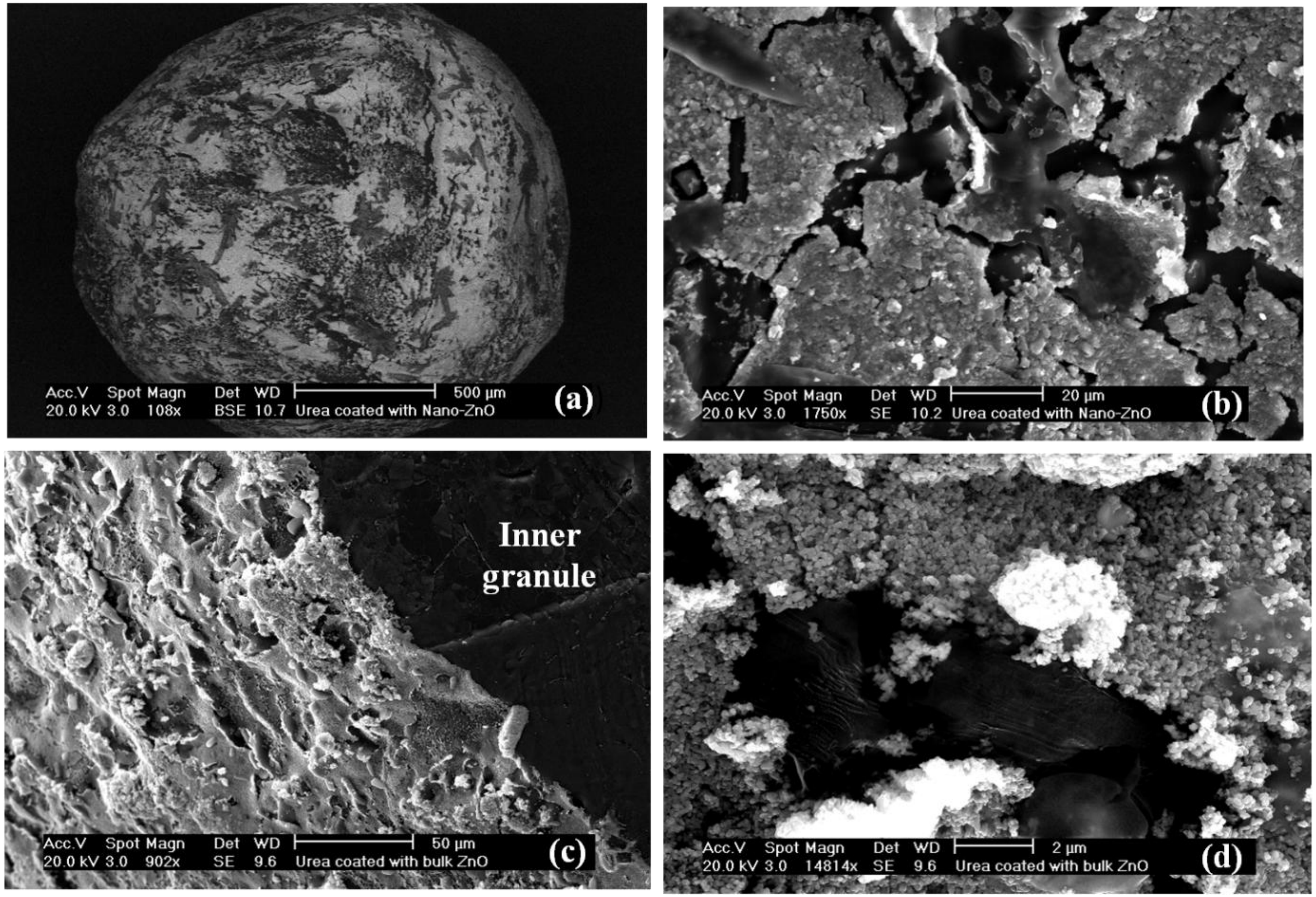

Figure 6.3- Scanning electron microscopy (SEM) images of (a) the distribution of ZnO NPs at the surface of urea granule coated with ZnO NPs, (b) surface coating of urea granules with nanoparticulate ZnO in a more focused image, (c) cross- sectioned surface of urea granule coated with bulk ZnO illustrating surface coating and inner granule without coating and (d) a focused image of the coating of urea granules with bulk ZnO.

