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Ecotoxicology of Nanoparticles: Effects on Plant Growth and Soil Processes

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Ecotoxicology of Nanoparticles: Effects on Plant Growth and Soil Processes

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Abstract

The uptake and impact of CdS and ZnO NPs in/on maize roots and shoots was investigated and compared with their soluble bulk materials ($ZnCl_2$ and $CdCl_2$). The plants were grown in Eutric Cambisol soil for 21 days. The soil was treated with seven concentrations (0.1–1.25 mg kg^{-1}) for each metal. The Tolerance Index (TI), the Agronomical Efficiency (AE), the Bio–Concentration Ratio (BCR), the Relative Increase Percentage (RI), uptake and uptake % were calculated for maize roots and shoots. The concentration of metals in maize roots and shoots following treatments of soil with either NPs or bulk materials increased relative to control samples. In addition, the concentration of all metals was higher in maize roots than in shoots across all metals concentrations studied. The uptake of Cd and Zn by maize roots and shoots grown in soils treated with bulk compounds was higher than for those grown in equivalent treated soil with NPs. The majority of NPs and their bulk materials had no significant negative effects on maize growth parameters. However, CdS NPs, $CdCl_2$ and ZnO NPs had negative effects on the length of maize roots and shoots at the highest metal soil ratio (1.0 and 1.25 mg kg^{-1}). The calculated maize growth parameters (TI, AE, BCR, RI, uptake and uptake %) were varied in maize roots and shoots depending on the plant part, growth period and metal treatments. The toxic effects of CdS NPs (0–100 mg L^{-1}) and ZnO NPs (0–1000 mg L^{-1}) on the germination and the development of maize root were studied for 8 days. The results indicated that the concentration of Zn in maize seeds and roots was higher than Cd for equivalent initial NPs concentrations. Most of the NP concentrations studied had a negative effect on the length and dry weight of maize roots. Germination of maize seed was reduced by the ZnO NPS (68.6%) more than that of CdS (58.1%). The uptake of CdS, ZnO, and CuO NPs was also investigated for maize plants grown in Eutric Cambisol soil and hydroponic culture over 21 days. High NPs concentrations were used across both growth mediums (0.01–1.0 g kg^{-1}/g L^{-1}). The TI, AE, BCR, RI, uptake and uptake % were also calculated for maize roots and shoots. The concentration of all NPs showed a similar trend of accumulation behaviour in maize roots and shoots to those found in low concentrations of NPs (< 1.25 mg kg^{-1}). The concentration of all metals in maize roots and shoots grown in nutrient solution containing NPs was higher than those grown in the NP treated soil. In addition, the impact of all NPs indicate that CuO and CdS NPs has negative effect on the length of maize roots and shoots at the highest concentrations in both cultures. Moreover, the dry weight of maize shoots was decreased by CdS NPs at the highest concentration in hydroponic culture. The calculated maize growth parameters were also varied in maize roots and shoots depending on the

plant part, growth period and metal concentrations. The adsorption kinetics and desorption % of CdS, ZnO, and CuO NPs was studied on the surface of four soils using the batch method. Adsorption isotherms were evaluated by Freundlich and Langmuir model. The results of study suggest that the adsorption of all NPs increased as a function of increasing NPs concentrations until the adsorption equilibrium was reached across all soils. The relative mean adsorption of NPs in four soils was found to follow the following order: Cu > Cd > Zn. Results also indicated that the highest adsorption of NP on soils was as follows: Libyan sandy soil > Eutric Cambisol soil > Sandy soil > Haplic podzol soil. The adsorption results for all NPs were best modelled using Freundlich equation across all soils. The kinetic behaviour of all studied NPs toward four soils showed the pseudo–second order rather than pseudo–first order kinetics. The mean desorption % of NPs in four soils was found to follow the following order: Zn > Cd > Cu. The effect of CdS, CuO, and ZnO NPs on the rate of nitrogen mineralization was investigated in Eutric Cambisol, Haplic podzol, and Sandy soil over 28 days, three concentrations of each metal NP were used (0.01–1.0 g kg^{-1}). The influence of all test NPs on soils respiration rate was also examined for 48 hours using the same NPs concentrations above. The results of nitrogen mineralization revealed that, the concentration of nitrate (NO_3^-) accumulated readily in three soils; however, the concentrations of ammonium (NH_4^+), dissolved organic nitrogen (DON) and free amino acids had low levels of accumulation across all of test soil and NP types. The comparison results of NPs impact indicated that the large majority of NPs failed to reveal any significant effect upon nitrogen mineralization under any of the NP concentrations save that for amino acid concentrations. Results of soil respiration revealed that no negative significant impacts for all NPs on soil respiration across all NPs and soil types.

Dedication

I dedicate this thesis to my mother, and to my father's soul.

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I am very thankful to Almighty Allah for making this work possible for me. I would like also to express great thanks to my supervisors, Dr Lorrie Murphy and Professor Davey Jones, for their guidance and support all through my study, and for the encouragement they gave me in hard times. I would also like to express enormous thanks for Dr Paula Roberts for her excellent mentorship during my research, I will never ever forget the important advices I received in meetings with my supervisors.

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List of abbreviations

NPS– Nanoparticles

MNPs–Manufactured nanomaterials

TiO_2 – Anatas

DW–Dry weight

PCR–DGGE–Polymerase Chain Reaction –Denaturing Gradient Gel Electrophoretic

FCMHPCV–(Flow Cytometric Histograms Peak Coefficient of Variation)

TI–Tolerance Index

AE–Agronomical Efficiency

BCR–Bio–concentration Ratio

RI–Relative Increase Percentage

g– Gram

mg–Milligram

kg–Kilogram

M– Molar

mM–Millimolar

NOM–Normal organic matter

CEC– Cation exchange capacity

WHC– Water holding capacity

OM– Organic matter

EC– Electrical conductivity

Na_2EDTA –Disodium ethylenediamine tetraacetate

$C_7H_5NaO_3$–Sodium salicylate

OPA–MET reagent– α–phthaldialdehyde and β–mercaptoethanol

$(Na[Fe(CN)_5NO].{}_2H_2O)$ – Sodium nitroprusside

Aqua Regia – HNO_3 and HCl

RH– Relative humidity

ANOVA–Analysis of variance

HSD– Honestly significant difference

DON– Dissolved organic nitrogen

AA–Amino acid

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Chapter 1: The objective of present study

In the presented work, three soils were collected from Henfaes Research Centre (Wales), Libyan sandy soil was obtained from Janzur site located in the Gefara Plain, the maize seeds were provided from the Environmental Centre, Bangor University. The objective of this study is to investigate the effects of low and high manufactured nanoparticles (MNPs) concentrations on maize plant grown in Eutric Cambisoil soil and hydroponic culture. The study also investigates the adsorption and desorption of MNPs on the surfaces of these soils. In addition, the present work evaluates the impact of these MNPs on the rate of nitrogen mineralization using the first three soils over 28 days.

1.1. The plan of thesis

The thesis is divided into eight chapters as follows:

Chapter 2 provides the literature review. It details (1) the classification, properties, and environmental sources of manufactured nanoparticles (MNPs); (2) the fate and behaviour of natural and manufactured nanoparticles in aquatic and terrestrial systems; (3) the ecological impacts of manufactured nanoparticles to terrestrial biota; (4) the adsorption and desorption of manufactured nanoparticles on soil surfaces; (5) the interaction of nanoparticles with plants; and (6) the uptake, accumulation, and translocation of nanoparticles into edible plants.

Chapter 3 describes the general chemical and physical analysis of soil and maize plant samples.

Chapter 4 describes two main experiments. The first experiment determined the uptake of Cd and Zn NPs by maize roots and shoots compared with the uptake of their soluble bulk counterparts, maize seedlings were grown for 21 days in Eutric Cambisol soil. The soil was treated with different concentrations of CdS NPs, $CdCl_2$, ZnO NPs and $ZnCl_2$. The added concentrations of the NPs and their bulk counterparts were 0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg (metal) kg^{-1} soil for all the metals. It investigates the impact of all metals upon length and dry biomass of maize root and shoot compared to their soluble forms under low concentrations (0 –1.25 mg kg^{-1} soil) of these materials in test soil. It evaluates the effects of these metals on experimental maize parameters using the appropriate equations to calculate Tolerance Index (TI), Agronomical Efficiency (AE), the Bio–concentration Ratio (BCR), the Relative Increase Percentage (RI), and the Uptake Ratios in maize roots and shoots. The second experiment evaluated the toxic effects of CdS NPs and ZnO NPs on the germination and the development of maize root for eight days; different concentrations of CdS NPs (0–100 mg L^{-1}) and ZnO NPs (0–

1000 mg L^{-1}) were used. It investigates all metals availability and their bio–accumulation during the preliminary stage of seed germination compared with the full growth of this plant that was conducted in Experiment1.

Chapter 5 investigates the toxicity of CdS, ZnO, and CuO NPs in maize plants after 21 days of seedling growth in Eutric Cambisol soil and hydroponic culture; high doses of these nanoparticles were used for both growth media; these were 0, 0.01, 0.1, and 1.0 g kg^{-1} soil/g L^{-1} nutrient solution. The uptake of these nanoparticles by maize roots and shoots was determined after 21 days. It studies the impact of high NP concentrations on the length and dry biomass of maize roots and shoots in both cultures. The impact of these nanoparticles on experimental maize parameters was calculated using the appropriate equations including Tolerance Index (TI), Agronomical Efficiency (AE), the Bio–concentration Ratio (BCR), the Relative Increase Percentage (RI), and the Uptake Ratios in maize roots and shoots.

Chapter 6 assesses the adsorption kinetics and desorption % of CdS, ZnO, and CuO NPs on the surface of Eutric Cambisol, a Haplic podzol, a Sandy soil, and a Libyan sandy soil using the batch experiment. The adsorption of nanoparticles on these soils investigated at different times (0, 3, 6, 24, 48, 72, 96, 120, 144, and 168 hours) using solutions containing 0, 25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1} of each nano–metal. It determines adsorption isotherms and descriptive constants of these nanoparticles for test soils using the Langmuir and Freundlich equation. It studies the effect of pH on the desorption of experimental nanoparticles using a series of the pHs solutions containing 3.0, 5.3, and 10.0, including metal digestion using 6 M HCl.

Chapter 7 studies the effects of CdS, CuO, and ZnO NPs on the rate of nitrogen mineralization for Eutric Cambisol, Haplic podzol, and Sandy soils over 28 days; three concentrations of each metal nanoparticle used, representing scale of dosing (0.01, 0.10 and 1.0 g kg^{-1}). It studies the impact of these nanoparticles on the breakdown of soil organic nitrogen to dissolving organic nitrogen and then ammonium ions and finally to nitrate ions. It illustrates the influence of experimental nanoparticles on the rate of soils respiration for 48 hours using the same concentrations above; the NPs concentrations were in the range 0–1.0 g kg^{-1}.

Chapter 8 it highlights main conclusions and identifies areas for future work.

Chapter 2: Literature review

2.1. Introduction

Nanoparticles are widely accepted as materials with at least one dimension between 1 nm and 100 nm with large surface area, which confer specific physico–chemical properties such as strength, electrical, and optical characteristics that are not observed in the bulk materials.[1,2] Nanoscale science and technology have seen amazing growth in their use and application in the last decade, to the extent that thousands of tonnes of manufactured nanomaterials are now a used in variety of commercial goods, ranging from sensors to sunscreens.[3,4] However, there are regulatory and public concerns regarding the potential adverse impacts that release of NPs into the environment, including the health of ecosystems and humans.[3] Large quantities of NPs are already present in the environment; these NPs may arise from natural sources (e.g., colloids in aquatic systems and soils, volcanic dusts, and natural brush fire products in the air), and anthropogenic sources (e.g. motor vehicle discharges, industrial emissions, and smoking exhausts).[3,5–7]

NPs in the size range 3–7 nm may account for more than 36–44% of the total number of NPs in certain urban air samples from The UK; the total size of particles is in the range < 10–10,000 nm.[8] The impact of these NPs on human health has been extensively studied in the environmental atmospher; the smaller particles, which can reach deep into the lungs, are of particular concern. Natural NPs occurring in terrestrial and aquatic environments have also been extensively researched,[9] but few studies have focused on manufactured particles. MNPs can have distinct physical and environmental properties when compared with naturally occurring materials; these are products of other activities not designed at nano–production.[10] The term *NPs* can also refer to materials with surfaces has unnecessary nanofeatures in the nature, or to substances with nanometre – sized voids. The small size of nanomaterials imparts unique properties when compared with bulk materials with similar formulations.[11,1,2] The optical, magnetic, and electrical properties can differ in nanomaterials; these are subject to the quantum physics laws rather than classical physics. Sizes in the nano – range give NPs a large surface to mass and volume ratio, and potentially greater mobility and reactivity. The surface areas of some nanomaterials can reach 1000 m^2/g; this is far higher compared with conventional catalysts, for instance nanomterials can lose their distinctive nano–properties by agglomerating into larger microparticles, even though manufacturers now stabilise NPs with specially devised coatings.[3,12]

The small sizes of these materials may potentially allow them to be more bioavailable, and provide the ability to penetrate biological membranes and to enter plant cells by *endocytosis* (engulfment by the cell wall).[3] Small sizes of NPs are expected to be transported with the water phase through soil pores and to easily interact with organisms.[12] The particle size and specific surface area are more appropriate indicators of phytotoxicity than nominal concentrations of NPs.[3,12] In addition, the pore sizes of plant walls are typically in the range of 3–8 nm, this allow small particles to penetrate through plant walls. However, cell internalization of NPs of different sizes and compositions has been observed for different plant species. Thus, NPs aggregates with a size smaller than the largest pore are expected to pass through and reach the membrane of plasma and the larger particle aggregates will not enter into plant cells.[3,12]

The interaction and behaviour of MNPs with plants and soils has raised concerns regarding their discharge effects on the environment, Therefore, it is essential to assess their risk to ecosystems.[3] The effects of MNPs on plant species depends on their size, large specific surface area, concentration, structure, and diverse other physical and chemical properties. The effect of MNPs on the plants growth at different development stages have received much attention.[3,5] The bioaccumulation of MNPs might be caused by their uptake through the plant's roots and their subsequent transfer around the plant's shoots through its vascular system. Few studies in this area have been documented, therefore; many questions arise concerning the mechanism and behaviour of MNPs in plant and soil systems, the effect of their surface activity on phytotoxicity, the ways NPs enter plant tissues.[3,5,12] Adsorption and desorption reactions on the surfaces of soils are factors that control the concentration of metals in soil solutions.[12] thus increase the availability of MNPs in soil might affect the plant growth, in addition any change brought about by the release of MNPs to the microbial diversity and function could potentially influence the quantity of plant available nitrogen in the soil matrix.[3,5,12]

This introductory chapter describes current understanding of NP fates and of NP effects in the environment. It particularly focuses on terrestrial and aquatic systems include plants.

2.2. Classification of nanoparticles

As already indicated, NPs are defined as having at least one dimension on the order of 1–100 nm, but some debate still continues over the terminology ascribed to NPs, which related to their size.[13] The size range of NPs does not indicate any specific set of theoretical or chemical properties that occur at these parameters.[14] NPs are also described as nanostructures,

nanomaterials, ultrafine particles, and nanospheres; the term also embraces colloidal fractions when describing naturally–occurring NPs in ecosystems. These contrasting terminologies arise from different morphologies and the multidisciplinary nature of nanomaterials research. Park.[15] recommended that NPs could be classified into three categories as follows:

- *Naturally–occurring NPs*: These are particulates of colloidal size.
- *Adventitious NPs*: These are anthropogenically sourced particulates, including soot from the combustion of fossil fuels.[16] They are materials that have not been created for a specific purpose; instead, they by–products of other processes.
- *Manufactured NPs*: These are NPs synthesised for commercial purposes.[10] There are several classifications of MNPs. The first is metal oxide NPs; these are in common use in their bulk forms, but are now produced in nano–sized forms that take advantage of the enhanced properties of NPs. ZnO, for example, has long been used as a sunscreen because its UV–absorbing properties and its ability to scatter light in the wavelength range of 200–700 nm.[17] See Table 2.1.

As nanoparticulate structures, ZnO NPs retain the ability to absorb UV radiation and retain much of the light energy that is transparent to the eyes, albeit over a narrower spectrum. ZnO NPs are used to coat clear glass beer bottles to stop UV–degradation of the materials, although the glass bottles appear visible to the consumers.[3,17] Other commonly used metal oxides include titanium and cerium dioxides, mixed compounds of metals–indium–tin oxide (ITO), for instance, is presently used in polishing agents for semiconductor wafers, as formulas for sunscreens, and for scratch–resistant coatings for glass.[20] These uses all increase the potential for release of these nanoproducts into the environment, either accidentally or deliberately (spillages and discharges, respectively), and therefore increase the opportunities for negative environmental effects.[3]

Fluorescent semiconductor nano–crystals can be defined as quantum dots (QDs) and are classified as a fourth type of MNPs. Semiconductor materials include CdSe/ZnS, CdTe, CdSe, PbSe, and InP; the sizes of these compounds are in the range 10–50 nm.[21] The synthesis of CdS has caused interest in semiconductor that possesses unique photochemical and photophysical properties.[22–24] CdS NPs used in technological applications; this range through microelectronics to non–linear optics, catalysis, optoelectronics, and optical windows for photo–electrochemistry and solar cell.

Table 2. 1. Classification of manufactured nanomaterials (MNPs).

Class	Component	Use
Metal oxides	Zinc oxide	Cosmetic, sunscreens, UV coatings, paints, plastics and packaging.[3,17]
	Copper oxide	Gas sensors, catalysts, superconductors, and ceramic pigments.[18,19]
	Titanium dioxide	Cosmetics , paints and coating.[12]
	Cerium dioxide	Automobile catalysts
Carbon products	Fullerenes	Catalysts ,Plastics, battery and fuel cell electrodes, super–capacitors, cosmetics ,water purification systems, conductive coatings, orthopaedic implants, composites, adhesives, and sensors, and components in electronics, aircraft, and aerospace and automotive industries
	Single–walled and multi–walled carbon nanotubes	
	Amorphous carbon	Photocopier ,toner Inks, and automobile tyres
Metals	Silver	Air filters, Bactericide in wound dressings, socks and other textiles, baby products, toothpaste, vacuum cleaners and washing machines
	Iron	Groundwaters remediation, soils and sediments
	Gold	Electronics in flexible conducting inks or films, and as catalysts
	Copper	Microelectronics.[12]
	Bimetallic NPs (Fe–Pd, Fe–Ni, Fe–Ag)	Remediation of organics in waters; usually supported NPs
Quantum dots and semiconductors	CdTe, CdSe/ZnS, PbSe and CdS	Photovoltaics,Medical applications, security inks, photonics and telecommunications

Source: Adapted from Batley and McLaughlin.[3]

At present, there is much interest in exploring the optoelectronic applications of CdS semiconductors, for example, photoelectrocatalysis, telecommunications, and biology. The optoelectronic characteristics of CdS NPs are largely a function of their morphologies and structures.[23,24]

2.3. Properties of nanoparticles in the environment

NPs are characterised by their physical properties; these include surface area, shape, size scale, chemical composition, and molecular weights (polymeric particles). Measurement of these parameters is difficult because of NPs behaviour differs under altered conditions. A major problem lies in determining the environmental properties of these NPs (e.g., through interactions

with soil and aqueous solutions). The majority of techniques used for NP characterisation require that the NPs be separated into individual particles (e.g., through electron microscopy); NP properties are plausibly perturbed in the natural environment.[3,12]

2.3.1. Manufactured nanomaterials (MNPs)

The properties of MNPs are determined by their synthesis; they vary for different commercial purposes. Consequently, their interaction with ecosystems also varies widely once the NPs are released; they interact with other chemical compounds in the environment, and might undergo other transformation processes (e.g., dissolution and aggregation).[3,12] NPs dissolution is a significant property that impacts their action mode (e.g. characteristics, toxicity, medicinal applications, antimicrobial and environmental impact). Dissolution is a dynamic process in which the dissolving solid from constituent molecules migrate from the surface to the bulk solution through a diffusion layer. This thermodynamic parameter that controls this process is described as solubility and along with the NPs concentration between the bulk solution and the particle surface acts as the driving force of particle dissolution. Both speed rate of dissolution and solubility are dependent on a surface properties and particle's chemical, in addition to size, and the surrounding media.[3,12] These processes (dissolution and aggregation) can subsequently affect biological interactions under different conditions encountered such as field and simulated field media. The toxicity of MNPs is therefore not a useful concept at this stage, unless toxicity measurements can be linked with the media that the NPs encounter.[12]

Metal speciation in aquatic and terrestrial systems can be determined as total dissolved metals as a first stage and then by the measurement of the bioavailable fraction, followed by measurements of actual size. However, in real life the situation is difficult; this is because other additives are often included the formulations of manufactured NPs; these can alter the behaviour of NPs in certain media.[3]

2.3.2. Natural nanoparticles (NNPs)

As indicated, there are a range of natural NPs occurs in soil, water, and the atmosphere, for instance air can contain aerosols, including very fine particles that can be associated with volatile emissions from different types of vegetation (e.g., tress and other plants) and other sources that provide these particles (e.g., bush fires); the natural particle sizes are usually less than 1 μm; much smaller particles can exist before agglomeration occurs.[3,12]

Clays are regarded as a natural significant source of nanoparticulates in several soils. Certain natural materials such as manganese, iron oxides and other high molecular weight of mineral phases, along with dissolved organic matter, can be found in water of soil pore. Therefore, both soil pore and natural waters contain colloidal particles that include clays, manganese, iron oxides, and organic matter. This can provide more information about the expected behaviour of MNPs from the behaviour of natural particles, which is already known.[3,12]

In natural waters, macromolecules and colloids include humic and fulvic acids, fibrillar colloids (exopolymers), that are produced from algae and other microorganisms (these include some proteins and large polysaccharides), manganese and aluminium oxides, and hydrous iron.[25] The size range of natural aquatic colloids runs from 1 to 1000 nm; the degree of aggregation plays a significant role in their classification.[9,25,12] The proteins and humic and fulvic acids have sizes ranges below 10s of nm; metal oxides and polysaccharides are larger–iron oxide colloids, for instance, can extend across the full nanoscale range. Consequently, these natural materials do not exist as separate particles, so they are classified as heterogeneous mixtures of organic and inorganic species.[10,25] In natural waters, the microbial communities can be permanent sources of macromolecular nanomaterials (e.g., polysaccharides).[11,26]

Colloid particles vary widely in size; however, the small particles have the highest percentage of total surface area; against this, large particles have the maximum percentage of mass.[4] Many factors affect colloid aggregation; these include surface charge, particle size, chemical properties, and density.[9,27] Particle collisions due to natural Brownian motion cause aggregation and result in settling of differently sized particles. In the case of colloid mixtures, where each fraction size has the same volume, the small colloids (< 100 nm) disappear first through aggregation. The large ones settle by sedimentation; this leaves a distribution of particle size in the range 100 nm–1 μm.[26]

The interactions between natural colloids may be controlled by their bonding and charge (covalent against electrostatic). At the typical pH of natural waters, the surface charge of clay is normally negative. The bulk of the natural organic matter also carries a negative charge as a result of ionisable functional groups such as hydroxyl and carboxylic acids. At pH values where the surface charge is zero (pH 8–9), aluminium and iron oxyhydroxides have a positive charge, but they assume a net negative charge when they bind with natural organic matter in natural waters.[28]