6.1.3 Zinc content of coated fertilizer granules by weight (%)

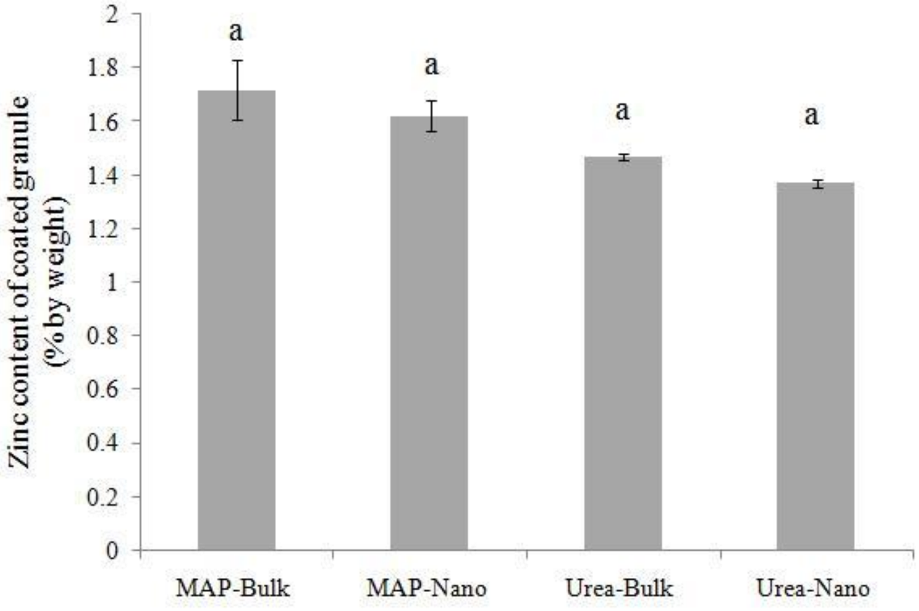

Figure 6.4- Zinc content of coated fertilizer granules by weight (%).

6.1.4 Cumulative Zn release from coated fertilizers and standard Zn compounds

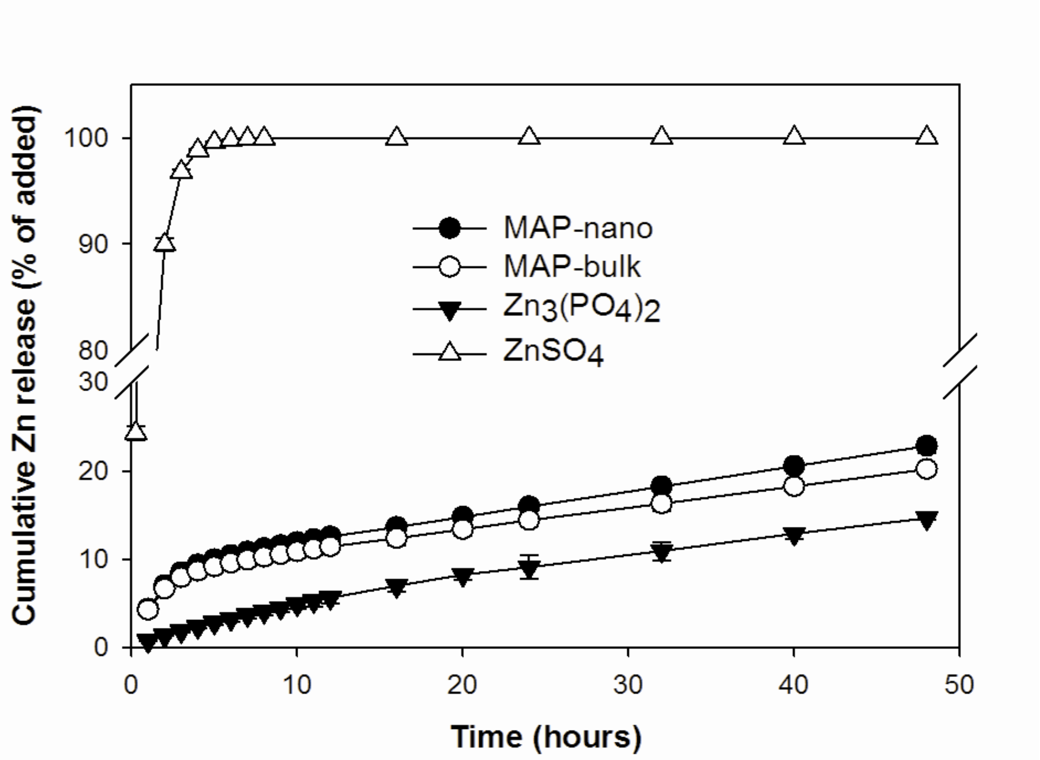

Figure 6.5- Cumulative Zn release (based on percent of added Zn) from MAP granules coated with bulk ZnO or ZnO NPs, $ZnSO_4$ and $Zn_3(PO_4)_2$.

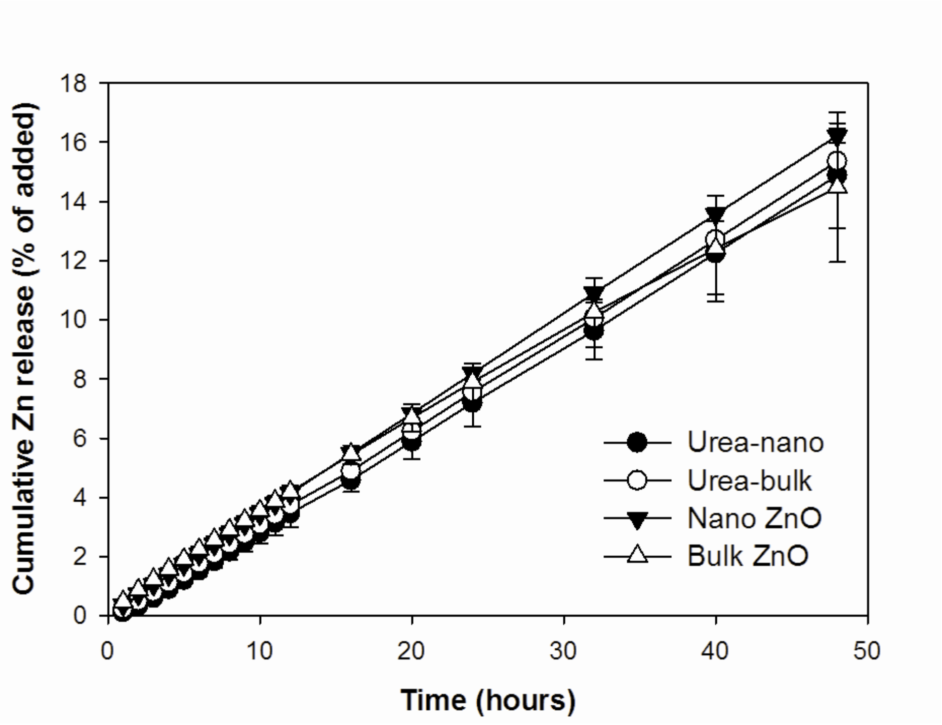

Figure 6.6- Cumulative Zn release (% of added Zn) from urea granules coated with bulk ZnO or ZnO NPs and ZnO powders (bulk or nanoparticulate).