Measuring the surface charge of a colloid is difficult, but can be estimated by the zeta potential (the potential between the surface of colloid particle and its solution). As a indicate of colloid particle stability, a range of zeta potential between +30 mV and –30 mV is classified as instability charge with aqueous dispersions solution.[3] Particles with close to neutral charges aggregate more quickly than do other particles. In natural system, interactions of colloidal particles and organic macromolecules lead to lose aggregations or flocculation (flocs), where structure depends on different factors (e.g., the relative concentrations and density of each particle in the mixture, macromolecular flexibility, and particle shape). Ultimately, the stability is a function of the nature of their bonding and of their relative charges. The aggregates may be stabilised at small size but these particles will not sediment.[3,12]

Larger aggregates of particles form more slowly than small ones. Predicting their behaviour is difficult because complicated mixtures tend to vary in stability and reaction rates. On the other hand, in low ionic strength solutions, particles show more substantial stability depending on the range of size (100 nm–1 μm), as described by Buffle and Leppard.[26] At higher ionic strengths, for example in seawater, the aggregation of particles can be rapid compared with their behaviour in fresh water, where colloids are able to reach the state of natural stabilisation with organic macromolecules.[29] Buffle and Leppard indicated that the increasing ionic strength in estuarine waters results in increased aggregation and particle charge of colloidal particles.[9] Liang and Morgan provided evidence that the precipitation and aggregation of colloidal iron above 15% salinity is more than 75%, complete within 30 minutes; they also showed that particles larger than 1.2 μm are formed.[30] High salt content (ionic strength) in soils encourages flocculation of particles; in addition soil pore waters that are high in calcium and low in sodium ions.[31]

Figure 2.1 provides a schematic diagram of their aggregate formation including natural colloids.[32] The diagram does not involve considerations of living components (e.g., bacteria); doing so would add a further layer (or layers) of complexity. The interactions of natural particles with NPs are therefore an important pathway when considering NPs' fates in the environment. These natural materials are known to occur regularly in high quantities both in soil pore waters and in natural water system. The nature of the behaviour of natural colloids in soils has long been broadly recognize.[33]

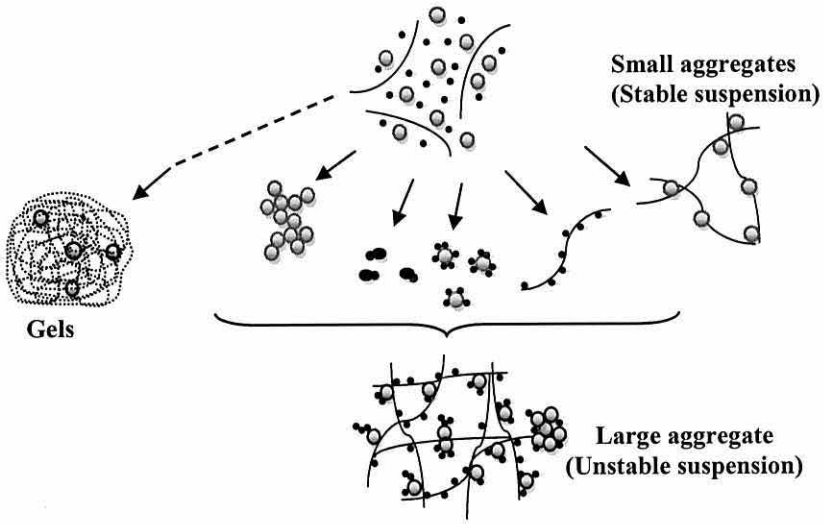

Figure 2. 1. Main types of aggregations shaped in the three of colloidal component system. This comprises fulvic compounds (or aggregated refractory organic material), small dots; inorganic colloids, circles; rigid biopolymers, lines. Both fulvics and polysaccharides may also form gels, these are characterized here as grey areas into which inorganic colloids may be embedded. Source: redrawn from Batley and McLaughlin.[3] and Lead *et al.*[12]

2.4. Ecological sources of manufactured nanoparticles

Anthropogenic nanoparticle sources require management to reduce the release of NPs into aquatic systems and the atmosphere, as illustrated schematically in Figure 2.2. NPs that are released into the atmosphere or aqueous media are potential risks to ecosystems and human health. Sources include stack emissions and vehicle exhausts. Stack emissions and motor vehicles are problematic sources of fine particles; this has been an issue for industry, especially the power industry, for several years.[3] However, nanoparticulate forms of chemical should be treated as new chemicals for regulatory purposes and new researches are needed to identify or to determine their routes of exposure, fate, transport and toxicity.[3,12] The potential risk of NPs in aqueous solutions is currently unidentified. The manufacturing processes of NPs can result in both liquid and solid wastes. The disposal of municipal water treatment from plants may have the ability to add NPs to the sewage system; however there are little studies on the capability of treated water to increase the NPs contamination. Specially, uncharged and anionic NPs could pass through sewage wastes without retaining in sewage biosolids.

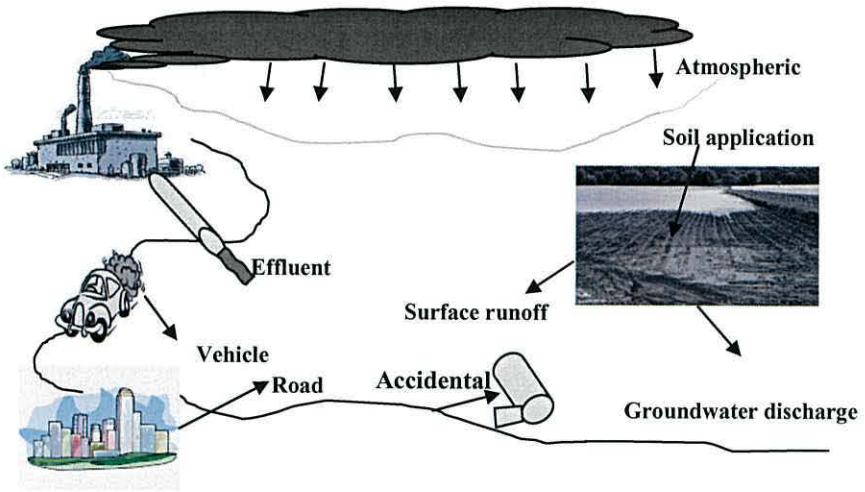

Figure 2. 2. Possible sources of manufactured nanoparticles in the ecosystem. Source: redrawn from Batley and McLaughlin.[3] and Lead *et al.*[12]

The interaction of potential nanomaterials with bacteria in sewage treatment plants has been indicated in several studies. Choi *et al.*[34] indicated that the tested bacteria were affected by Ag NPs; Ag NPs showed toxicity and impacts on nitrification, and this suggests that other microorganisms in wastewater treatment may also be affected. Kwak *et al.*[35] showed that titanium dioxide NPs are toxic to *Escherichia coli* in the presence of ultraviolet light, and prevent the fouling of water treatment membranes. However, very few studies have shown their interactions with plants and soil. Accidental release represents another source of NPs contamination in the environment. Spillage of containers, for instance, can be a source of entry of solid or liquefied nanomaterials into water systems or the soil.

2.5. Fate of nanomaterials in aquatic systems

2.5.1. Key pathways

Figure 2.3 summarises main physicochemical pathways that determine the fate of NPs in aquatic systems. These factors consist of sedimentation, aggregation, dissolution, adsorption to surfaces of other particulates, stabilisation via surfactants, and binding to dissolved organic matter in natural systems.[3] dissolution, adsorption and binding NPs are significant resources that control their toxicity, and environmental impact. Other pathways include biological degradation, which can be classified as aerobic and anaerobic processes, and degradation by hydrolysis and

photolysis, which are classified as abiotic pathways.[3] Oxidation and reduction reactions play significant roles in various environments for certain nanomaterials. However, these materials are most likely to accumulate in sediments at the bottom of aquatic systems.[12] However, studies of the fate of MNPs in aquatic systems are few at present, so understanding of the MNPs fate is incomplete; likewise, MNP fate in soil systems is not well understood.[3] However, the available information concerning natural colloids in aquatic systems provides some predictions as to the fate of MNPs in soils. The interactions of MNPs with natural colloids may play an essential role in their fate.[3]

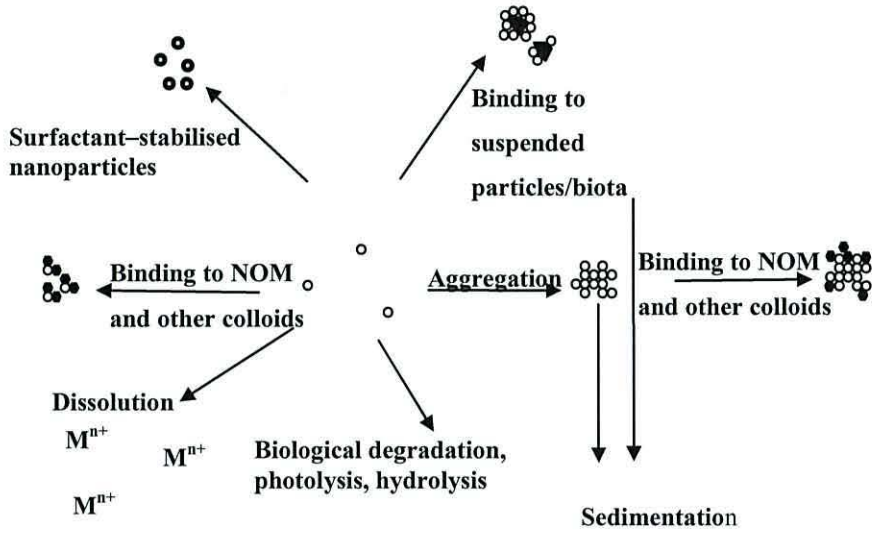

Figure 2. 3. Pathways for metal oxide nanoparticles in natural water systems. NOM represents normal organic matter and M^{n+} represents the dissolution of M ions. Source: redrawn from Batley and McLaughlin.[3] and Lead *et al.*[12]

2.5.2. Behaviour of manufactured nanoparticles

It difficult to predict the behaviour of NPs in the environment; however, as indicated, the two significant factors that play important roles in the environmental effects of NPs in water system are aggregation and dissolution, as shown in Figure 2.3.

2.5.2.1. The aggregation and solubility of manufactured nanoparticle

As described in Section 2.3.2, the behaviours of NPs in aqueous systems imitate natural colloid behaviours. NPs in aqueous solution have a natural tendency to grow in size. Although manufactured NPs are classified as nanosized, they are often aggregated in their suspension

solution at neutral pH (Figure 2.4); thus the aggregation size of these particles is typically larger than the 100 nm that is the maximum range of NPs. However, in the case of NPs, factors such as surface charge and electrolyte ion concentration control the aggregation; this is due to electrostatic forces, as described for natural colloids. Thus, interactions of NPs with natural colloids (inorganic colloids, organic macromolecules or heterogeneous aggregates) might also show similar behaviour, as discussed in Section 2.3.2.[36] Adams *et al.*[37] reported that ZnO NPs show a strong tendency to aggregate in freshwater. The initial size of ZnO NPs is 67 nm, and the aggregation size ranges from 420 nm to 640 nm. Brant *et al.*[38] showed that n–C_{60} fullerenes aggregate strongly (25-500 nm) within weak electrolyte solutions (< 0.001 M ionic strength) and that these aggregations are stable for more than 15 weeks.[39]

In general, the slow aggregation of the particles suggests the potential for interaction with biota; this suggests a need for studying the rates of aggregation for manufactured NPs. Unfortunately, this field has been poorly investigated, even though sufficient data are available on natural colloidal nanomaterials.[3,12] The majority of metal–based NPs are classified as hydrophilic metals, although they often have low solubility .[3] However, n–C_{60} fullerenes eventually settle out of suspension or adsorb to particles or immobilised on surfaces. The impact of basic water chemistry (salinity, pH, redox potential and hardness) on NPs had been poorly understood.[39]

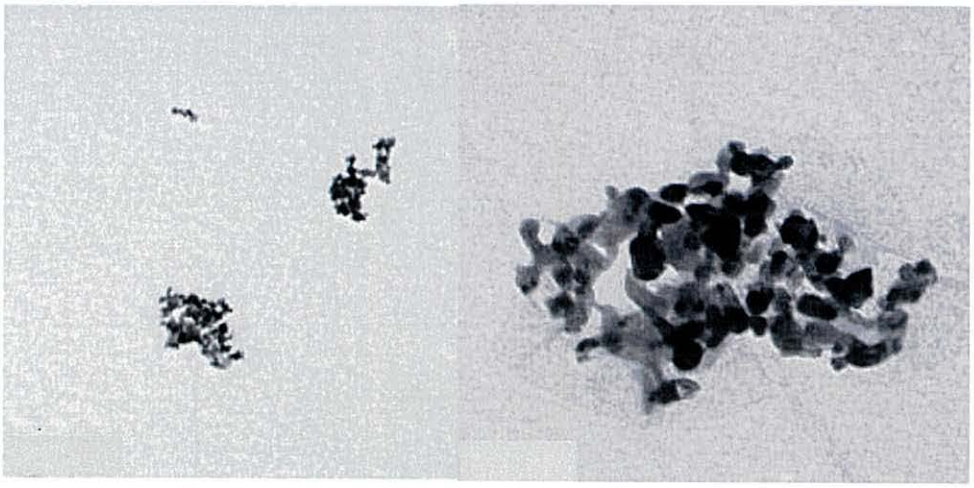

Figure 2. 4. The images of electron micrographs show aggregations of ZnO NPs. These come from a dispersion of a ZnO nanopowder (30 nm) in a medium of freshwater algal, pH 7.5. Source: Batley and McLaughlin.[3]

The most toxic fraction to aquatic biota is soluble metal ionic materials; thus, it is important to measure the materials' solubility. Franklin *et al.*[40] studied the biological effects of ZnO NPs; results indicated that, although ZnO is insoluble in water, ZnO NPs are rapidly dissolved, and

this results in 6 mg L^{-1} of the Zn being dissolved within 6 hours and 16 mg L^{-1} of the Zn being dissolved within 72 hours at a buffered solution (pH 7.5) algal medium. This is in excess of the 5 mg Zn L^{-1} that is considered toxic to the majority of aquatic biota.[40] Cadmium selenide (CdSe) semiconductor quantum dots have a significant tend to release Cd as ion as a result of selenide oxidation. The solution of 250 mg L^{-1} results as high as 80 mg Cd L^{-1}.[41]

Those NPs with a small radius of positive curvature are energetically disfavoured and are prone to preferential dissolution; this comes from the prediction of the Gibbs–Thompson effect (the reduction of local chemical potential due to nano–scale curvature).

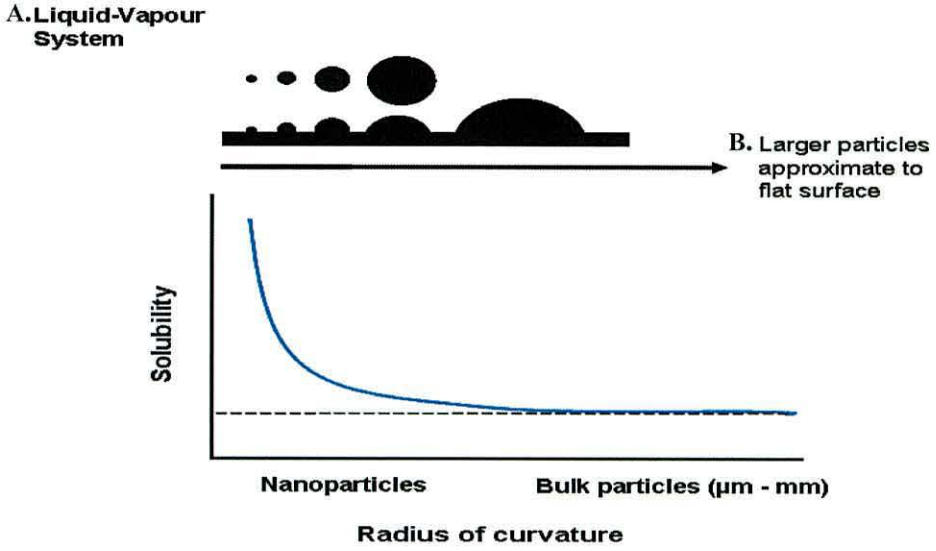

Figure 2. 5. Showing the solubility of amorphous silica as a function of the curvature radius. A represents the solubility media, B represents larger particles over time. Source: Adapted from Borm *et al.*[42]

This effect suggested that NPs of small radius dissolve at lower electrochemical potentials than bulk materials. The NPs have a higher equilibrium solubility compared to macroparticles, as shown in Figure 2.5.[42] The solubility of these materials can exceed saturation condition in some instances, leading to increase NPs growth and their precipitation, this phenomenon known as Ostwald ripening (When smaller particles in solution dissolved and deposit on large particles in order to reach a more thermodynamically stable state wherein the surface to area ratio is minimized), where with time, the growth of larger particles with lower solubility reduced the rapid initial dilution and super–saturation solubility.[42]

2.5.2.2. Role of nanomaterial formulations and impurities

The synthesis of nanomaterials in many industries involves additives such as surfactants that are required to minimise aggregation and to modify surface properties.[3,12] These modifications possibly result in different solubility characteristics, but the impacts on aggregation and solubility of several commercial formulations of NPs is not known. For instance, formulations of ZnO show an equilibrium water solubility of ZnO; for instance, nanoparticulates in sunscreens have different solubility from that the raw NPs.[40]

Many nanomaterials include impurities.[43,44] Carbon nanotubes, for instance, may contain metal catalyst impurities.[43] Plata *et al.*[44] indicated that metal and carbonaceous impurities can reach levels of up to 10%; this is higher than the weight of Multi–Walled Carbon Nanotubes (SWCNT) formulations–for example Ni (up to 22%), Y (6%), Co (2–10%), Mo (0.7%), and Fe (0.5%); there may also be traces of Pb, Cr, and Cu. These impurities may alter the reactivity, and ecotoxicology of the NPs surface charge, as well as their transport and distribution.

2.5.2.3. Fate in natural water systems

Experimental studies,[3] have explored NPs behaviour in water, but their behaviour is expected to differ in natural waters; this is due to the opportunity for interaction with colloids comprising dissolved (and particulate) natural organic matter (NOM). This interaction cannot be ignored. For instance, in freshwater systems, the concentrations of colloidal organic matter stabilize in the range of 1–10 mg L^{-1}. By contrast, concentrations of manufactured NPs plausibly lie in the range1–100 μg L^{-1}.[45]

Hyung *et al.*[46] indicated that the dispersion of SWCNTs in Milli–Q water is enhanced significantly by the addition of standard Suwannee River humic acid; similar impacts were also detected in suspensions of samples of Suwannee River water. The dispersion of this material was greater than that found in a solution of sodium dodecyl sulfate. Iron oxide NPs were also stabilised by humic acids;[47, 48] however, natural colloids that have very small size–approximately 1 nm (fibrillar)–are expected to increase the aggregation of these materials as a result of different characteristics of their binding compared with the humic substance mechanism of charge stabilisation.[32] The majority of studies suggest that in natural water systems NPs may have a greater stability than in synthetic (NOM–free) waters, particularly in the higher ionic strength of marine waters.[3]

2.5.2.4. Nanoparticles as vectors for contaminant transport:

As indicated, NPs can be significant sources of environmental contamination, with potential impacts on living organisms.[3] However, NPs have also been suggested to act as vectors, as they have excellent binding sites for many soluble contaminants.[3] Therefore, NPs may plausibly deliver toxic materials, and the properties of NP surfaces might be important in determining this type of binding. Thus, for example, Hu *et al.*[49] indicated that suspensions of fullerene have effective adsorption sites for polycyclic aromatic hydrocarbons (PAHs), and the enhancement of adsorption is increased by addition of humic acid. Thus, NPs might affect the level and behaviour of diverse hydrophobic organic contaminants within natural waters, including soil systems, river, lakes, and ground waters. As indicated, natural colloids have significant roles in the adsorption and transport of trace metals.[28]

2.6. Fate of manufactured nanomaterials in terrestrial systems

Little information is available regarding the behaviour and environmental risk of MNPs to terrestrial ecosystems.[3,12] Their fate, environmental effects, and transformation in soils most likely depend on the physicochemical characteristics of these nanometals in the soil matrix.[3] As indicated, marine and fresh water are different from soils. This because soils (sinks) contain large reactive sites (e.g., hydroxyl and carboxylic groups) within the solid phase for NPs; these functional groups increase cation exchange capacity of soils which have a significant impact on the availability of several metals in soil solution and for plants. Thus, the availability of NPs in soil may increase to toxic levels for soil biota. However, it is difficult to determine the level of NPs among the huge numbers of natural NPs in the soil.[3]

2.6.1. Key pathways

Figure 2.6 shows factors that may affect the bioavailability and behaviour of NPs in soils. The high reactivity of NP surfaces is important; this unique property depends on coating and surface charge and the adhesion of NPs to reactive surfaces of soil; both can be strong with these materials. However, few publications have focused on contamination of soil by NPs;[3] the adsorption of NPs in soil is discussed in a subsequent section. Diverse studies of NP transport in soil colloids suggest that the coating of NP surfaces is an important indicator of their mobility and can enhance their transportation within soils,[36,50,51] similar results have been found for NPs used in the remediation of groundwater.[52]

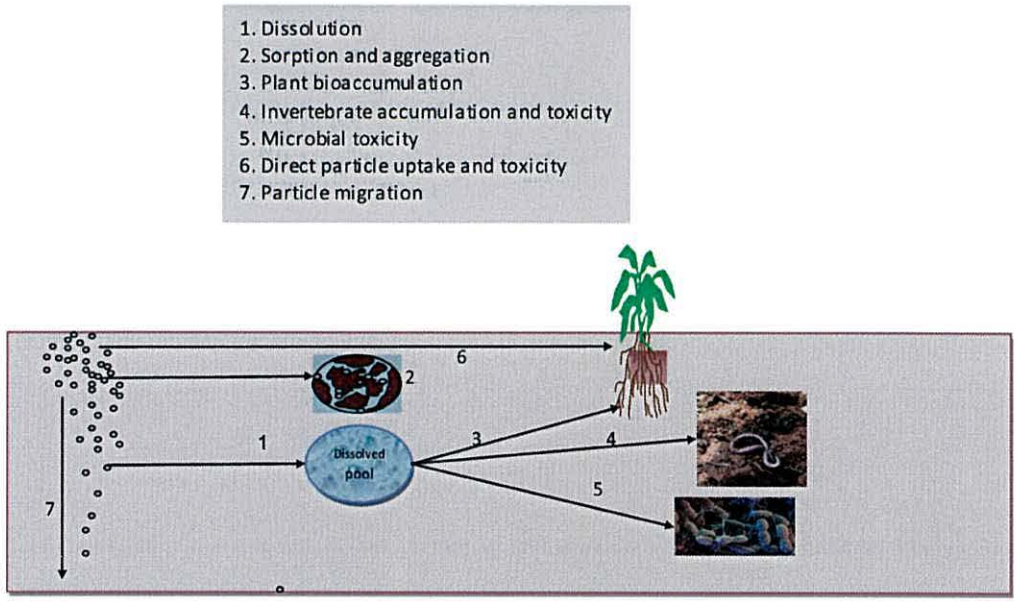

Figure 2. 6. Processes in soil relating to transformation and potential risk from manufactured nanoparticles Source: redrawn from Batley and McLaughlin.[3]

Eight NPs were tested for their transportation by Lecoanet *et al.*[53] who examined the association of fullerol (C_{60}–OH_m), SWCNTs, silica (57 nm), alumoxane, silica (135 nm), n–C_{60}, anatase (TiO_2), and ferroxane NPs (γ-FeOOH) with spherical glass beads; the efficiencies of NP attachment were in the order listed. Another study used sand columns and showed that the surface coatings of zerovalent iron NPs is an important determinant of transport,[36] nonetheless, more transport studies may be required, especially for different soil types–there appears little research on the subject.

2.6.2. Behaviour of natural colloids and manufactured nanoparticles in soils

The macromolecules and natural colloids found in soils are similar to those in freshwater systems, as the soils can provide these materials to water systems in different ways. Soils contain materials such as organic matter, clays at nano and micro scales, iron oxides, and diverse kinds of minerals; these resources play important roles in the biogeochemical interactions of soil systems. Soil colloids have been tested for decades as regards their impact on soil progress, pedogenesis (e.g., soil evolution), and their influence on the behaviour of the soil structure, such as crusting and dispersion.[33]

The aggregation of natural colloids is controlled by a number of factors; these include particle size, surface charge, exchangeable ions, and the chemical composition of soil pore water. It is plausible that soils that have a high ionic strength and high calcium levels tend to promote aggregation, and that soils that have a low ionic strength and more sodium tend to promote dispersion of colloids. A high flow of water within soils may tend to mobilise colloids, whereas a slow flow of water may tend to allow soil minerals to interact and bind the colloids and organic matter.[31] Some studies indicate that the behaviour of NPs is similar to that of natural colloids.[36,54] As described in Section 2.6, the fate of NPs in a soil system can be controlled by a number of properties. These factors include partitioning between the soil solid phases and solution and the aggregation of the NP materials.

2.6.2.1. The solubility and aggregation of nanoparticle

Nanoparticle dissolution in aqueous systems was discussed in Section 2.5.2.1. Although the factors affecting soil solubility are different from those found in water systems, the exchange capacity of cations and anions, together with the large surface area of the soil, can encourage greater dissolution of NP compounds and attract these dissolved materials (this is the so called *soil sink*). Soil pH plays a significant role in the solubility of NPs; this is because low pH provides more protons to enhance compound dissolution. However, to date it appears that no studies have yet tested the dissolution of NPs in soils or compared their dissolution with that of their bulk materials.[3]

No practical research has tested the aggregation of MNPs within soils; however, the behaviour of natural colloids can reliably predict NPs behaviour, as described in Section 2.6.2. The behaviour of NPs aggregation in aquatic systems has been discussed in Section 2.5.2.1. A similar performance is expected in soil systems; however, in soil systems, NPs aggregation might be larger than that in most surface waters because of the high ionic strength (high level of dissociated ions from soil salts) of soil pore waters.[3] Soil aggregation also causes particle entrapment within the soil pores through which the dispersed NPs might have passed, thereby restricting mobility.[54]

2.6.2.2. Partitioning

No studies have examined partitioning of NPs; however, the charges and large surface areas of several hydrophilic NPs may promote strong binding with the negative charges on surfaces of organic matter and soil minerals,[55] and this process may be a function of the charge nature.

Particles with net positive charge may be retained strongly, whereas those with net negative charge may be highly mobile, at least in most soil systems.[36] Therefore, if the NPs bind strongly to the surfaces of organic matter in soil, this will inhibit the availability and mobility of NPs and thereby limit the toxicity to organisms. Another important factor in the fate of NPs in soils concerns the coating surfaces used with many NP compounds. This coating or sorbed species may affect dissolution, this effect due to the stabilizing particular crystal surfaces.[12] A reactive surface may interact with different material surfaces in the soil, such as organic matter and minerals.[3]

2.7. Adsorption and desorption of manufactured metal nanoparticles on soil surfaces

Many studies have evaluated adsorption and desorption in terms of different soils; however, there is not much information as regards the adsorption and desorption of NPs; so, as indicated in Section 2.6.1., more research is needed to clarify their fate within soils. It is necessary to know the distribution of metals between the solution and the solid phases of a soil when assessing the availability and mobility of the metals in soil systems.[56] The concentration of the metal solution phase controls the transportation of metals into groundwater or within the soil profile.[57] Thus, the reactions of adsorption and desorption on soil surfaces or oxides are important (possibly the most important) processes in determining metal concentrations within soil solutions.[58–61]

Several factors contribute to changeability in the adsorption affinity of soil surfaces for metals; these include charge density, electronegativity, the solubility of metals within solutions, and Lewis acidity.[62–64] Consequently, as pointed out in Section 2.6.2, the composition of soil can be an important determinant of the toxicity of metals within soils; pertinent factors include the type and amount of clay minerals or colloids, iron and manganese oxides, and organic matter in soil systems.[65–67] The mobility and bioavailability of these metals may largely be a function of their physicochemical forms.[68]

Data analysis of adsorption is important for characterisation of the retention of chemicals by soils and is conducted using the most common equations for isotherms in soil science. Simple equations, for instance the Langmuir and Freundlich isotherms, are usually used to describe the data adsorption for metals in different soils.[69]

$$\frac{C}{q} = \frac{1}{kb} + \frac{C}{b}$$ Langmuir equation 2.1

Where: q is the amount of sorbed NPs by different soils (mg g^{-1}), C is the equilibrium concentration in (mg L^{-1}), k is the Langmuir constant (L mg^{-1}), and b is the maximum adsorption capacity (mg g^{-1}).

$$Log\ q_e = log\ K + \frac{1}{n} log\ C_e \qquad \text{Freundlich equation 2.2}$$

Where, k and $1/n$ are the Freundlich constants related to the adsorption capacity and intensity respectively or as Kf, which represents the Freundlich adsorption coefficient and gives an estimate of the adsorptive capacity. $1/n$ describes the isotherm curvature and gives an estimate of the adsorptive intensity.

Langmuir and Freundlich isotherms of adsorption are commonly used to distinguish chemical retention in soils. These equations are also incorporated into programs for chemical speciation on absorbent surfaces.[70] Isotherm parameters can also assess the mobility and transport of chemicals as input. However, in some cases the equations do not describe isotherms accurately; moreover, they can be difficult to use and their output can be difficult to interpret.[71]

Giles *et al.*[72] classified the isotherms of adsorption in terms of their curvatures and initial slopes. The authors divided all isotherms into four main classes: high affinity (H), Langmuir (L), constant partition (C), and sigmoidal–shaped (S) isotherms. Each class is divided into subgroups, as shown in Figure 2.7.

Sheela *et al.*[73] argued the expectations discussed in Section 2.5.2.4 as regards the ability of NPs to carry contaminants via vector transport. The authors used ZnO NPs as an adsorbent to remove Zn^{2+}, Cd^{2+} and Hg^{2+} ions from an aqueous solution. The results evaluated their data by kinetic and thermodynamic studies to assess the properties of ZnO adsorption of metal ions; these included adsorption equilibrium, adsorption kinetics, and temperature and pH effects. Results showed that the Langmuir equation describes the adsorption isotherms well, with a correlation coefficient (R^2) of more than 0.99. The capacity of maximum adsorption was measured at 303 K (related to the affinity of the binding sites); the Zn^{2+} ion showed the highest adsorption capacity; this was followed by the Cd^{2+} and Hg^{2+} ion. The mechanism and the rate constant of metal adsorption were determined by using pesudo–first and second order kinetic models. The mechanism for adsorption of metal ions followed the pseudo–second–order rate model, which provided the best fit for the adsorption data with a high correlation coefficient. An increase in temperature decreased the adsorption capacity, and an exothermic process was suggested for the adsorption of metal ions, this by using thermodynamic calculations.

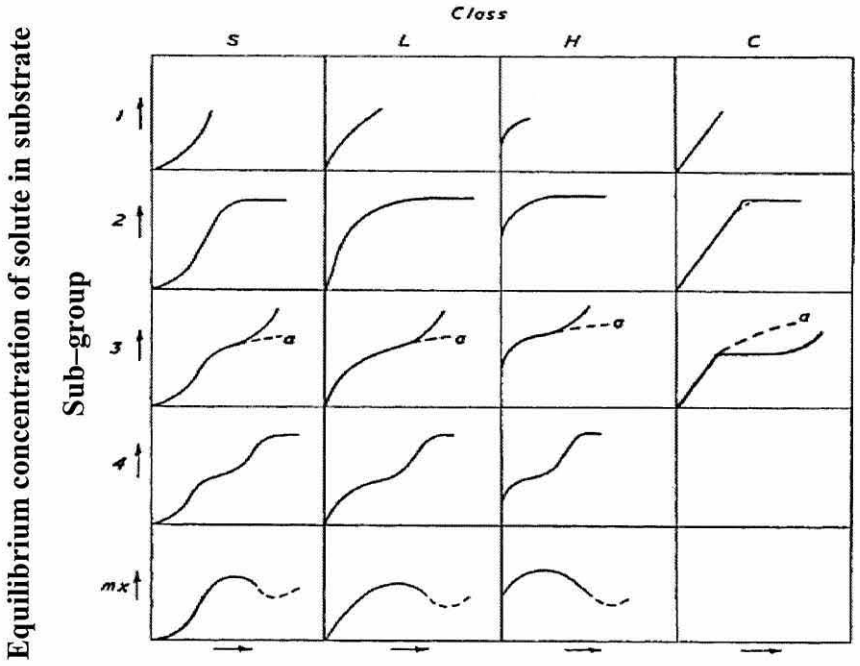

Figure 2. 7. Classifications of adsorption isotherms.The figure shows differently transformed coordinates. High affinity (H), Langmuir (L), constant partition (C) and sigmoidal–shaped (S) isotherm classes. Source: Giles *et al.* (1974).[72]

In the case of bulk materials for heavy metals, Ma *et al.*[74] reported the adsorption and desorption of Cu^{2+} and Pb^{2+} from upland red soil, and from paddy soils that originated from the upland soil, after cultivation for 8, 15, 35, and 85 years in China. The authors used the Langmuir and Freundlich equations. The soils were evaluated using the batch term and the term of desorption. The paddy soil cultivated for 15 years showed the highest adsorption for Pb^{2+}; this was followed by the 35 year paddy soil. These soils (15–35 year) also adsorbed more Cu^{2+} compared with the other upland and paddy soils. The results also indicated that the content of organic matter and cation exchange capacity (CEC) of the soils are important factors in the control of the adsorption and desorption of the metals. In the case of the desorption term, paddy soil cultured for 15 years showed a high percentage of desorption for both metals. However, the 85–year paddy soil showed lower desorption percentages than did the other soils; this appeared related to the low content of organic matter in this particular soil; the low content increased the stability of these metals because they formed complexes with organic matter.

Abat *et al.*[75] studied Cu and Zn in terms of their adsorption and desorption in the peat soils of Sarawak, Malaysia. Soils deficient in the micronutrients were assessed by adsorption and desorption reactions. Three soils of untreated and limed tropical peat sample were tested. Compared with control soils, limed soils showed a high adsorption of Zn; this was followed by Cu ions. The Freundlich constant (KF) value for Cu was higher than for Zn in control and limed peat soils; this was because the Cu adsorbed more strongly to solid phases than did Zn in both soils. The desorption percentage for Cu was higher than Zn, using 5 mM DTPA because of the higher critical constant of Cu with DTPA. It appeared that liming of peat soils played an important role in decreasing the bioavailability of these ions in soil solutions.

Another experiment was conducted by Echeverria *et al.*[76] who studied competitive adsorption of Cd, Zn, Ni, Pb, and Cu on Paralithic Xerorthent, Calcixerollic Xerochrept, and Lithic Haplumbrept soils in Navarra (Spain). These soils represented a range of chemical and physical properties. Competitive and monometal adsorption isotherms were established at 25°C. A factorial analysis design was used to clarify the individual impact of ions separately. Results suggested that the majority of the adsorption isotherms were type L and subtype 2 of the Giles classification. The most capacity of adsorption isotherm was in monometal compared with competitive capacity; this was in basic soils compared with acidic soil; the authors used Langmuir monolayer and Freundlich distribution coefficient. In calcareous soils, calcium (Ca) appeared to play a significant role in adsorption isotherms; this appeared to be due to the cationic exchange of Ca with other metal ions. Thus, the use of a factorial design corroborated the idea that the presence of the cations reduces the quantity of these metals retained, although the presence of Pb and Cu in the soils appears to depress the amount of Ni, Cd, and Zn adsorption. The mobility of cations appeared to improve when the equilibrium concentration increased; the impact appeared high in soils saturated with Ca cations.

2.8. Ecological risk assessment of manufactured nanoparticles

Many questions need to be addressed when assessing the environmental risk of manufactured nanomaterials; this is because the release of NPs is expected to have potentially serious effects on our environment. NPs production is expected to occur in huge amounts in forthcoming years; nevertheless, if commercial production arises more from many widely–dispersed of small scale activities than from relatively few large scale activities, the risk may possibly be lower.[3]

Owen and Handy argued that formulation is critical;[77] therefore, the potential for diffuse releases into the ecosystem must be assessed. In cases in which NPs are incorporated into stable solid–phases (e.g., ZnO NPs used as UV protection on glass coatings), the potential for release of dispersed NPs is low. In contrast, when zero valent iron is used in a dispersed form for the remediation of groundwater, there is a much higher potential for movement than in ZnO. Regulators worldwide are seeking to assess the environmental risks of manufactured NPs to evaluate any effects of their manufacture and use.[77] Initial fears that all MNPs are environmental hazards appear to have been exagerrated.[78]

2.9. Modelling exposure of manufactured nanoparticles

Existing models have resulted in initial achievements in the prediction modelling of the kinetics of NP aggregation and their suspension stability. However, these models provide poor information as regards testing in soils or real systems, and the majority of models now in use that address exposure to soluble contaminants may have little relevance to the manufacture of NPs.[79]

Boxall *et al.*[45] attempted to predict the extent of manufactured NPs that might be found in an ecosystem using set of simple algorithms to show the expected amounts of nanomaterials in soils and waters. For example, in the case of soils, they considered five ways that NPs could enter the soil: through the application of pesticides, the excretion of nanomedicines, diverse forms of aerial deposition, the use of sewage sludge in agriculture as a fertiliser, and the application of remediation technologies. In their predictions, they focussed on paint, cosmetics, and personal care products; these limited data were derived from European countries. Three scenarios were modelled. The models allowed for 10%, 50% and 100% of each product type containing NPs. Table 2.2 shows the predicted applications for the 10% model. The expected concentrations of ZnO NPs were at a higher level than those of other NPs. Despite some limitations, the concentrations can be compared with known toxic levels–this to determine whether they fall into similar ranges.

Table 2. 2. The Prediction of manufactured NPs concentrations in UK waters and soil

Particle type	Application	Water, µg/L	Soil, µg/kg
Aluminium oxide	Paint	0.002	0.01
Cerium dioxide	Catalysts ,scratch resistant coatings	<0.0001	0.01
Fullerenes	Face powder, anti–inflammatory cream, eyeliner, lipstick ,foundation, mascara, moisturizing cream, perfume	0.31	44.7
Gold	Face cream	0.14	20.4
Organosilica	Scratch resistant coatings	0.0005	0.07
Silver	Shampoo, biocidal coatings, soap, toothpaste	0.01	1.45
Titanium dioxide	Sunscreen ,paint	24.5	1030
Hydroxyapatite	Toothpaste	10.1	422
Latex	Laundry detergents	103	4310
Zinc oxide	Paint, scratch resistant coatings, sunscreens	76	3190

Source: Adapted from Boxall *et al.*[45]

2.10. Ecological impacts of nanoparticles

The published evidences to date for the broad ecological impacts of MNPs on terrestrial organisms are limited and remains unclear.[3]

2.10.1. Sediment toxicity

In aquatic systems, sediments are known to be the ultimate receptor of NPs; therefore, NP effects on benthic organisms are possibly of more concern than are effects on organisms in the overlying water.[3,12] Aggregation of NPs is expected to be high in the sediment medium; thus, the toxic properties of NPs that are associated with the size of NPs are not likely to be present in the sediment. Little information is available for nanomaterials in sediments, however. For instance, Kennedy *et al.*[80] indicated that MWCNTs may be a hazard to several species of amphipod in entire sediment bioassays, although the nanomaterials were supplied at impractical treatment levels exceeding 100 g kg^{-1}. Further research is necessary to fully assess the properties of NPs (surface area, aggregation) and NP toxicity, bioavailability, and adsorption in more complex sediment environments.[3,80]

2.11. Toxicity to terrestrial biota

There are few studies to evaluate the toxicity of nanomaterials on the terrestrial organism.[3,13]

2.11.1. Ecotoxicity to individual species

The environmental risks of NPs to terrestrial environments have not been well studied,[81] at present, the scientific literature is poor as regards NP toxicity to soil organisms. Few researchers have studied ecotoxicity in soil biota; although aqueous environments have been tested.[82–85] The presence of NPs in soil system has not been assessed.

Yang and Watts.[83] indicated that alumina NPs (coated with and without Phenanthrene, 13 nm) shows toxic effects on root elongation in five plant species (carrot, cabbage, corn, soybean, and cucumber); the authors exposed the plants to high concentrations (2,000 mg L^{-1}) of aqueous suspensions of NPs. Results indicated that loading of the alumina NPs with Phenanthrene decreases NP toxicity. Franklin *et al.*[40] found similar results as regards ZnO NPs exposure to aquatic biota. Lin and Xing evaluated the toxicity of MWCNTs, Al, Al_2O_3, Zn, and ZnO to seed germination and early root growth for six plant species in aqueous media at a close to neutral pH of 6.5–7.5.[85] Results indicated that the Zn–based NPs negatively affect seed germination and root elongation.

2.11.2. Ecotoxicity to microbial communities

A few studies have examined the impacts of NPs on terrestrial soil organisms; each used fullerenes.[86,87] Tong *et al.*[86] evaluated n–C_{60} toxicity in aqueous suspension and in granular shape to soil bacteria and protozoa by analysing soil respiration, microbial biomass, and enzyme activities as endpoints; this was in addition to phospholipid fatty acids. The DNA mutation of the microbial community was also tested in this study. All results were obtained under laboratory conditions. The authors found that n–C_{60} had no adverse impacts at any endpoint in the soil (pH 6.9, silty, 4% organic matter, clay loam). They suggested that these findings might be related to the strong binding of carbon NPs to organic matter within the soil matrix.

Johansen *et al.*[87] performed similar experiments. They studied the influence of n–C_{60} added to neutral soil (pH 6.7, 1.5% organic matter) on soil respiration, microbial biomass, the abundance of bacteria and protozoans, and the PCR–DGGE profile of soil microbial DNA. Soil respiration, microbial biomass, and protozoan abundance were not affected by the exposure to n–C_{60}; however bacterial abundance was reduced according to the counts of the community colony. The

n–C_{60} also affected a small shift in bacterial and protozoan DNA. A further study by Tong *et al.*[86] reported a small change in the structure of the microbial community, which agrees the earlier results,[86] Similar findings were reported by the same group using anaerobic bacteria that are typical of wastewater sludge treatment systems.[88]

2.12. Interaction of nanoparticles with plants

The environmental impacts of NPs have been studied in diverse organisms, including, protozoa microorganisms, vertebrates and invertebrates.[89–94] Nonetheless, there are relatively few investigations into interactions between NPs and plants (or between organisms similar to plant cells–e.g., algae); thus the impact of NPs exposure to plants is unclear,[95] This lack of information results in an imperfect understanding of how NPs might be accumulate or transferred in diverse levels of the crop food chain.[96]

2.12.1. Phytotoxicity of nanoparticles using morphological and physiological indicators

The influences of TiO_2, Fe_3O_4, and C NPs were investigated in cucumber plants, results suggested negative impacts on the rate of seed germination, the germination index (GI), and root elongation.[97] In addition, the effects of five types of NPs (Ag, Cu, ZnO, Si, and MWCNTs) were assessed in *Cucurbita pepo* growing in suspension solutions up to 1000 mg L^{-1}, and revealed different morphological impacts on seed germination, root elongation, and dry biomass that depended on the physicochemical properties of the NPs.[98] in this study, germination rates were not reduced following exposure to NPs, while Cu NPs decreased root emergence. Exposure to Ag NPs reduced the dry biomass and transpiration rate. The authors concluded that "standard phytotoxicity tests such as germination and root elongation may not be sensitive enough or appropriate when evaluating NP toxicity to terrestrial plant species".[98]

In a different study, Zhang *et al.*[95] studied the relative phytotoxicity of Yb_2O_3 NPs to cucumber plants compared with bulk Yb_2O_3, and $YbCl_3 \cdot 6H_2O$ NPs. Their study focused on accumulation and biotransformation and the toxicity of these materials in plant roots. Exposure to nano–Yb_2O_3 resulted in deposition of $YbPO_4$ within the cytoplasm in root cells. Other research was conducted on ZnO NPs in *Allium cepa* in root cells, where ZnO showed genotoxic and cytotoxic impacts, comprising decreases in the mitotic index, enhanced lipid peroxidation, and increases in the chromosomal aberration indexes and the formation of micronuclei. ZnO toxicity to *A. cepa* could be induced by dissolved metal ions from the ZnO.[99]

Ma *et al.*[100] studied the effects of CeO_2, La_2O_3, Gd_2O_3, and Yb_2O_3 NPs on root elongation in seven species of higher plants. Plants responsive to the different NPs showed negative impacts on root elongation; the impact varied according to plant species, with lettuce being the most sensitive. NP surface modification may also play an important role in the phytotoxicity of these materials. A recent study evaluated SiO_2 NPs and their interaction with an alga (*Pseudokirchneriella subcapitata*, L.). Alumina coated SiO_2 NPs appeared less toxic than did bare SiO_2 NPs.[101]

In contrast, relatively few studies have indicated any significant negative effects on plants in response to NPs. For instance, Al_2O_3 NPs (~ 150 nm) at high treatments up to 4000 mg L^{-1} had no negative toxic effects on root elongation, germination rate, or leaves number in *Arabidopsis thaliana*, but no positive effects either[102] In contrast, results of another study suggested positive effects of carbon nanotubes (~ 5 nm) when applied at 10–40 mg L^{-1}, where tomato plant growth and seed germination were increased significantly.[103] The authors proposed that carbon nanotubes are able to enter the seed coat and increase water uptake, giving rise to the effects observed in the plant, even though exact mechanisms for this were not reported. Therefore, some uptake of NPs by plants is very possible. However, little is known about the maximum NP size amenable for plant uptake. Similar behaviour was seen in response to TiO_2 NPs, which improved conversion efficiency and energy utilization in the D1/D2/Cyt b559 complex of spinach plants,[104] indicating that TiO_2 had positive influences on the spectral responses and photochemical activities of test complex of spinach.

Larue *et al.*[105] Studied absorption of TiO_2–NPs NPs by plants and found no effect on germination or root elongation in *Triticum aestivum*, *Brassica napus*, and *Arabidopsis thaliana*. Nonetheless, the authors recommended that NP toxicity should be more comprehensively studied, especially regarding the interaction of NPs with plants. Rico *et al.*[106] mentioned that, because the majority of NPs studies suggested the negative effects of few NPs on seed germination, or 25 day old seedlings (early growth stage), and several NPs illustrate their biotransformation in few food crops. However, the possibility of NPs transmission to next plants generation that exposed to NPs is still unknown.