6.1.5 Activity of Zn in eluents from coated fertilizers and standard Zn compounds

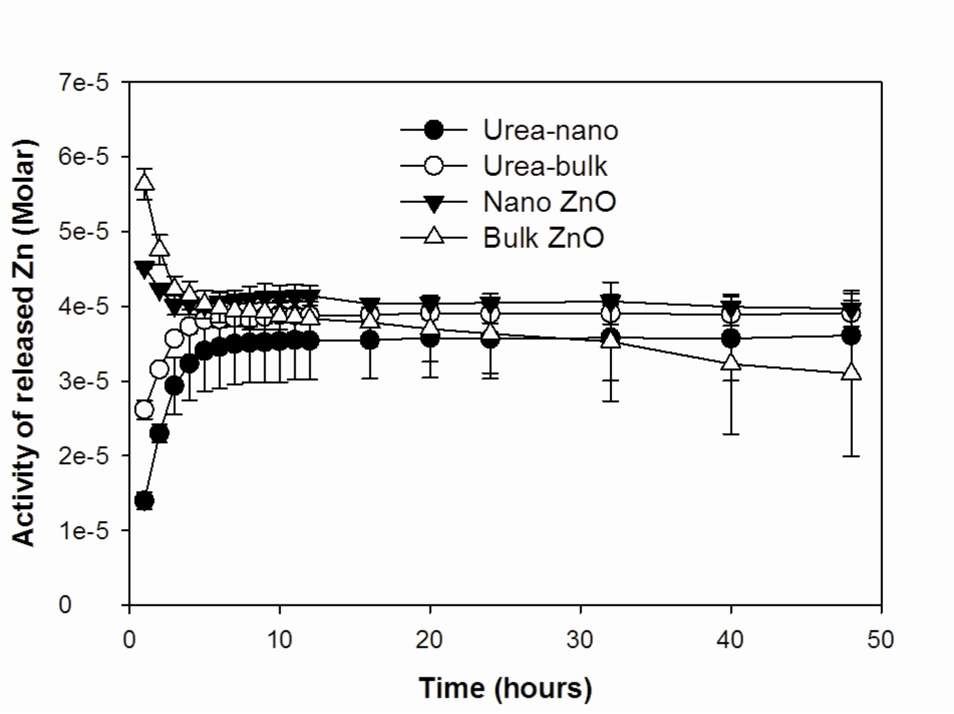

Figure 6.7- Dissolution rate of Zn from columns treated with bulk or nanoparticulate ZnO powders and urea granules coated with bulk ZnO or ZnO NPs.

6.2. Supporting information of Chapter 4: Retention and lability of manufactured zinc oxide nanoparticles in natural soils

6.2.1 Preparation of stable suspension of ZnO NPs

A preliminary experiment was conducted with NanoAmor ZnO NPs to investigate the preparation of a stable suspension of ZnO NPs for use as a spike solution in the retention experiments. The effects of modification with trisodium citrate, centrifugation or filtration on the average size of particles were examined. Organic acids such as citric acid are typically present in soil solution as a result of root exudation (Jones et al., 2003). Extremely rapid biodegradation of citrate with half-lives of 2-3 h depending on the soil type (Jones, 1998) suggested that citrate can be effectively used as an organic stabilizer for ZnO NP suspensions. Centrifugation for 15 min at 4000 g was expected to settle ZnO NPs larger than 0.2 µm based on the Stokes-Einstein equation (Kuhn et al., 2009). Moreover, filtration using a syringe microfilter with a 0.22 µm particle size cut-off (Millipore, Ireland) was used to remove large aggregates of ZnO NPs. A 400 mg L^{-1} suspension of NanoAmor ZnO NPs in ultrapure deionised water (Milli-Q, Millipore) or 0.5 mmol L^{-1} trisodium citrate solution was prepared. The suspension was manually stirred for 1 min. and then sonicated for 3 min. at 1500 W L^{-1} using an ultrasonic probe (VirtisVirsonic). Immediately, suspensions were filtered or centrifuged. The z-averaged hydrodynamic diameter and zeta potentials of suspensions were estimated using dynamic light scattering (DLS; Zetasizer Nano ZS, Malvern Instruments Ltd., U.K.). Concentrations of Zn in each suspension were measured using ICP-MS following a HNO_3 digestion method developed by Cornelis et al. (2010). The results showed that filtration or centrifugation of ZnO NP suspensions in citrate solution as well as centrifugation of aqueous suspensions of ZnO NPs effectively reduced the average particle size to < 100 nm (Table 1-SI). However, centrifugation of aqueous suspension of ZnO NPs was selected as the preferred methods as no modifier was introduced to the suspension in the process of stabilisation.

Table 6.1- Z-averaged hydrodynamic diameter of NanoAmor ZnO suspensions and associated Zn concentration in ultrapure water or trisodium citrate solution measured after sonication only, sonication followed by 0.22 µm filtering or centrifugation at 4000 g for 15 min.

Treatment	Ultrapure water (MQ)		Trisodium citrate	
	z-average (nm)	Zn conc. (mg L^{-1})	z-average (nm)	Zn conc. (mg L^{-1})
None	312 ± 0.05	41.2 ± 0.40	324 ± 0.03	40.43± 1.11
Filtration (0.22 µm)	ND ^{A}	0.38 ± 0.06	109 ± 0.93	1.61± 0.03
Centrifugation (4000 g, 15 min)	98.0 ± 2.02	0.74 ± 0.02	134 ± 0.71	1.07 ± 0.04

^{A} not detectable with ICP-OES

The stability of NanoAmor and NanoSun ZnO NP suspensions prepared with the selected method was examined over time. Suspensions of NanoSun ZnO NPs were nearly stable whereas suspensions of NanoAmor ZnO NPs revealed instability over the 48 h period of the experiment (Figure 6.8). Moreover, Zn concentrations in the NanoAmor suspensions centrifuged for 15 min at 4000 g were not high enough to be used as spike solutions in the retention experiments. Therefore, another preliminary experiment was conducted to evaluate the effect of modification of NanoAmor ZnO NPs with Suwannee River fulvic acid (SRFA) on average size of ZnO NPs and Zn concentrations in the suspension. For this reason, 0.08 g Zn as NanoAmor ZnO NPs was suspended in 50 mL of ultrapure water, 4 mg L^{-1} or 40 mg L^{-1} of SRFA. The suspensions were sonicated for 3 min at 90 W L^{-1} using an ultrasonic probe (VirtisVirsonic) followed by centrifugation at 2250 g for 15 min. z-averaged hydrodynamic diameters and zeta potentials of ZnO NPs were measured using DLS. Suspensions were digested in nitric acid outlined previously and Zn concentrations were determined in digest solutions using ICP-OES. Addition of SRFA at 40 mg L^{-1} spike rate reduced the average hydrodynamic size of particles (Table 6.2). At the same time, it stabilised ZnO NPs in the suspension as evidenced by the much higher concentrations in the suspensions. The recovery of Zn in suspension increased from 0.03% in aqueous suspension of NanoAmor ZnO particles to nearly 60% in suspension of ZnO NPs prepared in 40 mg L^{-1} SRFA solution. Adsorption of fulvic acids at the surface of ZnO NPs likely stabilised the suspensions of ZnO NPs through electrostatic attraction or ligand exchange (dominantly between carboxyl (COOH) groups and ZnO surface) (Yang et al., 2009) as was confirmed with measured decreases in the zeta potential.

Table 6.2 –z-averaged hydrodynamic diameter , zeta potential and concentration of Zn in suspensions of NanoAmor ZnO NPs following treatment with ultrapure water, 4 or 40 mgL^{-1} Suwannee River fulvic acid (SRFA).

Treatment	Hydrodynamic diameter	Zeta Potential	Zn concentration
	nm	mV	mg L^{-1}
MQ water	1817 ± 590	-16.1	0.04 ± 0.02
4 mg L^{-1} SRFA	2198 ± 758	-28.0	0.59 ± 0.39
40 mg L^{-1} SRFA	106.4 ± 1.80	-27.9	95.3 ± 9.12

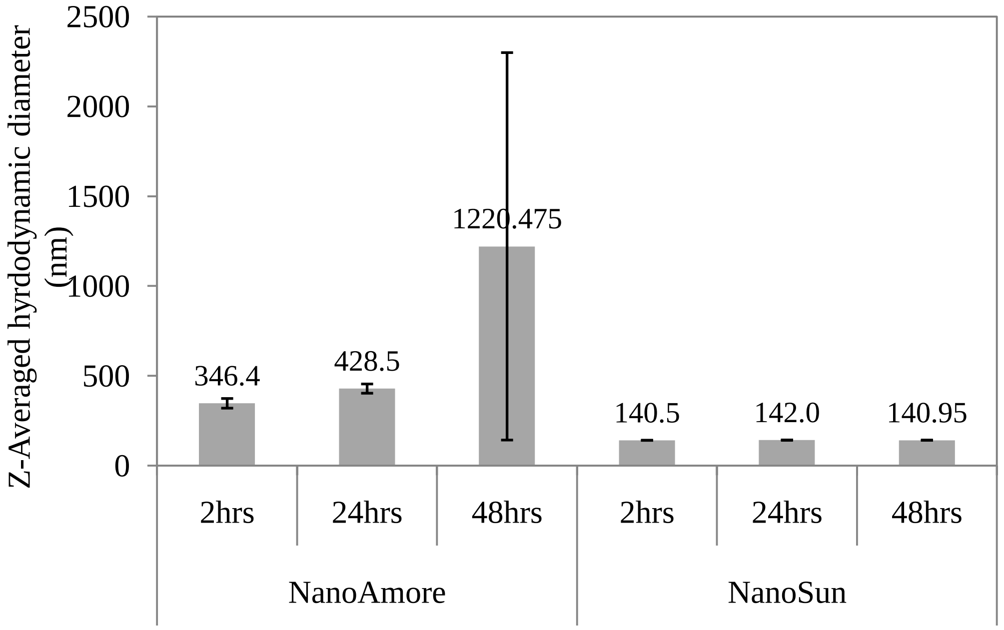

Figure 6.8 -Time dependent changes in the z-averaged hydrodynamic diameter of particles in aqueous suspension of NanoAmor and NanoSun ZnO NPs.