This area clearly requires more studies to determine the mechanisms underlying the effects of NPs on plants; one needs to understand the uptake, accumulation, and phytotoxicity of NPs. Knowledge of how nanoscale materials can affect food crops is also important; unfortunately,

most of recent studies have focused on root elongation and seed germination but have not evaluated NPs toxicity in mature terrestrial crop plant species.[107]

2.12.2. Genotoxicity of nanoparticles in plants

Few studies have examined the genotoxicity of NPs in plants, but some data have been generated in the two last years. For example, Atha *et al.*[108] reported that CuO NPs damages DNA in *Raphanus sativus*, *Lolium perenne*, and *Lolium rigidum*; this was the first report of NP effects in some grassland and agricultural plants. The plants were treated with different concentrations of CuO NPs (10 – 1000 mg L^{-1}). The results revealed that CuO NPs seemed to promote the significant accumulation of oxidatively modifie compounds (7,8-dihydro-8-oxoguanine; 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 4,6 diamino-5 formamidopyrimidine) that resulted in mutagenic DNA lesions (8-OH-Gua, FapyGua and FapyAde) and led to inhibition of *Raphanus sativus*, *Lolium perenne*, and *Lolium rigidum* growth under controlled laboratory conditions. The putative genotoxicity of the different NPs types at different concentrations needs more study to evaluate their effects in plants. Another issue concerns the analysis of genotoxic endpoints of NP genotoxicity. For instnace, comets, FCMHPCV (Flow Cytometric Histograms Peak Coefficient of Variation), and micronuclei plausibly have similar genotoxicity to that of metals in plants.[107]

2.13. Uptake, accumulation and translocation of nanoparticles into edible plants

As pointed out in Section 2.11.1, a few studies have been performed on the toxicity of nanoparticles in crop plants; for instance, corn (*Zea mays*), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), rape (*Brassica napus*), and cucumber (*Cucumis sativus*), amongst others plants.[87,109] The absorption, accumulation, and translocation of MNPs–metal based (MB) and carbon based (CB) in edible plants are the most recent studies.[106,110]

Studies of the uptake of MB and CB NMs by plants are recent. Most publications have looked at the germination and cell cultures,[106] because quantification of NPs within plant cells is as yet imprecise. The best studied materials are the fullerene C_{70}, the fullerol (C_{60} $(OH)_{20}$), and CNTs; in contrast, the best studied MB NMs are TiO2, CeO_2, Fe_3O_4, and ZnO NPs.

2.13.1. Uptake mode of nanoparticles by plants

The uptake, accumulation, and translocation of NPs can be controlled by different factors such as plant species and the chemical composition, type, size, functionalization, and NPs stability.[106]

The CB NMs fullerene C_{70} and fullerols are easily accumulated in plants. The majority of MB NPs also appear to be accumulated and assimilated in plants, although result of some studies conflict. Figure 2.8 shows the selective uptake of various NPs and their biotransformation and translocation by a model plant.

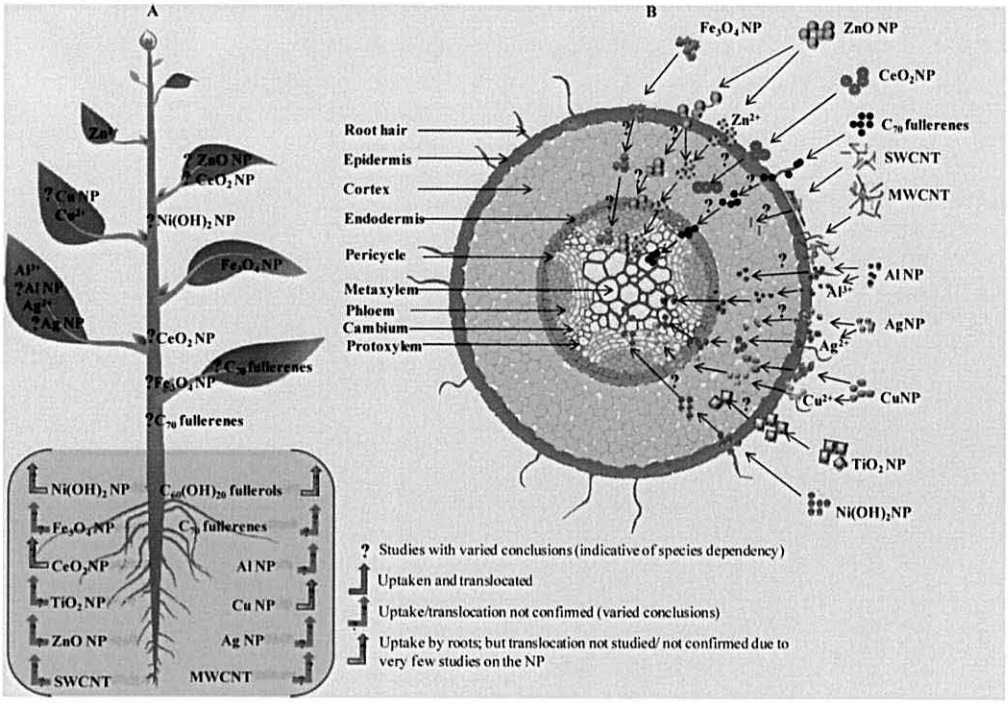

Figure 2. 8. Uptake, translocation, and biotransformation pathway of various nanoparticles in a plant system. (A) plant showing the selective uptake and translocation of NPs; (B) transverse cross section of the root absorption zone showing the differential NP interaction on exposure. Source: Adapted from Rico *et al.*[106]

As seen in Figure 2.8 A, the absorption and biotransformation of fullerols, $Ni(OH)_2$, and Cu NPs NPs within living plants was are so far the only conclusive studies on NPs.[111,112] the uptake of different types of NPs are presented in Figure 2.8B. The data for nanoparticles uptake by plants are ambiguous. Several pathways have been proposed for the NPs uptake by plant cells (see Figure 2.8). NPs can bind to carrier proteins to penetrate the plant cells, or can penetrate through ion channels, endocytosis or aquaporins, or by creating new pores of NPs (this is possible for CNTs). Another pathway is by binding to organic compounds in the environment (soil and aqueous media). Another important factor for NPS binding is the huge surface area to their mass ratio compared with their bulk materials; thus NPs can show more reactivity phases with their surroundings more than their counterparts.

The NPs might also form organic complexes within root exudates, organic acids are the primary components of root exudates (e.g. citrate) and by far the most reactive components with soil metals. This can be produced through the vascular systems as reported for some nanoparticles.[113,114] The physical and chemical interactions between the root exudates and NPs could explain differential NPs accumulations in the rhizosphere. The NPs might form organic complexes with membrane transporters and be transported into the plant tissues.[113,114] The majority of the metal based NPs that have been addressed as assimilated materials by plants comprise elements for which ion transporters have been recognized.[115] Once NPs are inside the plant cells, they may be transported symplastically or apoplastically. They may possibly be transported from cell to the other through their plasmodesmata. Although, the mechanism for uptake of several NPs are still unknown in some plant species and continue to be explored.

2.13.2. Uptake of metal oxide nanoparticles by plants

Few studies have reported the uptake of NPs within plant tissues; the majority tend only to test the toxicity of NPs such as ZnO and CuO NPs. The uptake, biotransformation, and translocation of CdS NPs do not yet appear to have been evaluated.

As in the case of other nano–materials, the uptake, accumulation, and translocation of ZnO NPs are not yet well understood in food crops. Also, for the most part, studies have been conducted only during the germination period; therefore, information is limited, the plant roots and vascular system were incompletely developed in the tested plants. However, the uptake and accumulation of ZnO NPs (8 nm) in soybean (*Glycine max*) seedlings have been studied by Lopez–Moreno *et al.*[115] following treatment with a range of ZnO NPs (500–4000 mg L^{-1}). The uptake of Zn by soybean seedlings was significantly higher at 500 mg L^{-1}; the authors explained this as being due to a lower aggregation of NPs at this application level, while at higher applications (1000–4000 mg L^{-1}), the probability of aggregation increased. Therefore, a substantial aggregation of NPs appears to make the NPs less likely to cross the walls of cell pore, thus reducing NPs uptake and accumulation. These authors determined the uptake and accumulation of ZnO NPs in the treated samples using X–ray absorption spectroscopy (XAS). The XAS measurements showed Zn^{2+} within the plant tissues, although the spectrum of XAS suggested that there was more zinc acetate and nitrate [$Zn(OAc)_2$ and $Zn(NO_3)_2$] than ZnO NPs inside the test plants. Zn^{2+} ions are expected to be a source from ZnO NPs, which increases the level of Zn^{2+} within tested tissues; therefore, it was difficult to determine whether Zn^{2+} accumulation arose from the

biotransformation of the ZnO NPs on or in roots. Root exudates may possibly have ionized the ZnO NPs on the surface of root–traces of ZnO NPs were not detected within the XAS spectra. Nonetheless, more studies are needed to determine the mechanism of ZnO NP biotransformation and the factors affecting ionization. In ryegrass (*Lolium perenne*), scanning electron microscopy (SEM) studies have evaluated the aggregation and adsorption of the ZnO NPs on the root surface.[116] The images of ryegrass roots were obtained from transmission electron microscopy (TEM). The TEM provides a high magnification images and information on element and compound structure. However, the plant samples could be affected by the sample preparation technique and the vacuum conditions. The root sections showed the presence of particles (dark dots) in the endodermis and vascular cylinder treated with ZnO NPs. The distribution of ZnO NPs was in the cytoplasm, apoplast, and the vascular cylinder, as well as in the nuclei of the endodermal cells; these particles were presumed to be ZnO. Unfortunately, the results of X–ray absorption techniques revealed no presence of NPs within the plant tissues.

Lee *et al.*[111] evaluated mung bean (*Phaseolus radiata*, L.) and wheat (*Triticum aestivum*, L.) uptake and translocation of Cu NPs in a agar. The Cu NPs were able to pass through the cell membrane and agglomerate within the cells. The bioaccumulation factors of mung bean and wheat plants exposed to 1000 mg L^{-1} of Cu NPs were 8 and 32 mg kg^{-1}, respectively.

2.14. Storage of nanoparticles in plants

Information is poor regarding the accumulation of NPs within plants. An important question concerns where and how the absorbed NPs are stored within plants. Recent studies do not appear to address this question.[106] The literature is unclear as to whether NPs are established within plant cells and tissues, although one often–mentioned study, conducted with fluorescent SWCNTs (FITC–SWNTs) indicated their presence in vacuoles of tobacco plant cell suspensions in addition to cytoplasmic strands (SWCNT–DNA).[117]

Results of a study on the accumulation and reduction of Ag ions into NP form in alfalfa (*Medicago sativa*, L.) indicate that Ag NPs can accumulate on root cell surfaces of organelle root cells.[118] In addition, results of a study by Gardea–Torresdey *et al.*[119] suggested the presence of Ag NPs in alfalfa stems. Other issues concern the transmission of NPs to next generation plants and the NP's accumulation in edible plants. Lin *et al.*[120] indicated that the discovery of C_{70} NPs, albeit at a low frequency, in the leaf tissues of a second generation of rice plants. If NPs occur in second–generation plants, it is possible that the plants become adapted, becoming more

accumulating and more responsive to the NPs. Another issue concerns the bioavailability of the accumulated NPs in the next trophic level–for example in humans and ruminants. NPs in algae and tobacco have been shown to be transmitted to the next trophic level.[121,122]

2.15. Factors affecting the toxicity of nanoparticles in edible plants

Physiological and visual measures of the effects of NPs in plants might not be a sensitive indicator of their toxicity. More studies are needed at the genomic, proteomic, and metabolic levels.[106] Several studies on NPs toxicity in edible plants have indicated that different factors impact on the toxicity in food crops; however, no general tendency of the toxicity of NPs could be found. The main factors that influence toxicity in food crops appear to be as follows: (1) concentration of NPs; (2) specific surface area and particle size; (3) physicochemical properties of NPs; (4) plant species and plant age; (5) growth media (soil and hydroponic); and (6) stability of NPs and the dilution agent.[123]

The physical and chemical properties of soil control the availability of heavy metals, which are taken–up by plants. Physical properties such as the water holding capacity (WHC) of soil are important: soils that hold generous amounts of water are less subject to leaching, and therefore to loss of nutrients. Because most plants extract water directly from the soil, the soil's physical characteristics influence the quantity and availability of water for plants. Microbiological measurements are often made after adjusting the water content to a constant value for all soils, as availability of water is crucial for their growth and metabolic activity.[124]

Soil pH is the major factor that determines the availability of heavy metals in the soil; this is because soil pH affects all adsorption mechanisms and the speciation of metals in solution. Acidic conditions in the soil often enhance the solubility of heavy metals (e.g., Cu, Zn, Pb, and Cd). An increase in the dissolved concentration of heavy metals may cause toxicity and contamination in soils.[125] Alloway.[126] reported that the addition of CaO to soils reduces the uptake of Cd by fodder rape (*B. napus*,) because of an increase in pH and competition between their respective ions. Organic matter plays an important role in the chemical behaviour of several heavy metals ions dissolved in soil water. Active groups on organic molecules have the ability to retain heavy metal ions in complexes and chelated forms. Fulvic acid in soils has a greater affinity for binding Cd, Zn, and Pb ions than has humic acid.[125]

Cation exchange and soil texture have been shown to have a significant impact on the availability and release of several metals for plants. Cation exchange has been demonstrated to

have important influence on microsized particles release.[12] In particular, deposited particles can be released when divalent cations on the exchanger phase are replaced by monovalent cations. The impact of cation exchange on particle release is still incomplete for NPs.[12] However; the influence of cation exchange on release of bulk heavy metals has widely studied. Haghiri reported a decrease in the amount of bulk Cd assimilated by oat shoots when the cation exchange of the soil was increased by adding organic matter.[127] Nevertheless, over a broad range of soils with varying CEC, the effects of other soil chemical properties tends to distort any consistent pattern in the absorption of Cd in relation to increased cation exchange. For example, varying concentrations of Pb and Cd were incorporated into a sandy loam soil with a relatively low CEC and positive relationships were observed between Langmuir parameters for the percentages of clay and organic matter.[128] Vincent *et al.*[129] showed that Cd retention is greater in fine texture soils with high cation exchange capacities than in coarse texture ones with lower exchange values.

Plants could be more sensitive to exposure to NPs depending on the size of the plants' seeds.[130,131] The reasoning here is that species with large seeds (e.g. cucumber) have lower surface to volume ratio than that of small seeded species (e.g. tomato). Research suggests that the toxic effects of SWNTs are larger in small seeded (such as lettuce, onion, and tomato) than in large seeded species (cucumber).[130,132] Although, it is as yet unclear how much size of seeds is relevant to the toxicity of NPs to plants.[87, 111,133]

Results from Lee *et al.*[111] suggested that mung bean plants are more sensitive than wheat plants to Cu NP toxicity. They concluded that this difference is most likely because of differences in root anatomy, as xylem structures control the speed of transport water or nutrient solution; moreover different xylem structures may display different NPs behaviour of transport kinetics within the vascular system.[134] The mung bean is classified as a dicot–it has one large primary root and several smaller lateral roots. In contrast wheat is a monocot–it has numerous small roots but no primary root. Nevertheless, it is as yet difficult to generalise as regards whether toxicity depends on the classification of dicot or monocot.[87,127,125]

Another factor is the concentration of NPs in food crops; high concentrations of NPs in plants might result in toxic effects on the consumer (human or animal). The macroscopic standard tests for phytotoxicity (root elongation–germination or vigour test) indicate that high concentrations of NPs (1000–4000 mg L^{-1}) negatively impact tested food crops; toxic effects begin to be

obvious at the critical level.[106] For example, the germination of ryegrass (*Lolium perenne*) is completely inhibited by Fe NPs (Zero–valent), as is germination in flax (*Linum usitatissimum*) and barley (*Hordeum vulgare*) at high concentrations (2000 and 5000 mg L^{-1}).[106] Similarly, ZnO NPs at 1000 mg L^{-1} causes the death of roughly all plant cells at the root tip of ryegrass.[120] Wheat root and seedling growth are reduced by Cu NPs at a relatively high concentration (< 200 mg L^{-1}). When toxicity studies are conducted in different soil media, however, high amounts of NPs appear necessary to induce toxicity in plants.[125,135,136]

2.16. Site Description of soils

Sandy and Eutric Cambisol (clay loam) soils were collected from lowland–lying sheep–grazed pastures at an altitude of 15 m. The soil samples were freely draining and had been lightly to heavily grazed by sheep. The soils *in situ* had received normal fertilization (i.e., 120 kg N, 60 kg K and 10 kg P yr^{-1}). Meteorologically, the soils samples has been exposed to a coastal climate, a mean annual rainfall of roughly 1250 mm a year and soils surface temperatures in the range 8–10°C to a depth of 10 cm. Three main plant species covered the sampling location: *Trifolium repens* (clover), *Lolium perenne* (ryegrass) and *Cynosurus cristatus* (crested dog's tail). The Haplic podzol soil was collected from a free–draining upland slope at an altitude of 200 m. The sampling location was predominantly covered in *Festuca ovina* (sheep's fescue) and *Agrostis capillaries* (bent grass) with a mean annual rainfall of approximately 1700 mm.[137] This soil sample had been exposed to a similar climate and land management to that of the Sandy and Eutric Cambisol soil.

Libya is classified as southern mediterranean country with approximately 1,900 km of shoreline. Janzur soil (sandy soil) is found in the Gefara Plain. The plain occupies a total area of 17000 km^2.[138] The Gefara Plain receives more than 80% of the country's total agricultural activity and is the location from which the soil sample was collected.[138] The plain's dominant climate ranges from arid in the west to semi–arid in the middle and dry sub–humid in the east. Its annual rainfall is about 50 mm; however, in the south it may remain rainless for years.[139] The average winter temperature along the coastal region of the Gefara Plain is in the range10–12°C; in the summer it is in the range26–29°C.

2.17. Assessable parameters of plants

2.17.1 Tolerance index (TI)

The evolution of heavy metals tolerance in the natural system of plants grown in contaminated soils has been extensively studied over the last 20 years.[140] The tolerance of plants to toxic metals is frequently measured by comparing rates of root growth in culture solutions with and without the addition of the metal as a control; however, many variants of the technique are available depending on the experimental conditions. Control growth rates may be measured beforehand on the same roots or in parallel on a duplicate set. Nevertheless there are some complications with stimulation at low concentrations. There is evidence that differences of plant tolerance are largely related to genetic origins.[141] For instance, there is a great genotypic variation in Cd tolerance among plants of the same or different species. The reasons for this are not well understood. It is, however, well known how plants have developed the tolerance mechanism to reduce Cd^{2+} ion influx at the cellular level.[142, 143]

2.17.2. Agronomical efficiency (AE)

Efficiency is defined as the amount of product produced per unit of resource used. This means nutritional efficiency is the amount of dry matter produced per unit of nutrient applied or assimilated.[144] According to Graham,[145] nutrient efficiency can be defined as the relative yield of genotype on deficient soil compared with its yield at optimum nutrition. Cooke defined efficiency as the increase in yield of the harvested fraction of the crop per unit of nutrient supplied by fertilizer.[146] Thus, agronomic efficiency (AE) is also called the economic efficiency. This measurement is the best way to test nutrient use efficiency. If the efficiency is determined under greenhouse conditions, it will be expressed in $g\ g^{-1}$.

2.17.3. The Bio–concentration ratio (BCR)

The accumulation of heavy metal contaminants in the environment has become a concern because of potential health risks to humans and animals. Heavy metals are elements that cannot be degraded by microbial or chemical processes and tend to accumulate in soils and aquatic sediments.[147] Toxic elements, such as Cd, Cu, and Zn, are accumulated at elevated levels mainly through different human activities.[148]

Plant–to–soil and plant–to–air bio–concentration ratios (BCRs) are used to relate chemical concentrations measured in different vegetation tissues to concentrations in the soil supporting

that vegetation. In spite of continuing laboratory and field studies, the role of terrestrial vegetation in transferring chemicals from soil or air into specific plant tissues (stems, leaves, roots, etc.) is not well understood. The lack of chemical, plant, and site specific BCR measurements has led to a reliance on empirical and process–based models to explain and predict the fate of chemicals in air, plant, and soil systems.[149] There are different ways to define a plant's BCR relative to chemical concentrations in the soil solids (dry mass) or to their concentrations in soil solution.[149]

2.17.4. Relative increase percentage (RI)

The growth of whole plants or their parts is frequently used as an easily measurable parameter to monitor the effects of various stressors.[150] Changes in growth are often the first and most obvious reaction of plants under stress, particularly in those organs that have the first direct contact with noxious substances–normally the roots in contaminated soils. These roots show rapid and sensitive changes in their growth characteristics.[151]

There are different ways to measure plant growth. Changes in biomass are quantified by weighing. The measurement of dry weight changes over time describes the overall growth of a plant. Another biological indicator is the length of roots and shoots and the number of leaves in plants. One of the most often used derived quantities in plant growth analysis is the Relative Growth Rate or Relative Increase Percentage in Plant Growth (RGR), which is expressed in units such as day^{-1}.[152] The RGR reflects plant productivity and gives the rate of biomass increase relative to the productive mass of plant. Trace metals can be grouped according to their effects on plants, namely: essential micronutrients, non–essential and toxic elements.[153] The absence of an essential elements cause abnormal growth or failure of the plant life cycle and these elements can not be substituted by others in their biochemical role. The plants have three response stages for essential elements: deficiency, tolerance and toxicity. However, non–essential elements have no deficiency phase and the tolerance plateau extends to zero dose.[153]

Plants evolved different mechanisms to handle exposure to toxicants (e.g., heavy metals) from the amount that is assimilated from the surrounding, to strategies of inactivation and sequestration in sub cellular compartments or even to the ability of tolerating putative deleterious impacts of heavy metals. However, some of the most common and often unspecific symptoms, of metals phytotoxicity are: nutrient imbalance, disturbances in the ion and water regime and growth inhibition (lethal level or endpoints). Plant biological endpoints (germination, tissue

contaminant content, and dry matter growth) are used as indicators of soil phytotoxicity.[153] It is important to ensure that the variation in plant response is due to the bioavailability of contaminants and not due to uncontrolled variables such as problems with soil quality (increase of pH or salinity, water availability etc.) or deficiencies of plant nutrient.[153]

2.18. Maize plant

Maize (*Zea mays*) is the third most important cereal crop in world. The principal maize cultivation countries are the United States, China, Brazil and Mexico which provide 70% of global maize production. India has 5% of total corn acreage and contributes 2% of world production.[154,155] The plant's productions have multible uses, including for human nutrient, an essential crop of animals feed and raw material for many of manufacture industrial products. These products comprise starch, oil, maltodextrins, syrup, alcohol and corn products of fermentation and distillation industries.[155] In addition, it has been used recently as biofuel. The plant belongs to the tribe Maydeae of grasses family (*Poaceae*).[154–157]

Maize is an adaptable cereal crop grown over a range of agricultural climatic zones. In fact the suitability of maize plant to varied environments is incomparable by any other cereal crop. it is also grown at latitudes varying from 58°N to 40°S, with critical photoperiod of 12.5 hours/day, from sea level to altitudes over 3000 m, under areas with 250 mm to more than 5000 mm of rainfall per year and with growing cycles ranging from 3 to 13 months.[154,156] However, the majority of maize production environment are located in temperate regions of the globe. The optimum temperature for maize development is 18 to 32 °C. It has ability to grow on a wide range of soil types. It makes a relatively heavy drain on the fertility of the soil. Maize plant grows successfully over a wide range of soil reaction, pH 5 to 8.[154,156]

Maize is a tall grass, determinate, monoecious, annual plant. It produced long large, narrow edges, opposite leaves, borne alternatively along the length of stalk (stem), and unusually big seed. It has a coarse fibrous root system which penetrates deeply and spreads widely.[154] All maize varieties follow same general pattern of development, although there are a specific period and interval between growth stages and total number of leaves developed may vary between different hybrids, location, seasons and time of planting. Despite, the numerous varieties, seven major kinds of maize are usually recognized. This classification was determined by the appearance, grain starch, and uses.[156] The corn types are flint, dent, sweet, flour, wax, pod, and

popcorn. Two of these, dent and flint, account for the bulk of world production; pop, sweet, and flour are used almost entirely for human consumption.[154,156,157]

This literature review thus suggests that the interaction of NPs with soil components needs more consideration and experimentation, including more assessments of adsorption–desorption, precipitation–dissolution, aggregation–dispersion, decomposition, mobility, and availability of NPs in the soil medium.

2.19. References

1. American Society for Testing and Materials. Standard terminology relating to nanotechnology. (2006). Designation: E2456–06. *ASTM International*, West Conshohocken, PA,USA.

2. Scientific Committee on Emerging and Newly Identified Health Risk. (2007). The appropriateness of the risk assessment methodology in accordance with the Technical Guidance Documents for new and existing substances for assessing the risks of nanomaterials. Brussels, Belgium: *European Commissions*. pp, 1–68.

3. Batley, G. E., and McLaughlin, M. J. (2010). Fate of manufactured nanomaterials in the Australian environment. CSIRO niche Manufacturing Flagship Report. *The Department of the Environment, Water, Heritage and the Arts*.pp:1–58.

4. Pratim, B., and Chang,Y. W. (2005). Critical review: nanoparticles and the environment. *Journal of the Air and Waste Management Association.* 55 (6): 708–746.

5. Nowack, B., and Bucheli, T. D. (2007). Occurrence, behaviour and effects of nanoparticles in the environment. *Environmental Pollution*. 150 (1): 5–22.

6. Tervonen, T., Linkov, I., Figueira, J. R., Steevens, J., Chappell, M., and Merad, M. (2009). Risk–based classification system of nanomaterials. *Journal of Nanoparticle Research.* 11 (4): 757–766.

7. Buzea, C., Pacheco, I. I., and Robbie, K. (2007). Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases.* 2 (4): 17–71.

8. Shi, J.P., Evans, D.E., Khan, A.A., and Harrison, R.M. (2001). Sources and concentration of nanoparticles (<10 nm diameter) in the urban atmosphere. *Atmospheric Environment.* 35 (7): 1193–1202.

9. Buffle, J., and Leppard, G.G. (1995a). Characterisation of aquatic colloids and macromolecules. 1. Structure and behaviour of colloidal material. *Environmental Science and Technology*. 29 (9): 2169–2175.

10. Daniel, M.C., and Astruc, D. (2004). Gold nanoparticles: Assembly, supramolecular chemistry, quantum–sized–related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical Reviews.* 104 (1): 293–346.

11. Yang, W.W., Li, Y., Miao, A.J., and Yang, L.Y. (2012). Cd^{2+} toxicity as affected by bare TiO_2 nanoparticles and their bulk counterpart. *Ecotoxicology and Environmental Safety.* 85: 44–51.

12. Lead, J. R., and Smith, E. (2009). Environmental and human health impacts of nanotechnology. *Chichester*, U.K.: Wiley.

13. RCEP (Royal Commission on Environmental Pollution). (2008). Novel materials in the Environment: The case of nanotechnology. *Crown Copyright*. 2.7.

14. Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., Mclaughlin, M.J. and Lead, J.R. (2008). Nanomaterials in the environment: Behaviour, fate, bioavailability And effects. *Environmental Toxicology and Chemistry*. 27(9). 1825–1851.

15. Park, B. (2007). Current and future applications of nanotechnology. In: Nanotechnology: consequences for human health and the Environment. Eds: Hester, R.E. and Harrison, R.M. *RSC Publishing*. pp1.

16. Su, D.S., Serafino, A., Muller, J.O., Jentoft, R.E., Schlogl, R. and Fiorito, S. (2008). Cytotoxicity and inflannatory potnetial of soot particles of low–emission diesel engines. *Environmental Science and Technology*. 42 (5). 1761–1765.

17. Ecotoxicology test protocols for representative nanomaterials in support of the OECD sponsorship programme. (2009). Toxicological review of nano zinc oxide. *Prospect, Global nanomaterilas safety*. pp 1–25.

18. Zhu, J., Li, D., Chen, H., Yang, X., Lu, L., and Wang, X. (2004). Highly dispersed CuO nanoparticles prepared by a novel quick–precipitation method. *Materials Letters*. 58 (26): 3324–3327.

19. Marino, E., Huijser, T., Creyghton, Y., and Van der Heijden. A. (2007). Synthesis and coating of copper oxide nanoparticles using atmospheric pressure plasmas. *Surface and Coatings Technology*. 201 (22–23): 9205–9208.

20. Arabe, K.C. (2003). Nanomaterials set for explosive growth.
(http://news.thomasnet.com/IMT/archives/2003/09/nanomaterials_s.html)

21. Kuno, M. (2008). An overview of solution–based semiconductor nanowires: synthesis and optical studies. *Physical Chemistry Chemical Physics*. 10 (5): 620–639.

22. Mubarak, A., D., Gopinath, V., Rameshbabu, N., and Thajuddin, N. (2012). Synthesis and characterization of CdS nanoparticles using C–phycoerythrin from the marine cyanobacteria. *Materials Letters*. 74: 8–11.

23. El–Tantawy, F. (2001). Influence of solvent transport on physico–chemical properties of crosslinked butyl rubber filled with TiC ceramic. *Polymer Degradation and Stability*. 73 (2): 289–299.

24. Faez, R., Martin, I. M., De Paoli, M. A., and Rezende, M. C. (2002). Influence of processing time and composition in the microwave absorption of EPDM/PAni blends. *Journal of Applied Polymer Science*. 83 (7): 1568–1575.

25. Lead, J.R., and Wilkinson, K.J. (2007). Environmental colloids and particles: Current knowledge and future developments. In: environmental colloids and particles: behaviour, separation and characterisation, Wilkinson, K.J., and Lead, J.R. (eds), John Wiley and Sons, *Chichester*, UK., pp 1–16.

26. Buffle, J., and Leppard, G.G. (1995). Characterisation of aquatic colloids and macromolecules. Key role of physical structures on analytical results. *Environmental Science and Technology*. 29 (9): 2176–2184.

27. Handy, R.D., Von der Kammer, F., Lead, J.R., Hassessov, M., Owen, R., and Crane, M. (2008). The ecotoxicology and chemistry of manufactured nanoparticles. *Ecotoxicolgy.* 17 (4): 287–314.

28. Kretzschmar, R., and Schafer, T. (2005). Metal retention and transport on colloidal particles in the environment. *Elements.*1 (4): 205–210.

29. Gustafsson, O., and Gschwend, P.M. (1997). Aquatic colloids: concepts, definitions and current challenges. *Limnoogyl and Oceanogrphy.* 42 (3): 519–528.

30. Liang, L., and Morgan, J, J. (1990). Chemical aspects of iron oxide coagulation in water: Laboratory studies and implications for natural systems. *Aquatic Sciences.* 52 (1): 32–55.

31. Rengasamy, P., and Olsson, K.A. (1991). Sodicity and soil structure. *Australian Journal of Soil Research.* 29 (6): 935–952.

32. Buffle, J., Wilkinson, K.J., Stoll, S., Filella, M., and Zhang, J. (1998). A generalized description of aquatic colloidal interactions; the three–colloidal component approach. *Environmental Scince and Technol.* 32 (19): 2887–2899.

33. Cameron, F.K. (1915). Soil colloids and the soil solution. *Journal of Physical. Chemistry.* 19 (1): 1–13.

34. Choi, O., Deng, K.K., Kim, N.J., Ross, L., Surampalli, R.Y., and Hu, Z. (2008). The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Research.* 42 (12): 3066–3074.

35. Kwak, S.Y., Kim, S.H., and Kim, S.S. (2001). Hybrid organic/inorganic reverse osmosis (RO) membrane for bactericidal anti–fouling. 1. Preparation and characterization of TiO_2 nanoparticle self–assembled aromatic polyamide thin–film–composite (TFC) membrane. *Environmental Science Technology.* 35 (11): 2388–2394.

36. Saleh, N., Kim, H.J., Phenrat, T., Matyjaszewski, K., Tilton, R.D. and Lowry, G.V. (2008). Ionic strength and composition affect the mobility of surface–modified Fe^0 nanoparticles in water–saturated sand columns. *Environmental Science Technology.* 42 (9): 3349–3355.

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

38. Brant, J., Lecoanet, H., Hotze, M., and Wiesner, M. (2005). Comparison of electrokinetic properties of colloidal fullerenes (n–C_{60}) formed using two procedures. *Environmental Science Technology.* 39 (17): 6343–6351.

39. Lyon, D.Y., Adams, L.K., Falkner, J.C., and Alvarez, J.J. (2006). Antibacterial activity of fullerene water suspensions: effects of preparation method and particle size. *Environmental Science Technology.* 40 (14): 4360–4366.

40. Franklin, N.M., Rogers, N.T., Apte, S.C., Batley, G.E. and Casey, P.E. (2007). Comparative toxicity of nanoparticulate ZnO, bulk ZnO and $ZnCl_2$ to a freshwater microalga (*Pseudokirchnerilla subcapitata*): the importance of particle solubility. *Environmental Science Technology.* 41(24): 8484–8490.

41. Derfus, A.M., Chan, W.C.W., and Bhatia, S.N. (2004). Probing the nanotoxicity of semiconductor quantum dots. *Nano Letters.* 4 (1): 11–18.

42. Borm, P., Klaessig, F.C., Landry, T.D., Moudgil, B., Pauluhn, J., Thomas, K., Trottier, R., and Wood, S. (2006). Research strategies for safety evaluation of nanomaterials, Part V: role of dissolution in biological fate and effects of nanoscale particles. *An Official Journal of the Society of Toxicology.* 90 (1): 23–32.

43. Haddon, R.C., Sippel, J., Rinzler, A.G., and Papdimitrakopoulos, F. (2004). Purification and separation of carbon nanotubes. *MRS Bulletin– Materials Research Society.* 29 (4): 252–9.

44. Plata, D.L., Gschwend, P.M., and Reddy, C.M. (2008). Industrially synthesized single–walled carbon nanotubes: compositional data for users, environmental risk assessments, and source apportionment. *Nanotechnology.* 19: 1–14.

45. Boxall, A.B.A., Chaudhry, Q., Sinclair, C., Jones, A., Aitken, R., Jefferson, B., and Watt, C. (2007). Current and future predicted environmental exposure to engineered nanoparticles. Central science laboratory report, *University of York*, prepared for the UK department of environment, food and rural affairs.

46. Hyung, H., Fortner, J.D., Hughes, J.B., and Kim, J–H. (2007). Natural organic matter stabilizes carbon nanotubes in the aqueous phase. *Environmental Science Technology.* 41: 179–184.

47. Tipping, E., and Higgins, D.C. (1982). The effect of adsorbed humic substances on the colloid stability of haematite particles. *Colloids and Surfaces.* 5 (2): 85–92.

48. Baalousha, M., Manciulea, A., Cumberland, S., Kendall, K., and Lead, J.R. (2008). Aggregation and surface properties of iron oxide nanoparticles: influence of pH and natural organic matter. *Environmental Toxicology and Chemistry.* 27 (9): 1875–1882.

49. Hu, X., Liu, J., Mayer, P., and Jiang, G. (2008). Impacts of some enviromentally relevant parameters on the adsorption of polycyclic aromatic hydrocarbons to aqueous suspensions of fullerene. *Environmental Toxicoogyl and Chemistry.* 27 (9): 1868–1874.

50. Kretzschmar, R., Robarge, W.P., and Amoozegar, A. (1995). Influence of natural organic–matter on colloid transport through saprolite. *Water Resources Research.* 31(3): 435–445.

51. Seaman, J.C., and Bertsch, P.M. (2000). Selective colloid mobilization through surface–charge manipulation. *Environmental Science Technology.* 34 (17): 3749–3755.

52. Hydutsky, B.W., Mack, E.J., Beckerman, B.B., Skluzacek, J.M., and Mallouk, T.E. (2007). Optimization of nano– and microiron transport through sand columns using polyelectrolyte mixtures. *Environmental Science Technology.* 41(18): 6418–6424.

53. Lecoanet, H.F., Bottero, J.Y., and Wiesner, M.R. (2004). Laboratory assessment of the mobility of nanomaterials in porous media. *Environmental Science and Technology.* 38 (19): 5164–5169.

54. Wang, Y.G., Li, Y.S., and Pennell, K.D. (2008). Influence of electrolyte species and concentration on the aggregation and transport of fullerene nanoparticles in quartz sands. *Environmental Toxicology and Chemistry.* 27 (9): 1860–1867.

55. Li, D., Lyon, D.Y., Li, Q., and Alvarez, P.J. (2008). Effect of soil adsorption and aquatic natural organic matter on the antibacterial activity of a fullerene water suspension. *Environmental Toxicology and Chemistry.* 27 (9): 1888–1894.

56. Jalali, M., and Moharrami, S. (2007). Competitive adsorption of trace elements in calcareous soils of western Iran. *Geoderma.* 140 (1–2): 156–163.

57. Brummer, G. W., Gerth, J., and Herms, U. (1986). Heavy metal species, mobility, and availability in soils. *Journal of plant nutrition and soil science.* 149 (4): 382–398.

58. Brummer, G. W., Gerth, J., and Tiller, K. G. (1988). Reaction kinetics of the adsorption and desorption of nickel, zinc and cadmium by goethite. I. Adsorption and diffusion of metals. *Journal of Soil Science.* 39(1): 37–51.

59. Ainsworth, C. C., Pilon, J. L., Gassman, P. L., and Vandersluys, W. G. (1994). Cobalt, cadmium, and lead adsorption to hydrous iron oxide: Residence time effect. *Soil Science Society of America Journal.* 58 (6): 1615–1623.

60. Backes, C. A., McLaren, R. G., Rate, A. W., and Swift, R. S., (1995). Kinetics of cadmium and cobalt desorption from iron and manganese oxides. *Soil Science Society of America Journal.* 59(3): 778–785.

61. McLaren, R. G., Backes, C. A., Rate, A.W., and Swift, R. S. (1998). Cadmium and cobalt desorption kinetics from soil clays: Effect of adsorption period. *Soil Science Society of America Journal.* 62 (2): 332–337.

62. McBride, M. B. (1994). Environmental Chemistry in Soils. New York: *Oxford University Press.*

63. Pardo, M. T (2000). Adsorption of lead, copper, zinc, and cadmium by soils: Effect of nitriloacetic acid on metal retention.*Communications in Soil Science and Plant Analysis.* 31(1): 31–40.

64. Sparks, D. L. (2003). Environmental Soil Chemistry (2nd ed.). *Academic Press*, New York.

65. Spark, K. M., Wells, J. D., and Johnson, B. B. (1995). Characterizing trace metal adsorption on kaolinite.*European Journal of Soil Science.* 46 (4): 633–640.

66. Elliott, H. A., Liberati, M. R., Huang, C. P. (1985). Effect of iron oxide removal on heavy metal adsorption by acid subsoils. *Water, Air,* and *Soil Pollution.* 27 (3–4): 379–389.

67. Gerritse, R. G., and van Driel, W. (1984). The relationship between adsorption of trace metals, organic matter, and pH in temperate soils. *Journal of Environmental Quality.* 13 (2): 197–204.

68. Kabata–Pendias, A. (1993). Behavioural properties of trace metals in soils. *Applied Geochemistry.* 8: 3–9.

69. Hinz, C. (2001). Description of adsorption data with isotherm equations. *GEODERMA.* 99 (3–4): 225–243.

70. Allison, J. D., Brown, D. S., and Novo–Gradac ,K. J. (1990). a geochemical assessment model for environmental systems: version 3.0 user's manual. Athens, Ga: Environmental Research Laboratory, *Office of Research and Development*, U.S. Environmental Protection Agency.

71. Kinniburgh, D. G. (1986). General purpose adsorption isotherms. *Environmental Science and Technology.* 20 (9): 895–904.

72. Giles, C. H., Smith, D., and Huitson, A. (1974). A general treatment and classification of the solute adsorption isotherm. I. Theoretical. *Journal of Colloid and Interface Science.* 47 (3): 755–765.

73. Sheela, T., Nayaka, Y.A., Viswanatha, R., Basavanna, S., and Venkatesha, T.G. (2012). Kinetics and thermodynamics studies on the adsorption of Zn(II), Cd(II) and Hg(II) from aqueous solution using ZnO nanoparticles. *Powder Technology.* 217: 163–170.

74. Ma, L., Xu, R., Jiang, J. (2010). Adsorption and desorption of Cu(II) and Pb(II) in paddy soils cultivated for various years in the subtropical China. *Journal of Environmental Sciences.* 22 (5) 689–695.

75. Abat, M., McLaughlin, M.J., Kirby, J.K., and Stacey, S.P. (2012). Adsorption and desorption of copper and zinc in tropical peat soils of Sarawak, Malaysia. *Geoderma.* 175–176: 58–63.

76. Echeverria, J.C., Morera, M.T., Mazkiaran, C., and Garrido, J.J. (1998). Competitive adsorption of heavy metal by soils. Isotherms and fractional factorial experiments. *Environmental Pollution.* 101 (2): 275–284.

77. Owen, R., and Handy, R., (2007). Formulating the problems for environmental risk assessment of nanoparticles. *Environmental Science and Technology.* 41(16): 5582–5588.

78. Dowling, A. (2005). Is nanotechnology "the next GM"?. *New Scientist.* P.19, ISSN, 2490.

79. Mackay, C.E., Johns, M., Salatas, J.H., Bessinger, B., and Perri, M. (2006). Stochastic probability modelling to predict the environmental stability of nanoparticles in aqueous suspension. *Integrated Environmental Assessment and Management.* 2 (3) : 293–298.

80. Kennedy, A.J., Hull, M.S., Steevens, J.A., Dontsova, K.M., Chappell, M.A., Gunter, J.C., and Weiss, C.A. (2008). Factors influencing the partitioning and toxicity of nanotubes in the aquatic environment. *Environmental Toxicology and Chemistry.* 27 (9): 1932–1941.

81. Science Policy Council (U.S.). (2007). Nanotechnology white paper. *US Environmental Protection Agency Report EPA.* 100/B–07/001, Office of the Science Advisor, Washington, DC, USA.

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

83. Yang, L., and Watts, D.J. (2005). Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicology Letters.* 158: 122–132.

84. Zheng, L., Hong, F., Lu, S., and Liu, C. (2005). Effect of nano–TiO_2 on strength of naturally aged seeds and growth of spinach. *Biological Trace element Research.* 104 (1): 83–91.

85. Lin, D., and Xing, B. (2007). Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environmental Pollution.* 150 (2): 243–250.

86. Tong, Z.H., Bischoff, M., Nies, L., Applegate, B., and Turco, R.F. (2007). Impact of fullerene (C–60) on a soil microbial community. *Environmental Science and Technology.* 41(8): 2985–2991.

87. Johansen, A., Pedersen, A. L., Jensen, K. A., Karlson, U., Hansen, B. M., Scott–Fordsmand, J. J., and Winding. A. (2008). Effects of C60 fullerene nanoparticles on soil bacteria and protozoans. *Environmental Toxicology and Chemistry.* 27 (9): 1895–1903.

88. Nyberg, L., Turco, R.F., and Nies, L. (2008) Assessing the impact of nanomaterials on anaerobic microbial communities. *Environmental Science and Technology.* 42 (6): 1938–1943.

89. Pelletier, D.A., Suresh, A.K., Holton, G.A., McKeown, C.K., Wang, W., Gu, B., Mortensen, N.P., Allison, D. P., Joy, D. C., Allison, M. R., Brown, S. D., Phelps, T. J., and Doktycz, M. J . (2010). Effects of engineered cerium oxide nanoparticles on bacterial growth and viability. *Applied and Environmental Microbiology.* 76 (24): 7981–7989.

90. Dimkpa, C.O., Calder, A., Britt, D.W., McLean, J.E., and Anderson, A.J. (2011). Responses of a soil bacterium, Pseudomonas chlororaphis O6 to commercial metal oxide nanoparticles compared with responses to metal ions. *Environmental Pollution.* 159 (7): 1749–1756.

91. Mortimer, M., Kasemets, K., and Kahru, A. (2010). Toxicity of ZnO and CuO nanoparticles to ciliated protozoa Tetrahymena thermophila. *Toxicology.* 269 (2–3): 182–189.

92. Federici, G., Shaw, B.J., and Handy, R.D. (2007). Toxicity of titanium dioxide nanoparticles to rainbow trout (Oncorhynchus mykiss): Gill injury, oxidative stress, and other physiological effects. *Aquatic Toxicology.* 84 (4): 415–430.

93. Zhao, C. M, and Wang, W. X. (2011). Comparison of acute and chronic toxicity of silver nanoparticles and silver nitrate to Daphnia magna. *Environmental Toxicology and Chemistry.* 30 (4): 885–92.

94. Valant, J., Drobne, D., and Novak, S. (2012). Effect of ingested titanium dioxide nanoparticles on the digestive gland cell membrane of terrestrial isopods. *Chemosphere.* 87 (1): 19–25.

95. Zhang, P., Ma Y., Zhang Z., He X., Ding Y., Guo, Z., Tai, R., Ding, Y., Zhao, Y., Chai, Z. (2012). Comparative toxicity of nanoparticulate/bulk Yb_2O_3 and $YbCl_3$ to cucumber (*Cucumis sativus*). *Environmental Science and Technology.* 46 (3): 1834–1841.

96. Kahru, A., and Dubourguier, H.C. (2010). From ecotoxicology to nanoecotoxicology. *Toxicology.* 269: (2–3): 105–119.

97. Mushtaq, Y.K. (2011). Effect of nanoscale Fe_3O_4, TiO_2 and carbon particles on cucumber seed germination. *Journal of Environmental Science and Health.* 46 (14): 1732–1735.

98. Stampoulis, D., Sinha, S.K., and White, J.C. (2009). Assay–dependent phytotoxicity of nanoparticles to plants. *Environmental Science and Technology.* 43 (24): 9473–9479.

99. Kumari, M., Khan, S.S., Pakrashi, S., Mukherjee, A., and Chandrasekaran, N. (2011). Cytogenetic and genotoxic effects of ZnO nanoparticles on root cells of *Allium cepa*. *Journal of Hazardous Materials.* 190 (1–3): 613–621.

100. Ma Y., Kuang L., He X., Bai W., Ding Y., Zhang Z., Zhao Y., and Chai Z. (2010). Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere.* 78 (3): 273–279. 101. Van Hoecke, K., De Schamphelaere, K. A., Ramirez–Garcia, S., Van der Meeren, P., Smagghe, G., and Janssen, C. R. (2011). Influence of alumina coating on characteristics and effects of SiO_2 nanoparticles in algal growth inhibition assays at various pH and organic matter contents. *Environment International.* 37 (6): 1118–1125.