6.2.2 Adsorption isotherms of NanoAmor and NanoSun ZnO NPs in soils

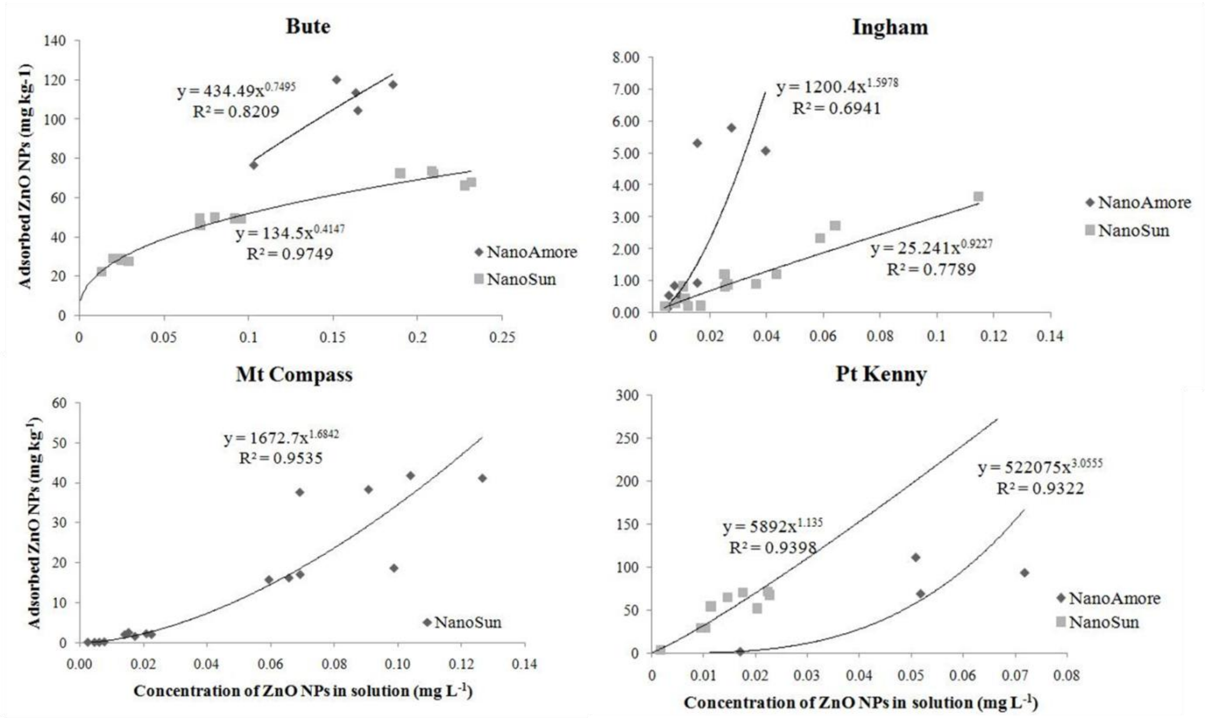

Figure 6.9- Adsorption isotherm of ZnO NPs in 4 soils. Strong retention of Zn in the clay-rich Emerald Black soil at the ZnO spike rate investigated restricted plotting adsorption isotherms for this soil.

6.2.3 The results of data analysis for pot experiment

Table 6.3- Analysis of variance table for the effect of Zn form, Zn spike rate, incubation time and soil on dry matter, Zn uptake and L value.

Souce	d.f	F pr.		
		Dry matter (mg pot^{-1})	Zn uptake (mg pot^{-1})	L- value (mg kg^{-1})
Zn form	2	0.30	< 0.001	0.058
Zn rate	1	0.90	< 0.001	< 0.001
Incubation	1	0.82	< 0.001	0.435
Soil	1	0.03	< 0.001	< 0.001
Zn rate × Zn form	2	< 0.001	< 0.001	0.012
Zn rate × soil	2	0.43	0.42	< 0.001
Zn form × soil	2	0.14	< 0.001	0.070
Zn rate × incubation	1	0.32	0.39	< 0.001
Zn form × incubation	2	0.23	0.13	0.002
Soil × incubation	1	0.54	0.03	0.019
Zn rate × Zn form × soil	2	0.58	< 0.001	0.085
Zn rate × Zn form × incubation	2	0.53	< 0.001	0.013
Zn rate × soil × incubation	1	0.28	0.89	0.203
Zn form × soil × incubation	2	0.57	0.12	0.004
Zn rate × Zn form × soil × incubation	2	0.44	0.02	0.825
M.S. Residual	69	0.14	0.007	0.006

6.2.4 Effect of Zn source and spike rate on Zn uptake (mg pot^{-1}) by durum wheat plants

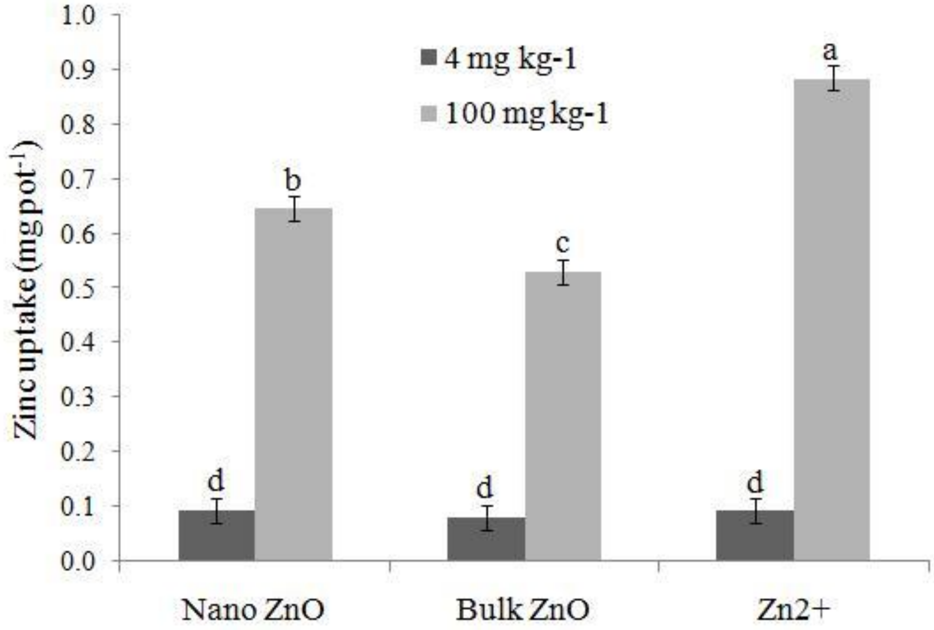

Figure 6.10- Changes in Zn uptake by durum wheat plants as a function of Zn source and different Zn spike rates in soils. The difference between treatments in Zn uptake was calculated based on a least significant difference value of 0.057. Columns appended by the same letter are not significantly different ($P \leq 0.05$). Control plants were not included in analysis in order to highlight the effect of different Zn treatments.