102. Lee, C.W., Mahendra, S., Zodrow, K., Li, D., Tsai, Y.C., Braam, J ., and Alvarez, P.J. 2010. Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*. *Environmental Toxicology and Chemistry.* 29 (3): 669–675.

103. Khodakovskaya, M., Dervishi E., Mahmood M., Xu Y., Li Z., Watanabe F., and Biris A.S. 2009. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano.* 3 (10): 3221–3227.

104. Su, M., Liu, H., Liu, C., Qu, C., Zheng, L., and Hong, F. (2009). Promotion of nano–anatase TiO_2 on the spectral responses and photochemical activities of D1/D2/Cyt b559 complex of spinach. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 72: (5) : 1112–1116.

105. Larue, C., Khodja, H., Herlin–Boime, N., Brisset, F., Flank, A. M., Fayard, B., Chaillou, S., and Carriere, M. (2011). Investigation of titanium dioxide nanoparticles toxicity and uptake by plants. *Journal of Physics: Conference Series.* 304 (1): 1–12.

106. Rico, C.M., Majumdar, S., Duarte–Gardea, M., Peralta–Videa, J.R., and Gardea–Torresdey, J.L. (2011). Interaction of nanoparticles with edible plants and their possible implications in the food chain. *Journal of Agricultural and Food Chemistry.* 59 (8): 3485–3498.

107. Rodriguez, E., Azevedo, R., Fernandes, P., and Santos, C. (2011). Cr(VI) induces DNA damage, cell cycle arrest and polyploidization: a flow cytometric and comet assay study in *Pisum sativum*. *Chemical Research In Toxicology.* 24 (7):1040–1047.

108. Atha, D. H., Wang, H., Petersen, E. J., Cleveland, D., Holbrook, R. D., Jaruga, P., Dizdaroglu, M., Xing, B., and Nelson, B. C. (2012). Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. *Environmental Science and Technology.* 46 (3): 1819–1827.

109. Barrena, R., Casals, E., Colon, J., Font, X., Sanchez,A., and Puntes, V. (2009). Evaluation of the ecotoxicity of model nanoparticles. *Chemosphere.* 75 (7): 850–857.

110. Chen, R., Ratnikova, T. A., Stone, M. B., Lin, S., Lard, M., Huang, G., Hudson, J. S., and Ke, P. C. (2010). Differential uptake of carbon nanoparticles by plant and mammalian cells. *Small*, 6: 612–617.

111. Lee, W.M., An, Y.J., Yoon, H., Kweon, H.S. (2008). Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum awstivum*): plant uptake for water insoluble nanoparticles. *Environmental Toxicology and Chemistry*. 27 (9): 1915–1921.

112. Kurepa, J., Paunesku, T., Vogt, S., Arora, H., Rabatic, B. M., Lu, J., Wanzer, M. B., Woloschak, G. E., and Smalle, J. A . 2010. Uptake and distribution of ultrasmall anatase TiO_2 alizarin red S nanoconjugates in *arabidopsis thaliana*. *Nano Letters.* 10 (7): 2296–2302.

113. Watanabe, T., Misawa, S., Hiradate, S., Osaki, M. (2008). Root mucilage enhances aluminum accumulation in *Melastoma malabathricum*, an aluminum accumulator. *Plant Signal Behavior* . 3 (8): 603–605.

114. Hall, J. L., and Williams, L. E. (2003). Transition metal transporters in plants. *Journal of Experimental Botany.* 54 (393): 2601–2613.

115. Lopez–Moreno, M.L., De la Rosa, G., Hernandez–Viezcas, J.A., Castillo–Michel, H., Botez, C.E., Peralta–Videa, J.R., and Gardea–Torresdey, J.L. (2010). Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (*Glycine max*) plants. *Environmental Science and Technology.* 44 (19): 7315–7320.

116. Lin D., and Xing, B. (2008). Root uptake and phytotoxicity of ZnO nanoparticles. *Environmental Science and Technology.* 42 (15): 5580–5585.

117. Parsons, J.G., Lopez, M.L., Gonzalez, C.M., Peralta–Videa, J.R., and Gardea–Torresdey, J.L. (2010). Toxicity and biotransformation of uncoated and coated nickel hydroxide nanoparticles on mesquite plants. *Environmental Toxicology and Chemistry.* 29 (5): 1146–1154.

118. Harris, A., and Bali, R. (2008). On the formation and extent of uptake of silver nanoparticles by live plants. *Journal of Nanoparticle Research.* 10 (4): 691–695.

119. Gardea–Torresdey, J. L., Gomez, E., Peralta–Videa, J. R., Parsons, J. G., Troiani, H., and Jose–Yacaman, M. (2003). Alfalfa sprouts: A natural source for the synthesis of silver nanoparticles. *Langmuir: the ACS Journal of Surfaces and Colloids*. 19 (4): 1357–1361.

120. Lin, S., Reppert, J., Hu, Q., Hudson, J.S., Reid, M.L., Ratnikova, T.A., Rao, A.M., Luo, H., and Ke, P.C. (2009). Uptake, translocation, and transmission of carbon nanomaterials in rice plants. *Small nano micro* (*weinheim an der bergstrasse, germany*). 5 (10): 1128–1132.

121. Navarro, E., Baun, A., Behra, R., Hartmann, N. B., Filser, J., Miao, A. J., Quigg, A., Santschi, P. H., and Sigg, L. (2008). Environmental behaviour and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. *Ecotoxicology*. 17 (5): 372–386.

122. Judy, J.D., Unrine, J.M., and Bertsch, P.M. (2011). Evidence for biomagnification of gold nanoparticles within a terrestrial food chain. *Environmental Science and Technology.* 45 (2): 776–781.

123. Keller, A.A., Wang, H., Zhou, D., Lenihan, H.S., Cherr, G., Cardinale, B.J., Miller, R., and Ji, Z. (2010). Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environmental Science and Technology.* 44 (6): 1962–1967.

124. Forster, J.C., and Zech, W., (1993). Phosphorus status of a soil catena under Liberian evergreen rain forest: results of ^{31}P NMR spectroscopy and phosphorus adsorption expirements. *Journal of Plant Nutrition and soil science.* 156 (1): 61–66.

125. Reddy, K. J., Wang, L., and Gloss, S. P. (1995). Solubility and mobility of copper, zinc and lead in acidic environments. *Plant and Soil.* 171 (1): 53–58.

126. Alloway, B. J. (1995). Heavy metals in soils. London: Blackie Academic and Professional.

127. Haghiri, F. (1974). Plant uptake of cadmium as influenced by cation exchange capacity, organic matter, zinc, and soil temperature. *Journal of Environment Quality.* 3 (2).

128. Abdou, M. F., Abdel–Sabour, M. F., Elwan, L. M., and Al–salama, Y. J. (2001). Effect of soil contamination due to irrigation with wastewater on Cs, Co, Cr and Zn retention and release in some soils. *Minufiya journal of Agriculture.* 26 (4): 1127–1141.

129. Camobreco, V.J., Richards, B.K., and Steenhuis, T.S. (1996). Movement of heavy metals through undisturbed and homogenized soil columns. *Soil Science.*161 (11): 740–750.

130. Canas, J. E., Long, M., Nations, S., Vadan, R., Dai, L., Luo, M., Ambikapathi, R., Lee, E. H., and Olszyk, D. (2008). Effects of functionalized and nonfunctionalized single–walled carbon nanotubes on root elongation of select crop species. *Environmental Toxicology and Chemistry.* 27 (9): 1922–1931.

131. El–Temsah, Y. S., and Joner, E. J. (2012). Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. *Environmental Toxicology.* 27 (1): 42–49.

132. Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., Sigg, L., and Behra, R. (2008). Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii. Environmental Science and Technology.* 42 (23): 8959–8964.

133. Lopez–Moreno, M. L., De La Rosa, G., Hernandez–Viezcas, J. A., Peralta–Videa, J. R., and Gardea–Torresdey, J. L. (2010). X–ray absorption spectroscopy (XAS) corroboration of the uptake and storage of CeO_2 nanoparticles and assessment of their differential toxicity in four edible plant species. *Journal of Agricultural and Food Chemistry.* 58 (6): 3689–3693.

134. Ma, X., Geiser–Lee, J., Deng, Y., and Kolmakov, A. (2010). Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. *Science of the Total Environment.* 408 (16): 3053–3061.

135. DOSHI, R. (2008). Nano–aluminum: Transport through sand columns and environmental effects on plants and soil communities. *Environmental Research.* 106 (3): 296–303.

136. Shah, V., and Belozerova, I. (2009). Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. *Water, Air, and Soil Pollution.* 197 (1–4): 143–148.

137. Roberts, P., Roberts J.P., and Jones, D.L. (2006). Behaviour of the endocrine disrupting chemical nonylphenol in soil: Assessing the risk associated with spreading contaminated waste to land. *Soil Biology and Biochemistry.* 38 (7): 1812–1822.

138. Ben Mahmoud, K. (1995). Libyan Soils. *National Authority for Scientific Research.* pp 215.

139. Elyaagubi, F. (2011). Effects of salinity on soils of the Gefara Plain–Libya. *PhD thesis.* pp 4–49.

140. Antonovics, J., Bradshaw, A. D., and Turner, R. G. (1971). Heavy metal tolerance in plants. London: *Academic Press. 7,*1.

141. Wilkins, D. A. (1978). The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist.* 80 (3): 623–633.

142. Prasad, M. (1995). Cadmium toxicity and tolerance in vascular plants. *Environmental and Experimental Botany.* 35 (4): 525–545.

143. Grant, C.A., Buckley, W.T., Bailey, L.D., Selles, F. (1998). Cadmium accumulation in crops. *Journal of Plant science.* 78: 1–17.

144. Fageria, N. K. (1992). Maximizing crop yields. 1st ed.New York: Marcel Dekker.V 23.

145. Graham, R. D. (1978). Nutrient efficiency objectives in cereal breeding. In Proceedings of the 8th international colloquium on plant analysis and fertilizer problems, 165–170. *New Zealand Division of Scientific and Industrial Research*, Information Series no. 134. Wellington, New Zealand: Government Printer.

146. Cooke, G.W. (1987). Maximizing fertilizer efficiency by overcoming constraints. *Journal of Plant Nutrient.* 10, 1357–1369.

147. Ali, N. A., Bernal, M. P., Ater, M. (2004). Tolerance and bioaccumulation of cadmium by Phragmites australis grown in the presence of elevated concentrations of cadmium, copper, and zinc. *Aquatic Botany.* 80 (3): 163–176

148. Ross, S. (1994). Toxic metals in soil–plant systems. *Chichester, West Sussex, England: Wiley.*

149. McKone, T. E., and Maddalena, R. L. (2007). Plant uptake of organic pollutants from soil: bioconcentration estimates based on models and experiments. *Environmental Toxicology and Chemistry.* 26 (12): 2494–504.

150. Prasad, M. N. V. (2004). Heavy Metal Stress in Plants: From Biomolecules to Ecosystems. Second edition. *Springer*, India.

151. Baker, A.J.M., and Walker, P.L., 1989. Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. *Chemical Speciation and Bioavailability.* 1: 7–17.

152. Smolders, E., Merckx, R., Schoovaerts, F. and Vlassak, K. (1991). Continuous shoot growth monitoring in hydroponics. *Physiologia Plantarum*.83 (1): 83–92

153. Derome, J., and Lindroos, A. J. (1998). Effect of heavy–metal contamination on macronutrient availability and acidifation parameters in forest soil in the vicinity of the Harjavalta Cu–Ni smelter, SW Finland. *Environmental Pollution*. 99 (2): 225–232.

154. Warrier, R., and Tripathi, K.K. (2011). Biology of *Zea mays* (Maize). *Ministry of Environment and forests, Government of India*. pp 1–30.

155. Dowswell, C. R., Paliwal, R. L., and Cantrell, R. P. (1996). Maize in the third world. Boulder, Colo: *Westview Press*. USA.

156. Miracle, M. P. (1966). Maize in tropical Africa. Madison, Wis, *University of Wisconsin Press*.

157. Schrimpf, K. (1966). Maize cultivation and fertilization. *Bochum*, Ruhr–Stickstoff AG.

Chapter 3: Experimental methods–General chemical and physical analysis of soil and plant samples

3.1. Introduction

This chapter describes the general chemical and physical experimental methods used to determine the basic characteristics of untreated soil and plant samples as well as those samples exposed to nanoparticles. Eutric Cambisol soil (clay loam) was used to grow maize plants treated with low concentration of CdS NPs, $CdCl_2$, ZnO NPs and $ZnCl_2$. this soil was also treated with high concentrations of CdS, ZnO and CuO NPs to grow maize plants. In addition Eutric Cambisol, Haplic podzol (loamy sandy), Sandy soil, and Libyan sandy soil samples were selected to study the adsorption and desorption % of CdS, ZnO, and CuO NPs at their surfaces. Finally, Eutric Cambisol, Haplic podzol, and Sandy soil were tested for the effects of the CdS, ZnO, and CuO NPs on nitrogen mineralization.

3.2. Materials and methods

3.2.1. Collection of soil samples

Eutric Cambisol, Haplic podzol and Sandy soil were collected from Bangor University's Henfaes Research Centre Abergwyngregyn (531140N 41010W) in North Wales.[1] The Eutric Cambisol, Haplic podzol and sandy soils has been exposed to a costal climate, and soils surface temperature was in the range 8–10°C to a depth of 10 cm.[1] Libyan sandy soil was collected from four farms located in the Gefara Plain, Janzur area (30° 00' NW, 35° 00' NE). The soil samples were taken in June 2010 at a prevailing mean annual temperature in the range 26–29°C.[2] All soil samples (four soils) were collected in humid field conditions.

3.2.2. Preparation of soil samples

Each of the four top–soil samples (Eutric Cambisol, Haplic podzol, Sandy soil, and Libyan sandy soil) was collected at a depth between 0 and 30 cm. A "W" sampling pattern was used .[3] The samples were prepared by air drying, homogenising, and passing each soil through a 2 mm sieve to remove stones and roots. The soil samples were stored at 4°C to avoid any changes to their physical and chemical properties. For the adsorption study, three portions of 0.5 kg of each moist soil were put into three glass bottles. The bottles were covered with aluminium foil and sterilized in autoclave–Certoclav® (CV–EL–12L) for 20 minutes at 100 °C. This procedure was to minimize microbial activity that might affect results.

3.2.3. Determination of water holding capacity (WHC) for soils

Field capacity is the amount of water content or soil moisture that is held on the soil's surface after excess water has drained through a known amount of moist field soil. The volume of water retained by the soil is calculated as its water holding capacity (WHC).[4] All soil samples were collected in humid field conditions. 20 g of each soil sample were placed in filter funnels lined with Whatman® 42 ashless filter paper followed by 100 g deionized water (dH_2O). The WHC of each soil was conducted three times for each soil. Soil samples were allowed to stand overnight. The funnels were covered with aluminium foil to prevent the evaporation of water from the tested soils. The necks of the glass funnels were tapped to remove the excess water into the flasks. The water that had percolated through the soil samples was weighted. The masses of drained water were recorded and the WHC % was calculated using Equation 3.1.

$$\% \, WHC = [(100 - Wp) + Wi] / dwt \times 100\% \qquad \text{Equation 3.1}$$

Where Wp is the weight of the percolated water in grams, Wi is the initial amount of water in grams contained in the samples and dwt is the soil dry weight in grams.[4]

3.2.4. Determination of soil moisture content and organic matter (OM)

For accurate results, measurement of soil moisture content should be followed by an organic matter percentage determination. To measure the soil moisture content, approximately 10 g of each soil sample (W2) was weighed in clean dry porcelain crucibles (W1); three replicates of each soil were performed, and samples were dried overnight in an oven at 105 °C. The samples were subsequently cooled in desiccators for 30 min and reweighed (W3).[5]

For the OM determination (total organic matter), the soil samples (W3) were placed overnight in an oven at 450°C. Subsequently, the samples were allowed to cool in desiccators and reweighed (W4).[5] The moisture content and organic matter as percentages were calculated using Equations 3.2 and 3.3.

$$Soil\ moisture\ (g/g) = [W2 - W3] / [W3 - W1] \times 100 \qquad \text{Equation 3.2}$$

$$Loss\ on\ ignition\ (g/g) = [W3 - W4] / [W3 - W1] \times 100 \qquad \text{Equation 3.3}$$

3.2.5. Soil electrical conductivity (EC) and soil pH

The sample soil solutions were prepared for EC and pH readings as follows: 5 g of each soil sample were placed in 50 mL centrifuge tubes, and 25 mL of deionised water (dH_2O) was added to each of the soil samples (1:5 solid: solution (w/v) ratio). Three replicates of each soil were performed for both measurements. The samples were shaken for 30 min at 250 rpm (SW2 Shaker Table, Edmund Buhler Swip). The EC of the soil solution was measured using a conductivity meter (CDM 210) at 23°C. The conductivity meter had previously been calibrated using a 0.01 M potassium chloride solution. This solution has an electrical conductivity of 1413 μScm^{-1}. The pHs of the soil solution were measured subsequent to their EC determination by using a pH electrode (ANNA, Model 410A) after it had been calibrated.[6]

3.2.6. Cation exchange capacity (CEC)

air dried soil samples (4 g) were placed into 50 mL centrifuge tubes; 33 mL of 1 M sodium acetate solution (CH_3COONa) were then added to each of the soil samples and shaken for 5 min. Triplicate samples were used for each soil. The soil samples were centrifuged at 1000 rcf until the supernatant was clear. The supernatant was discarded. This stage was repeated four times. 33 mL of 95% ethanol (W/V) was added to each soil sample and shaken for 5 min and centrifuged, discarding the supernatant liquid. This procedure was repeated three times. The EC of these solutions was below the 400 μScm^{-1} by the time of the third washing. The adsorbed sodium ions were replaced using 1 M ammonium acetate (NH_4OAc) solution by following the same procedure as that of the sodium acetate solution (above) save that the supernatant solutions were collected in a 100 mL volumetric flask and brought to volume with 1.0 M NH_4OAc solution with mixing. A range of standard solutions (20–200 mg L^{-1}) was prepared from stock solution (1000 mg L^{-1}, NaCl) for the calibration curve. The CEC of the soil samples was determined by flame photometry (Sherwood 410) in milliequivalents per 100 g soil.[6]

The cation exchange capacity (CEC) was calculated using Equation 3.4.

$$CEC\ (meq/100\ g) = meq/L\ Na\ (from\ calibration\ curve) \times A/Wt \times 100/1000 \quad \text{Equation 3.4}$$

Where: A = Total volume of the extract (ml), Wt = weight of the air–dry soil (g).

3.2.7. Determination of soils texture by sedimentation method

Soils were sieved (2 mm) and 40 g samples of each air–dried soil were weighed into a 600 mL beaker. Triplicate samples were used for each soil, 60 mL of a dispersing solution that consisted

of 700 mM sodium hexametaphosphate ($(NaPO_3)_6$) and 100 mM sodium carbonate (Na_2CO_3) was added to each soil sample. The beakers were covered with a watch–glass and left overnight; their contents transferred to soil stirring cups and filled about three quarters full with dH_2O. The suspensions were stirred at high speed for 3 min using a special stirrer and subsequently allowed to stand for one minute before being transferred into a 1 L calibrated cylinder and brought to volume with dH_2O. The same procedure was followed for the blank sample (without soil). The reading of blank (R_b) was taken after directly mixing the dispersing solution. The readings for silt and clay were recorded after mixing the suspensions in the hydrometer jar using a special paddle. The hydrometer was inserted immediately after mixing and the reading for the silt was taken 40 seconds after withdrawing the paddle (R_{sc}). The reading for clay was taken from the suspensions after 4 hours (Rc). After recording clay and silt readings, the soil suppositions were passed through a 50 μm sieve and washed until water passing the sieve was clear. The sand was transferred from sieve to a 50 mL beaker of known weight. The sand in the beaker was allowed to settle and any excess water was decanted. The sand was dried overnight in an oven overnight at 105°C and the mass of the dry sand was recorded in grams. The percentages of silt, clay, and sand were calculated using Equations 3.5–3.8.[6]

$$\%\ [Silt + Clay]\ (w/w) = (R_{sc} - R_b) \times 100/Oven - dry\ soil\ (g) \qquad \text{Equation 3.5}$$

$$\%\ Clay\ (w/w) = (R_c - R_b) \times 100/Oven - dry\ soil\ (g) \qquad \text{Equation 3.6}$$

$$\%\ Silt\ (w/w) = [\%\ Silt + Clay\ (w/w)] - [\%\ Clay\ (w/w)] \qquad \text{Equation 3.7}$$

$$\%\ Sand\ (w/w) = sand\ weight \times 100/Oven–dry\ soil\ (g) \qquad \text{Equation 3.8}$$

An error of +/– 0.4°C was allowed for when measuring temperature differences. The soil was classified using the USDA textural triangle.[6]

3.2.8. Determination of nitrate and ammonium in soil extraction

Soil samples (10 g of each soil) were placed in 50 mL centrifuge tubes. 25 mL dH_2O was then added to each of these soil samples (1:2.5 W/V ratio). Three replicates of each soil were used for ammonium and nitrate ion measurements. The soil samples were shaken for 30 min at 4000 rpm (SW2 Shaker Table, Edmund Buhler Swip). The soil extraction was filtered through Whatman® 42 ashless filter paper. The N method involved the recovery of soil solution by the centrifugal drainage technique using dH_2O. The mixed soil solutions were then centrifuged for 15 minutes

using a Hettich–Zentrifugen centrifuge (Rotanta, 460 R.) at 9050 rcf. The filtered soil solutions were stored frozen at –20°C to await analysis. A microplate reader using 96 well microplates was used to conduct the assay. For the determination of nitrate ion content, 100 µL of the sample was added to each microwell plate followed by 100 µL of 50 mM vanadium chloride, 50 µL of 10 mM N–(1–Naphthyl) ethyenediamine dihydrochloride (NEDD) and 50 µL of 20 mM sulphanilamide were added and mixed several times by micropipette. The mixed solution was allowed to stand for 20 to 30 min until a pink colour developed. A micro–plate spectrophotometer was set at a wavelength of 540 nm to read the absorbance on 96 micro–plates using the Biotek Power Wave XS, including a range of standard solutions (0.5–8 mg L^{-1}) was prepared from stock solution (1000 mg $NO_3^- L^{-1}$) for the calibration curve.

For the determination ammonium ion content, 150 µL of each sample solution were added to the well of the micro–plate, followed by 15 µL of 200 mM Ethelenediaminetetraacetic acid (Na_2EDTA). 60 µL of 500 mM sodium salicylate–5 mM sodium nitroprusside reagent; 30 µL of Buffered hypochlorite reagent (700 mM sodium hydroxide, 400 mM Sodium monohydrogen phosphate heptahydrate and sodium hypochlorite) were added as a final step and the pH was adjusted to 13. At each step the solution was mixed and then allowed to stand for 30 min in an incubator at 37°C. A range of standards (0.5–8 mg L^{-1}) was prepared from stock solution (100 mg $NH_4^+ L^{-1}$). The absorption of the ammonium ions was taken at 667 nm (wavelength) on the Biotek Power Wave XS.[7,8]

3.2.9. Determination of total amino acids concentration in soil

The solutions containing the extracted ammonium and nitrate ions (Section 3.2.8) were used to determine the total amino acid concentration of the soil samples by using a fluorescence spectrophotometer. 20 µL solutions containing the ions were added to a square fluorimeter cuvette and 200 µL of OPA–MET reagent (70 mM α–phthaldialdehyde and β–mercaptoethanol) were added. The excitation and emission wavelengths were set at 340 and 440 nm respectively and readings were taken 1 minute after the addition of the final reagent. 10 µM glycine was used to prepare a set of standards (5–30 mg L^{-1}).[9] Measurements were taken using a fluorescence spectroscopy (Perkin Elmer Corp., Boston, MA).

3.2.10. Determination of total and dissolved carbon and nitrogen in soils

0.1 g of oven–dried soil was used to determine the total organic carbon and nitrogen content of the soils using Elemental determinator carbon/nitrogen (CHN–2000 analyzer, Leco Corp, St

Joseph, MI). The total dissolved carbon and nitrogen was measured using a Shimadzu TOCV–TNM1 analyzer. Soils extraction (10 mL) was added to each vial of Shimadzu' autosampler. Standard solutions of total carbon were prepared from potassium hydrohen phthalate ($C_8H_5KO_4$) as stock solution (1000 mg L^{-1}) and standard solutions of total nitrogen were prepared from potassium nitrate (KNO_3) as stock solution (1000 mg L^{-1}). The calibration curves of total nitrogen and carbon were arranged from 5 to 50 mg L^{-1}. The results were recorded on data sheets.[10]

3.2.11. Digestion of plant samples

Prior to chemical analysis all plant materials were dried, ground, and digested using the following procedure:

Plant roots and shoots were oven dried at 80°C for two days in pre–weighed paper bags. Dry weights were recorded before all materials were ground to less than 500 µm in a stainless steel mill. Approximately 0.1 g of each ground sample were accurately measured and placed in 100 mL borosilicate digestion tubes. 5 mL of analytical grade concentrated nitric acid (HNO_3) were added to each tube and the samples were stood overnight. The tubes were placed in a digestion block at 60°C and covered with marbles and left to stand overnight in a backwashed fume hood. 3 mL of 9.7 M hydrogen peroxide (H_2O_2) were added to each of the plant samples and allowed to digest for 3 hours at 150 °C until the solutions were clear and the volume in the tubes was reduced to approximately 3 mL. The solutions were allowed to cool, then made up to a volume of 20 mL with dH_2O in a 20 mLvolumetric flask and filtered through Whatman® 42 ashless filter paper. Digested samples were shaken manually and refrigerated at 4°C prior to analysis. The same procedure was followed with blank samples (i.e. without plant material). The concentrations of the heavy metals in plant samples and their growing soil were determined by using an Inductively Coupled Plasma (ICP, Optical Emission Spectrometer).[11–13]

3.2.12. Digestion of soil samples

The total elemental concentrations (bulk materials and nanoparticles) in the soil samples were estimated using the same techniques that were applied to the plant samples. The different metals were extracted using Aqua Regia solution (HNO_3 and HCl). 1.0 g of dry soil samples was weighed after sieving (< 2 mm sieve) and homogenized. The soils were placed into 100 mL beakers followed by additions of 10 mL of concentrated nitric acid to each sample. The beakers were covered with watch glasses. The mixtures were heated for an hour using a block digestion.

5 mL of concentrated nitric acid were added to each sample mixture; heating was continued until no brown fumes formed on the walls of the beakers. Finally, 10 mL of concentrated HCl were added to each sample; heating was applied for another 30 min. The mixtures were allowed to cool and were then filtered through Whatman® 42 ashless filter paper. The volumes of the filtrates were made up to 40 ml using dH_2O.[14,15,1] The concentrations of metals in soils (Chapter 6) were measured using an Atomic Absorption Spectrometer (Model Varian–220 FS). The available forms of these elements were extracted as reported by Lindsay and Norvell.[16]

3.3. The study of assessable parameters for maize plants

The parameters and measurements that were obtained from the experimental maize plants (i.e. the dry yield matter of roots and shoots–g/pot) after treating plants with low concentration of CdS NPs, $CdCl_2$, ZnO NPs and $ZnCl_2$, these concentrations ranging from 0–1.25 mg kg^{-1}. The maize plants also treated with high concentrations of CdS, ZnO and CuO NPs, these concentrations ranging from 0–1.0 g kg^{-1}. Maize parameters were calculated using different equations (see Sections, 3.3.1–3.3.4).

3.3.1. Tolerance index (TI)

The tolerance index (TI) for maize plants was calculated at the Cd, Zn and Cu concentrations by dividing the dry weight of the plants exposed to different metal concentrations by that measured during growth in the control media using Equation 3.9.

(TI) = Dry matter yield of treated plant/Dry matter yield of untreated plant Equation 3.9

The formula comes from Wilkins.[17] The same equation was applied by Gaudet *et al.*[18] Abdel-Sabour,[19] and Bradshaw.[20]

3.3.2. Agronomical efficiency (AE)

The AE equation was used to evaluate the impact of nanoparticles on the development of maize plants under laboratory conditions.[21]

AE was calculated using Equation 3.10.[21–23]

$$AE = \frac{Dry\ matter\ yield\ of\ treated\ plant - Dry\ matter\ yield\ of\ untreated\ plant}{Added\ heavy\ metal\ mg/kg}$$

Equation 3.10.

3.3.3. The Bio–concentration ratio (BCR)

The current research, the bi–concentration of Cd, Zn, and Cu for maize plants grown in Eutric Cambisol soil is used to describe the ratio of the concentration measured in the tested plants to the concentration in the pot's soil and nutrient solution providing for that plant species. The bio–concentration ratio (BCR) was calculated by using Equation 3.11.[24–28]

$$(BCR) = \frac{Element\ in\ plant\ (\ \mu g/g\ dry\ weight)}{Element\ in\ soil\ \ \mu g/g\ soil} \qquad \text{Equation 3.11}$$

3.3.4. Relative Increase Percentage (RI)

The RGR is calculated by differential equations. The relative increase percentage (RI) is calculated using Equation 3.12.[29–32]

$$(RI) = \frac{\text{Dry matter yield of treated plant} - \text{Dry matter yield of untreated plant}}{\text{Dry matter of untreated plant}} \times 100$$

Equation 3.12.

3.4. References

1. Roberts, P., Roberts J.P., and Jones, D.L. (2006). Behaviour of the endocrine disrupting chemical nonylphenol in soil: Assessing the risk associated with spreading contaminated waste to land. *Soil Biology and Biochemistry.* 38 (7): 1812–1822.

2. National Atlas Libya, (1977). *Ministry of Planning.*

3. Peters, J. B., Laboki, C. A., and Bundy, L.G. (2007). Sampling soils for testing. The board of Regents of the *University of Wisconsin System.* pp 1–4.

4. Alef, K. (1995). Estimating of soil respiration. In: Methods in soil microbiology and biochemistry. *Academic Press*, New York. pp 464–470.

5. Faithfull, N. T. (2002). Methods in agricultural chemical analysis: a practical handbook. *Oxon*, UK: Cabi Pub.

6. Ryan, J., George, E., and Abdul, R. (2001). Soil and plant analysis laboratory manual. Second edition. Available from *ICARDA*, Aleppo, Syria. pp 1–146.

7. Mulvaney, R.L. (1996). Nitrogen– inorganic forms. In methods of soil analysis. Part 3. *Chemical Methods* (D.L.Sparks Ed, SSSA, Madison, WI, USA). pp 1123–1184

8. Miranda, K., Espey, M., and Wink, D. (2001). A Rapid, simple spectrophotometric method, for simultaneous detection of nitrate and nitrite. *Nitric Oxide: Biology and Chemistry.* 5 (1): 62–71.

9. Jones, D.L., Kielland, K. (2002). Soil amino acid turnover dominates the nitrogen flux in permafrost–dominated taiga forest soils. *Soil Biology and Biochemistry.* 34 (2): 209–219

10. Jones, D. L., Shannon, D, Murphy, D. V., and Farrar, J. (2004). Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. *Soil Biology and Biochemistry.* 36 (5): 749–756.

11. Temminghoff, E. E., and Houba, V.J. (2004). Plant analysis procedures. Dordrecht: *Kluwer Academic Publishers*, Second Edition. pp 1–66.

12. Shar, G. Q., Kazi, G. T., Shah, F. A., Shar, A. H., and Soomro. F. M. (2011). Variable uptake and accumulation of essential and heavy metals in maize (*Zea mays* L.) Grains of Six Maize Varieties. *Australian Journal of Basic and Applied Sciences.* 5 (2): 117–121.

13. University of Wisconsin – Madison. (2005). analysis of major, minor and trace elements in plant tissue samples with ICP–OES and ICP–MS.

14. Crompton, T. R. (2001). Determinations of metals and anions in soils, sediments and sludges. *Spon Press*, UK.

15. Alloway, B. J. (1995). Heavy metals in soils. London: Blackie Academic and Professional.

16. Lindsay, W. L., and Norvell, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal.* 42 (3): 421–428.

17. Wilkins, D. A. (1978). The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist.* 80 (3): 623–633.

18. Gaudet, M., Pietrini, F., Beritognolo, I, Iori, V., Zacchini, M., Massacci, A., Mugnozza G. S., and Sabatti, M. (2011). Intraspecific variation of physiological and molecular response to cadmium stress in Populus nigra L. *Tree Physiology.* 31 (12): 1309–1318.

19. Abdel-Sabour, M. F. (1991). Nickel accumulation parameters, coefficients of transfer, tolerance index, and nutrient uptake by red clover grown on nickel polluted soils. *International Journal of Environmental Studies.* 37 (1–2): 25–34.

20. Bradshaw, A. (1968). Evolution of old mine workings, biological effect of nickel species and their determination in plant and soil. *Plant and Soil.* 107, 285–292.

21. Fageria, N. K. (1992). Maximizing crop yields. 1st ed. New York: Marcel Dekker.V 23.

22. Sisworo, E. L., Eskew, D. L., Sisworo, W. H., Rasjid, H., Kadarusman, H., Solahuddin, S., and Soepardi, G. (1990). Studies on the availability of Azolla N and urea N for rice growth using ^{15}N. *Plant and Soil.* 128 (2): 209–220.

23. Fageria, N. K. (2008). The use of nutrients in crop plants. Hoboken: Taylor and Francis Ltd. (http://public.eblib.com/EBLPublic/PublicView.do?ptiID=360076).

24. McKone, T. E., and Maddalena, R. L. (2007). Plant uptake of organic pollutants from soil: bioconcentration estimates based on models and experiments. *Environmental Toxicology and Chemistry.* 26 (12): 2494–504.

25. Blum, W. H. (1997). Cadmium uptake by higher plants. *In: Proceedings of extended abstracts from the Fourth International Conference on the Biogeochemistry of Trace Elements*, University of California, USA. pp.109–110, Berkeley.

26. Pavan, M., Andrew, P. Worth and Netzeva, Tatiana I. (2006). Review of QSAR models for bioconcentration. *European Chemicals Bureau.* pp 1–125, I–21020 Ispra (VA) Italy.

27. Smical, A. I., Hotea, V., Oros, V., Juhasz, J., and Pop, E. (2008). Studies on transfer and bioaccumulation of heavy metals from soil into lettuce. *Environmental Engineering and Management Journal.* 7 (5): 609–615.

28. Ivanciuc, T., Ivanciuc, O., and Klein, D. (2006). Modeling the bioconcentration factors and bioaccumulation factors of polychlorinated biphenyls with posetic quantitative super–structure/activity relationships (QSSAR). *Molecular Diversity.* 10 (2): 133–145.

29. Prasad, M. N. V. (2004). Heavy Metal stress in plants: From biomolecules to ecosystems. Second edition. *Springer*, India.

30. Tantawy, M. F. (2004). Monitoring of environmental pollution with heavy metals in some delta soils and its phytoremediation. *phD thesis.* pp: 45–46.

31. Pallardy, Stephen G. (2008). *Physiology of woody plants.* Amsterdam: Academic Press.

32. Wang, H., Zhang, S., Zhang, W., Wei, C., and Wang, P. (2010). Effects of nitric oxide on the growth and antioxidant response of submerged plants hydrilla verticillata (L.f.) royle. *African Journal of Biotechnology.* 9 (44): 7470–7476.

Chapter 4: Uptake of Manufactured nanoparticles (MNPs) and partitioning in maize plants

4.1. Introduction

The uptake of MNPs by a few plants has received much attention in recent years, however, it is a very recent area of study and the majority of NPs data corresponded to the germination stage and hydroponic culture.[1–3] Plants are a potential pathway for NPs accumulation and transport food.[1] few studies have demonstrated that NPs toxicity and their effects on seedling growth. For instance, a pumpkin plant (*Cucurbita maxima*) showed that iron oxide NPs (Fe_3O_4) is absorbed in fact by plant tissues through roots system. Iron oxide was accumulated in the roots more than plant leaves.[4] The absorption and accumulation of carbon NPs were also recognized in rice plants (*Oryza sativa)*.[5] These NPs might have accumulated in the shoots having translocated through the roots system. On other hand, C_{70} NPs could be transferred from the leaves to the roots if had been absorbed through shoots of the plant. In another study, the absorption of ZnO NPs by ryegrass (*Lolium perenne*) showed that ZnO NPs accumulated on root surfaces and individual NPs entered the apoplast and symplast spaces of endodermis tissue and steles.[6]

Cu NPs also accumulate in wheat and bean plant biomass, demonstrating a positive relationship between increasing Cu NPs concentration in plant tissues and their growth media.[7] Another research used Ag NPs colloids. Their results suggested that *Arabidopsis thaliana* (thale cress) plants can take up Ag NPs through their roots and translocate them to their leaves. Most NPs (40 nm), however, adhered to the root cap and the Ag NPs accumulated in an unexpected place, namely the columella in the roots. The reason for this is unclear.[1] Harris and Bali reported that, although Ag NPs accumulates in the tissues of both *Brassica juncea* (leaf mustard) and *Medicago sativa* (alfalfa), concentrations in the latter were greater.[8] Whether these observations are true to subterranean roots is unknown. The examination of NPs uptake and their accumulation is still in its initial stage. Many studies are needed; therefore, this study aims to investigate the uptake of nanoparticles and to illustrate their potential concentration into maize roots and shoots grown in soil compared to their soluble bulk materials. The total uptake and partitioning of NPs are important in explaining differences in the plant NPs concentration of maize parts. To investigate these differences a plastic pot trial was undertake so that environmental conditions could be controlled. Although no previous studies have shown

differences between uptake of nanoparticles and their bulk materials into maize roots and shoots grown in soil.

4.2. Objectives

The above examples show that the use of MNPs gives rise to a number of environmental issues and can have a considerable effect on plant growth. Thus, the objectives of this work described in this chapter are as follows:

1. To investigate the impact of different concentrations of ZnO and CdS NPs on the parameters of length and dry biomass of maize roots and shoots after 21 days compared with that of their soluble non–nano counterparts ($ZnCl_2$ and $CdCl_2$).
2. To assess the distribution of ZnO NPs, CdS NPs, $ZnCl_2$ and $CdCl_2$ and their uptake by the roots and shoots of maize plants.
3. To illustrate the effects of nano (ZnO and CdS) and non–nanomaterials ($ZnCl_2$, and $CdCl_2$) on the development of and their availably and transport within maize parts (root and shoot) compared to those of soluble non–nano materials in the Eutric Cambisol soil.
4. To evaluate the effect of these metals on maize dry yield matter and length of maize roots and shoots (g/pot) using the appropriate equations to calculate the roots' and shoots'TI, AE, BCR, RI, and uptake ratio.

4.3. Materials and methods

4.3.1. The characterisation of nanoparticulate powders using X–ray Diffraction (XRD)

XRD is generally used to analyse the crystalline structure of a material and its chemical composition. CdS NPs were synthesised by chemical precipitation method according to the method of Singh and Chauhan.[9] The diffraction patterns of CdS and ZnO was obtained from pure dried powder.[9,10] The powder materials were dried for overnight at 80°C. The patterns of XRD were recorded on a Philips X'Pert diffractometer operating at 40 keV and 30 mA (Cu Kα radiation, $\lambda = 1.542$ Å).

4.3.2. Scanning electron microscopy (SEM) of nanoparticles powders

The powders of CdS and ZnO NPs were scraped gently and sprinkled onto carbon adhesive tape to obtain a very thin layer of powder.[11,12] All samples were prepared as coated and uncoated–gold on an SEM (HITACHI 4700 FE–SEM). The particle size for two NPs was calculated using

image analysis software (Image–J 1.43 for Microscopy, USA). The samples were observed at 20 keV.

4.4. Site description and preliminary preparation of the soil

The Eutric Cambisol soil was collected from Bangor University's Henfaes Research Centre, Abergwyngregyn (531140N 41010W). The site collection and the preparation of the Eutric Cambisol soil was previously described (see Chapter 3, Sections 3.2.1 and 3.2.2). Its total carbon and nitrogen content was determined (see Chapter 3, Section 3.2.10), as was its nitrate and ammonium ion contents (see Chapter 3, Section 3.2.8). The soluble nitrogen was extracted by the centrifugal drainage technique for soil solution. The pH and electrical conductivity of the soil (EC) were measured (see Chapter 3, Section 3.2.5). The cation exchange capacity (CEC) and the particle size distribution of the soil were tested (see Chapter 3, Sections 3.2.6 and 3.2.7). Its moisture and organic matter (OM) contents (%) were determined (see Chapter 3 Section 3.2.4.). The soil's Water Holding Capacity (WHC) was also evaluated (see Chapter 3, Section 3.2.3). Table 4.1 shows the soil composition.

Table 4. 1. Chemical composition and physical characteristics of the Eutric Cambisol soil

Measurement	Content
Texture	Clay loam
Moisture content (%)	28.92±0.03
pH	5.50±0.03
EC (mS cm^{-1})	0.64±0.004
Total carbon (g kg^{-1})	49.0±6.1
Total nitrogen (g kg^{-1})	7.7±0.1
NO_3^- (mg N L^{-1})	6.42±1.19
NH_4^+ (mg N L^{-1})	3.18±0.20
Water holding capacity (%)	70.43±0.06
CEC (mmol kg^{-1})	27.0±3.0
Total Zn concentration (mg kg^{-1})	39.06±2.37
Total Cd concentration (mg kg^{-1})	0.07±0.01

Values represent the means of three determinations (Mean ± SEM, n =3). CEC is cation exchange capacity, EC characterizes the electrical conductivity of the soil.

4.4.1. Chemical treatments

CdS NPs were synthesised at the School of Chemistry, Bangor University, UK. The ZnO NPs were purchased from IoLitec Nanomaterials Company, Germany. The zinc ions (Zn^{2+}) were prepared from $ZnCl_2$; the cadmium ions (Cd^{2+}) were obtained from $CdCl_2$. The soil pots were treated with different concentrations of these metals one week before planting the maize seeds. The particle solutions were suspended in deionised water and dispersed by ultrasonic vibration (100 W, 40 kHz) for 30 minutes.[13] The soil was treated with different concentrations of ZnO NPs and CdS NPs and their bulk counterparts ($ZnCl_2$ and $CdCl_2$). The same concentrations were applied for each metal in the Eutric Cambisol soil. The soil was artificially spiked with different concentrations of the NPs (CdS NPs and ZnO NPs) and their bulk counterparts ($ZnCl_2$ and $CdCl_2$). These concentrations were 0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg (metal) kg^{-1} soil for all the metals.

The soil pots were arranged in a randomised block design. The pots used in this experiment (84 pots) were classified into four main groups (21 pots for each main group). Each heavy metal soil concentration was repeated three times. Plastic pots 7.60 cm wide and 6.70 cm deep were used and each one was filled with 100 g soil. A layer of tissue paper was placed at the bottom of each free draining pot to prevent any loss of soil.[14,15] The parameters of the ZnO were measured at the IoLitec Nanomaterials Company, and the size of the CdS NPs was calculated using XRD and SEM (Sections 4.6 and 4.7). Table 4.2 lists properties of the NPs.

Table 4. 2. Properties of nanoparticles used for the phytotoxity experiment

Compound	Size (nm)	Purity (%)	Surface area (m^2/g)	Particular morphology	Molecular weight (g/mol)
ZnO	90–210	99.9	5–7	irregular	81.39
CdS	~ 7.6– 17.7	–	–	–	144.48

4.4.2. Plant material and growth

The maize seeds (*Zea mays*, L.) were obtained from the Environmen Centre Wales, Bangor University. Healthy and equal–sized seeds were chosen from a particular variety. Seed masses were in the range 0.23–0.25 g (±0.02 g). The seeds were soaked in distilled water for 24 hours with a provision of oxygen to allow for the respiration requirement of these seeds; then they were allowed to germinate in a dark room at 25°C for three days. Four seedlings were cultivated

in each experimental pot filled with 100 g of Eutric Cambisol soil. The seedlings were placed in a growth room (chamber) for 21 days under the following environmental conditions: a temperature of 26.7°C, a relative humidity (RH) of 70–80% and a photoperiod of 16 hours. The plants were watered every day; the first is with nutrient solution, the next with distilled water, the next with nutrient solution, the next with distilled water, and so on. Figures 4.1 and 4.2 show the maize plants 21 days after germination. Figure 4.3 shows the plants in their growing chamber 15 days after germination.

Figure 4. 1. The growth of maize plants after 21 days from germination and the experimental design of the soil pots dosed with CdS NPs (0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg kg^{-1}) (n = 3).

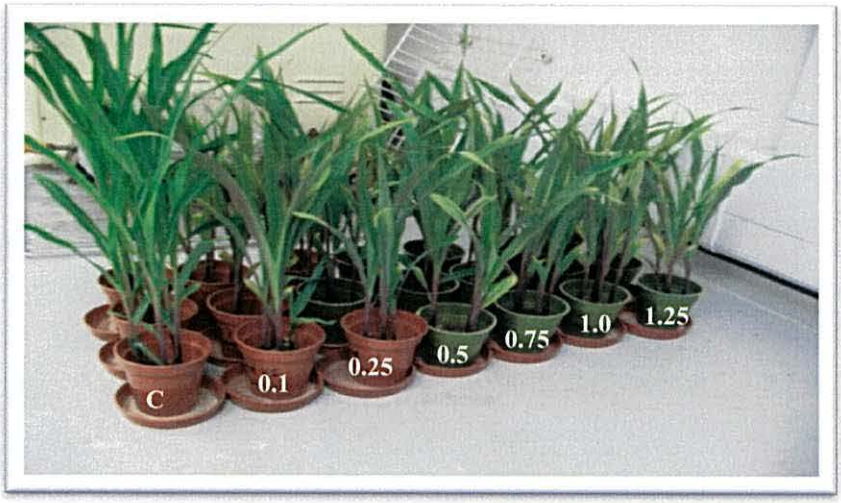

Figure 4. 2. The growth of maize plants after 21 days from seed germination and the experimental design of the soil pots dosed with ZnO NPs (0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg kg^{-1}) (n = 3).

Figure 4. 3. The growth of maize plant after 15 days from seed germination and the experimental design of the soil pots dosed with $CdCl_2$ and $ZnCl_2$ (0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg kg^{-1}) ($n = 3$).

4.4.3. Nutrient solution

Watering of the maize seedlings was started at the time of planting and completed at the end of the growth period (after 21 days). Deionised water was used to rinse the excess nutrient solution from the soil. The nutrient solution was diluted (100% to 25 % strength) from macronutrient solution to give final working concentrations as follows: 1 mM KNO_3, 2 mM $Ca(NO_3)_2.4H_2O$, 0.75 mM $MgSO_4.7H_2O$, 0.67 mM $NaH_2PO_4.2H_2O$, and 0.1 mM Fe EDTA.[15–17] The amount of Zn normally presents in the nutrient solution was removed such that the only Zn present was that added during the experiment to the Eutric Cambisol soil. Thus the effect of Zn on the maize plant was determined at the end of plant growth.