6.2.5 Effect of Zn source incubation time on Zn uptake (mg pot^{-1}) in Port Kenny and Mount Compass soils

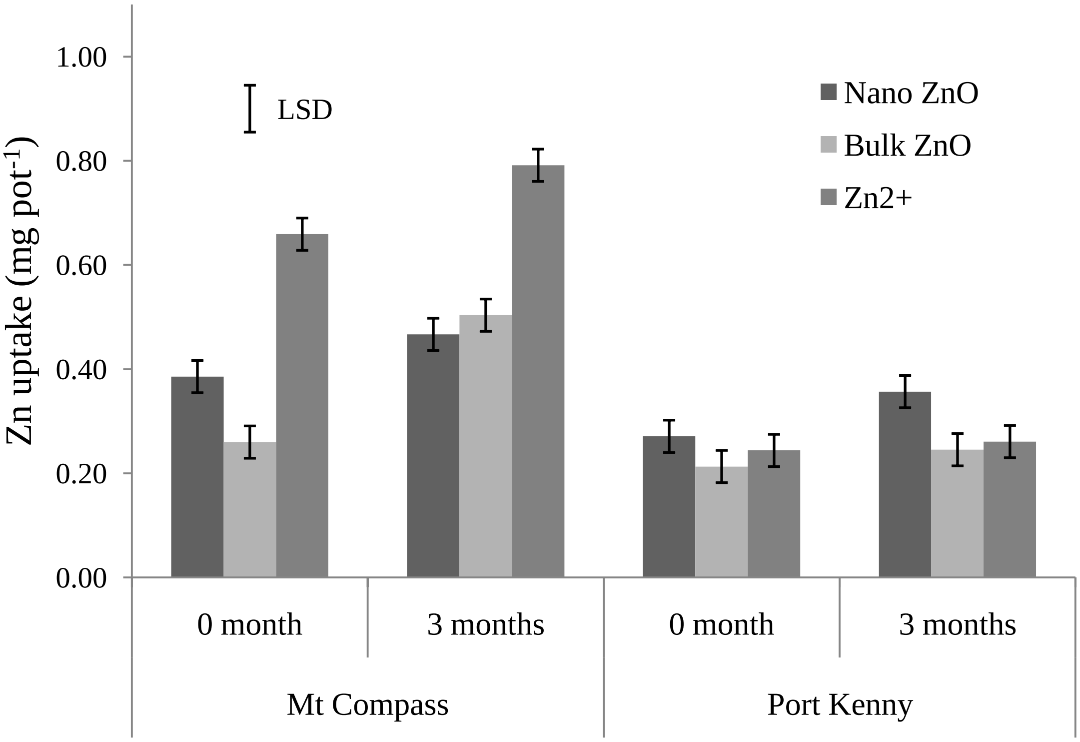

Figure 6.11 – Changes in Zn uptake as a function of Zn source and incubation time in Port Kenny and Mount Compass soils. Least significant difference of means (LSD; $P < 0.05$) was 0.09. Control pots were not included in the data analysis to highlight the effect of Zn source on Zn uptake.

6.2.6 Effect of Zn source on percentage of added Zn remaining labile after 0 mo and 3 mo of incubation in Port Kenny and Mount Compass soils.

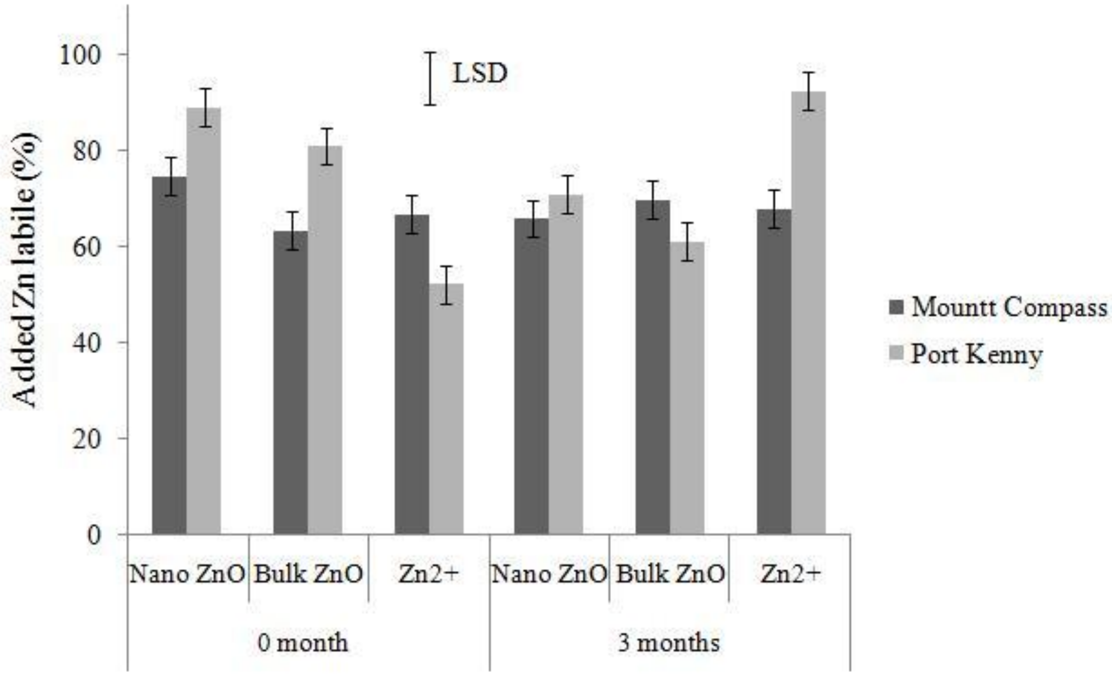

Figure 6.12 – Changes in the percentage of Zn remained labile in soils as a function of Zn source and incubation time in two soils investigated. Least significant difference of means (LSD; $P < 0.05$) was 11.1.

6.3 LITERATURE CITED

Jones, D.L. 1998. Organic acids in the rhizosphere - a critical review. Plant Soil 205:25-44.

Jones, D.L., P.G. Dennis, A.G. Owen, and P.A.W. van Hees. 2003. Organic acid behavior in soils - misconceptions and knowledge gaps. Plant Soil 248:31-41.

Kuhn, H., H.-D. Forsterling, and D.H. Waldeck. 2009. Principles of Physical Chemistry. 2nd ed. Wiley Hoboken, USA.

Yang, K., D.H. Lin, and B.S. Xing. 2009. Interactions of humic acid with nanosized inorganic oxides. Langmuir 25:3571-3576.