4.4.5. Plant harvest

Maize plants were harvested from their pots 21 days after planting. Each plant was rinsed three times with deionised water to remove any soil particles and metals ions from the surfaces of their roots and shoots. The roots and shoots of each plant were separated and their lengths and fresh weights were individually measured. Then the plant samples were oven dried overnight at 80°C. The dry weights of the roots and shoots were recorded. Each plant part was then ground to a fine powder in an agate mortar and stored at 4°C prior to analysis.

4.5. Plant analysis

The method used to analyse the heavy metals in the maize plants was described in Chapter 3, Section 3.2.11.

4.6. Soil analysis

The method used to analyse the heavy metals in the Eutric Cambisol soil was described in Chapter 3, Section 3.2.12.

4.7. Monitoring the effects of nanoparticles on maize plants using assessable parameters

The Tolerance Index (TI), the Agronomical Efficiency (AE), the Bio–Concentration Ratio (BCR), and the Relative Increase Percentage (RI) were calculated as described in Chapter 3, Sections, 3.3.1–3.3.4.

4.8. Statistical analysis

The concentrations of all test metals and the impact of these metals on maize parameters (dry weight and length of roots and shoots) were performed with three replicates of each concentration in Eutric Cambisol soil. Means and standard errors of each parameter were calculated using Microsoft Excel. All the data that pertained to the absorption of different metal concentrations (CdS, $CdCl_2$, ZnO, and $ZnCl_2$) in maize roots and shoots, the impact of these metals on plant parameters (length and dry weight of maize roots and shoots), the concentration of these metals in test soil and the results of TI, AE, BCR, RI and uptake ratios equations for assessing the effect of heavy metals on maize growth were subjected to a one way analysis of variance (ANOVA) and differences identified with a Tukey (HSD) test using the software package SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). Data normality was tested using Shapiro–Wilk test. Two–way ANOVA was used to test for significant differences between metal type (CdS and $CdCl_2$) and their concentrations (0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg kg^{-1}) for all Cd results as indicated above. Two–way ANOVA also tested the significant differences between metal type (ZnO, and $ZnCl_2$) and concentrations for all Zn results. Two–way ANOVA tested the significant differences between NP type (CdS and ZnO) and their bulk materils ($CdCl_2$ and $ZnCl_2$) as regards their concentrations for all the obtained data. *Post hoc* tests were performed using Tukey's HSD. Significant differences were accepted at the ($p < 0.05$) level all tests. Graphs were constructed using Sigma Plot 12.3 for Windows.[18,19]

4.9. Results and discussion

4.9.1. X–ray Diffraction peaks of cadmium sulphide and zinc oxide NPs

Figures 4.4 and 4.5 show the XRD plots of the diffraction peaks for the CdS and ZnO NPs.

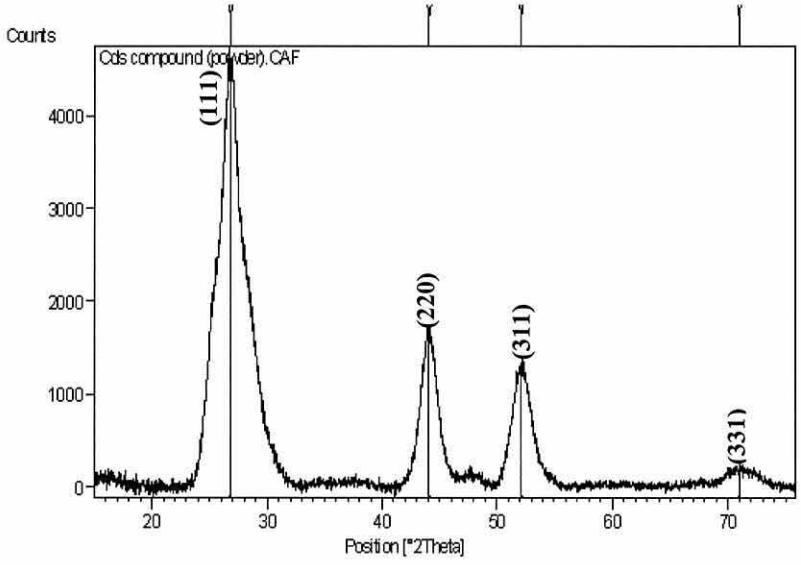

Figure.4. 4. The XRD spectrum of cadmium sulphide NPs (CdS) as a pure compound synthesised by chemical precipitation method.

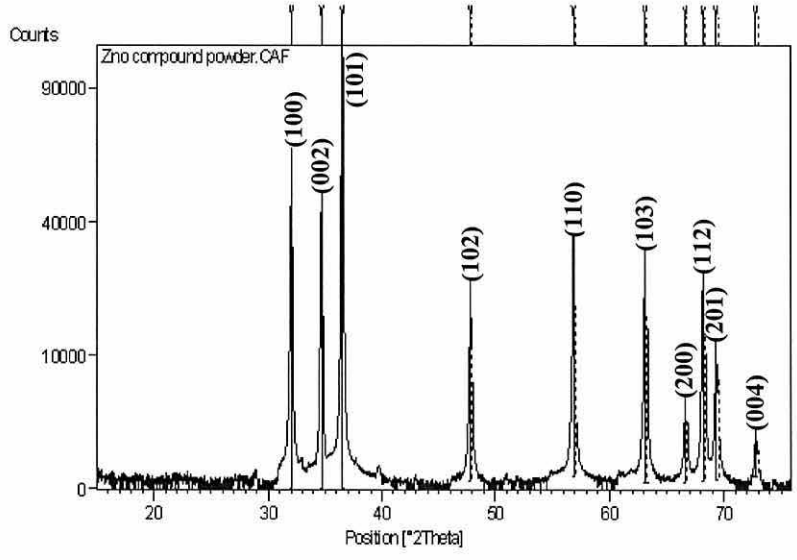

Figure 4. 5. The XRD spectrum of zinc oxide NPs (ZnO) as a pure compound.

The synthesized CdS NPs shows the expected reflections (111), (220), (311) and (331) for this compound (Figure 4.4). This result were consistent with XRD data of CdS NPs.[20,9]

The commericially bought ZnO NPs were also characterised via XRD, for example showing the (100), (002), and (101) reflections (Figure 4.5), which consistent with known XRD data for this compound.[21,22] No other impurities could be detected indicating the high quality of the CdS NPs and ZnO NPs samples.

By using theDebye – Scherrer equation, as shown in Equation 4.2, it is possible to estimate the size of the CdS and ZnO NPs.

$$D = 0.91\lambda / \beta \cos \theta \qquad \text{Equation 4.1}$$

Where D is the crystallite size, λ is the wavelength of the CuKα line (1.54 A°), θ is the angle between the incident beam and the reflection lattice planes, and β is the full width at half maxima (FWHM) of the diffraction peak in radian.[9]

The size of CdS NPs was found to range from ~ 7.6 nm to ~17.7 nm while the size of ZnO NPs ranged from ~ 85.1 nm to ~135.8 nm. However, the Debye Scherrer equation has size limitations that cause errors in size estimations for particles of less than 20 nm. In addition, there may be errors due to particle distribution problems within the sample.

4.9.2. SEM images of NP powders

The external sizes and shapes of different CdS and ZnO NP crystals were studied on an S–4700 scanning electron microscope (SEM). The SEM images of uncoated and coated CdS NP powder showed coagulated shapes or colloidal crystals (see Figure 4.6, A and B).

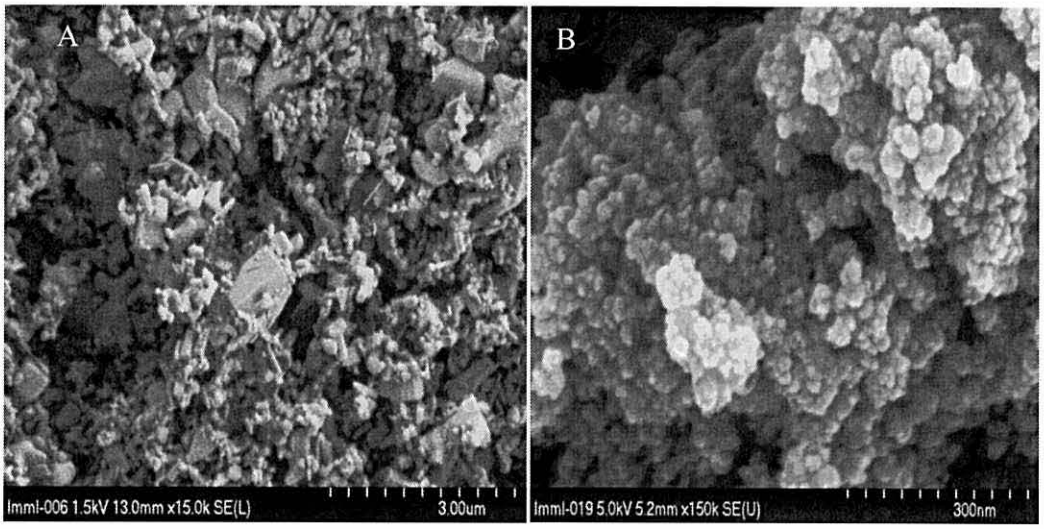

Figure.4. 6. SEM images of CdS NPs synthesized by chemical precipitation methods: (a) uncoated CdS NPs (the scale bar represents 3 μm) and (b) coated CdS NPs (the scale bar represents 300 nm).

The CdS NPs were seen to exist as agglomerates of mean sizes between 10.89 and 82.12 nm and seemed composed of irregularly shaped particles. The average size for coated CdS NPs was 26.20 nm ± 0.12 nm. The surface of the ZnO powder varied widely from rounded, ellipsoidal, and rod–like shapes (see Figure 4.7, A and B), with a size range of 6.14 to 98.27 nm.

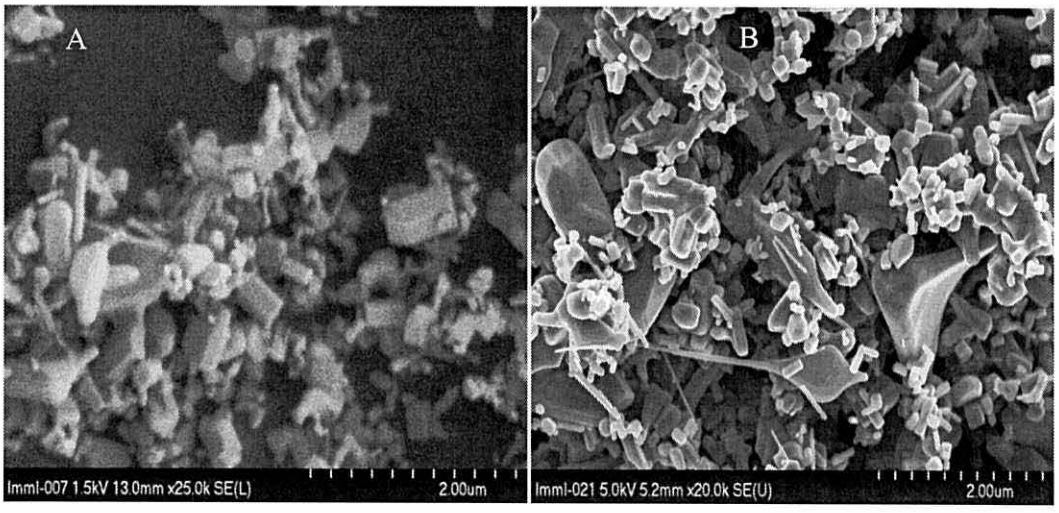

Figure.4. 7. SEM images of ZnO NPs as a powder: (a) uncoated ZnO NPs observed at 1.5 kV and (b) coated ZnO NPs observed at 5.0 kV. (The scale bar represents 2 μm).

The surface of the ZnO NPs had a mean size of 79.90 nm ± 3.07. This classification was performed within a NP range of 1 to 100 nm. The calculations for nanoparicle sizes were in the range of the XRD results (see Section 4.9.1), which agreed with the existing for SEM images.

4.10. Assimilated concentrations of cadmium in maize plants

The concentration of assimilated Cd gradually increased in maize roots and shoots, grown in the Eutric Cambisol soil, with increasing treatment concentrations of either CdS NPs or bulk $CdCl_2$ as shown in figure 4.8. The result for the bulk form of Cd agrees with that of Liu *et al.*[15] and that of Zhang *et al.*[23] in the present study the concentrations of both forms of Cd and their accumulation in roots was relatively similar to each other across the concentration of 0.1 and 0.25 mg kg^{-1}.

Analysis of ANOVA and *Post hoc* tests (Tukey's HSD) indicated significant differences ($p < 0.01$) for the majority of the experimental Cd concentrations for both CdS NPs and $CdCl_2$ compared to the controls across any of the applied concentrations when using a one way ANOVA and a *Post hoc* test (Tukey's HSD) for significance ($p < 0.05$).

Results of Tukey's tests indicated that the concentrations of Cd from each compound were highly significant ($p < 0.01$) in the maize roots at concentrations of 0.5, 0.75, 1.0, and 1.25 mg kg^{-1} compared with controls save the concentration of Cd NPs in maize roots had a significant difference ($p < 0.05$) at the concentration of 0.5 mg kg^{-1} compared with the control. ANOVA and *Post hoc* tests (Tukey's HSD) also showed a significant difference ($p < 0.01$) for concentrations of both Cd compounds (CdS and $CdCl_2$) in maize shoots at concentrations of 0.5, 0.75, 1.0 and 1.25 mg kg^{-1} compared with the control.

Statistical analysis (ANOVA) showed that the concentration of Cd NPs was significantly difference ($p < 0.01$) between roots and shoots as regards the treatment concentrations of CdS NPs. The concentration of Cd NPs was higher in roots (mean overall = 0.24 mg kg^{-1}; SE: ± 0.07); than that of shoots (0.15 mg kg^{-1}; SE: ± 0.05). The results of statistical analysis also show a significant difference ($p < 0.01$) between roots and shoots as regards the bulk Cd concentrations (0–1.25 mg kg^{-1}). The concentration of bulk Cd was higher in roots (mean overall = 0.39 mg kg^{-1}; SE: ± 0.13) than that of shoots (0.28 mg kg^{-1}; SE: ± 0.08).

Significant difference ($p < 0.01$) was observed between the concentrations of bulk Cd and Cd NPs in maize roots. There was significant difference ($p < 0.01$) between the concentration of bulk Cd and Cd NPs in maize shoots. Thus, the uptake of bulk Cd by the roots and shoots was higher than that of Cd NPs; this can be related to the high solubility of $CdCl_2$ in soil solution compared with that of CdS NPs.[24]

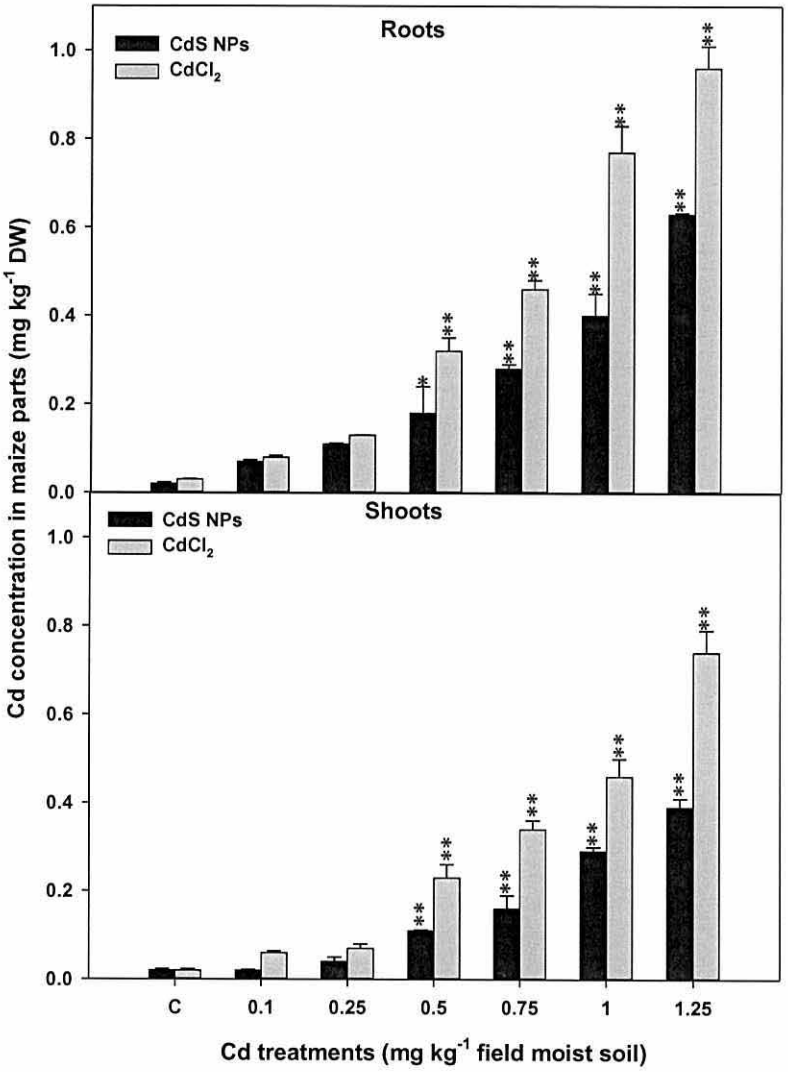

Figure 4. 8. Mean concentrations of Cd in maize roots and shoots grown in Eutric Cambisol soil. The soil was treated with 0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg Cd kg^{-1} soil from bulk ($CdCl_2$) or nanomaterial (CdS). C represents control samples. The values are given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Some of the error bars are too small to be visible.

The low concentration of Cd found in maize roots and shoots could be related to their low initial concentrations in the soil, and it is possible that aggregation and the presence of NOM strongly influenced their bioavailability within the Eutric Cambisol soil.[25] Results suggest that a high

concentration of both Cd compounds (CdS and $CdCl_2$) accumulate in the maize roots and that lower concentrations accumulate in the maize shoots.

Results also suggest that, total concentration of Cd NPs was 46% and 24% in the roots and shoots respectively in plants grown in soils inoculated with CdS NPs. Respective figures for bulk Cd (bulk $CdCl_2$) were 68% for roots and 40% for shoots respectively. Results of bulk Cd agree with those of Wang *et al* .[26] The finding that accumulation of Cd increases in the roots and shoots of maize plants with advancing age is related to their growth periods, as discussed by Perriguey *et al.*[14] In general, Cd accumulates more in the roots than in the shoots of maize plants accords with the results of Liu *et al.*[15]

4.10.1. Effect of cadmium from bulk and nanoparticulate sources on the growth characteristics of maize plants

Figures 4.9 and 4.10 show the impact of both Cd compounds on maize parameters. Statistical analysis observed that no observable negative impacts of Cd NPs and bulk Cd on the dry weight of maize roots and shoots compared with controls across all concentrations (0.1–1.25 mg kg^{1}) and compound types, (CdS and $CdCl_2$) as show in figure 4.10. Tukey's (HSD) test, however, $CdCl_2$ showed a significant effect ($p < 0.05$) on the length of maize root and shoots at a concentration of 1.25 mg kg^{-1} compared with the control (Figure 4.9). Furthermore, CdS NPs had a negative effect ($p < 0.05$) on the length of maize roots at concentrations of 1.0 and 1.25 mg kg^{-1} compared with control.

Statistical analysis showed that no significant difference between dry biomass of roots and shoots as regards the concentration of Cd NPs. The mean overall dry biomass of roots was (0.23 g/ pot; SE: ± 0.01); that of shoots was 0.23 g/ pot (SE: ± 0.01). The results of statistical analysis indicated no significant difference between dry biomass of roots and shoots as regards the concentration of bulk Cd. The mean overall dry biomass of roots was (0.25 g/ pot; SE: ± 0.02); that of shoots was 0.33 g/ pot (SE: ± 0.03). Statistical analysis also showed no significant difference between dry biomass of roots as regards the concentrations of Cd NPs and bulk Cd. The results of statistical analysis observed no significant difference between dry biomass of shoots as regards the concentration of Cd NPs and bulk Cd.

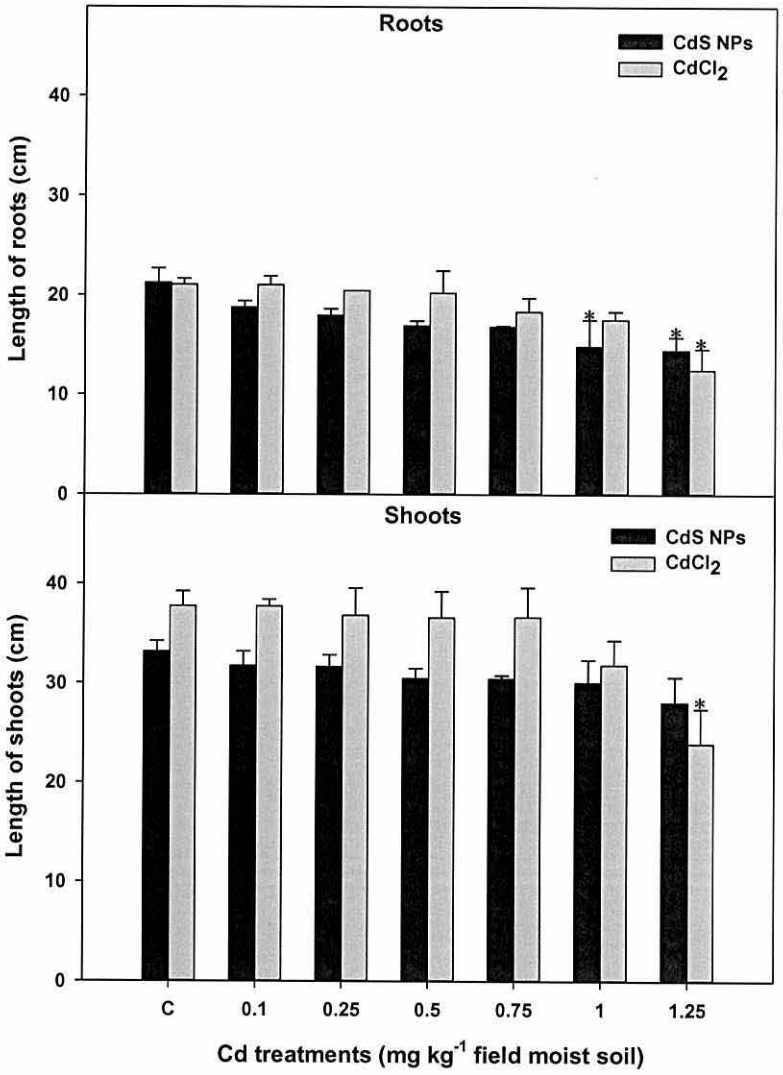

Figure 4. 9. The effects of Cd concentrations on the length of roots and shoots (cm) grown in Eutric Cambisol soil. The soil was treated with 0, 0.1, 0.25, 0.5, 0.75, 1.0 and 1.25 mg Cd kg^{-1} soil from bulk (CdCl2) or nanomaterial (CdS). C represents control samples. The values are given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Some of the error bars are too small to be visible.

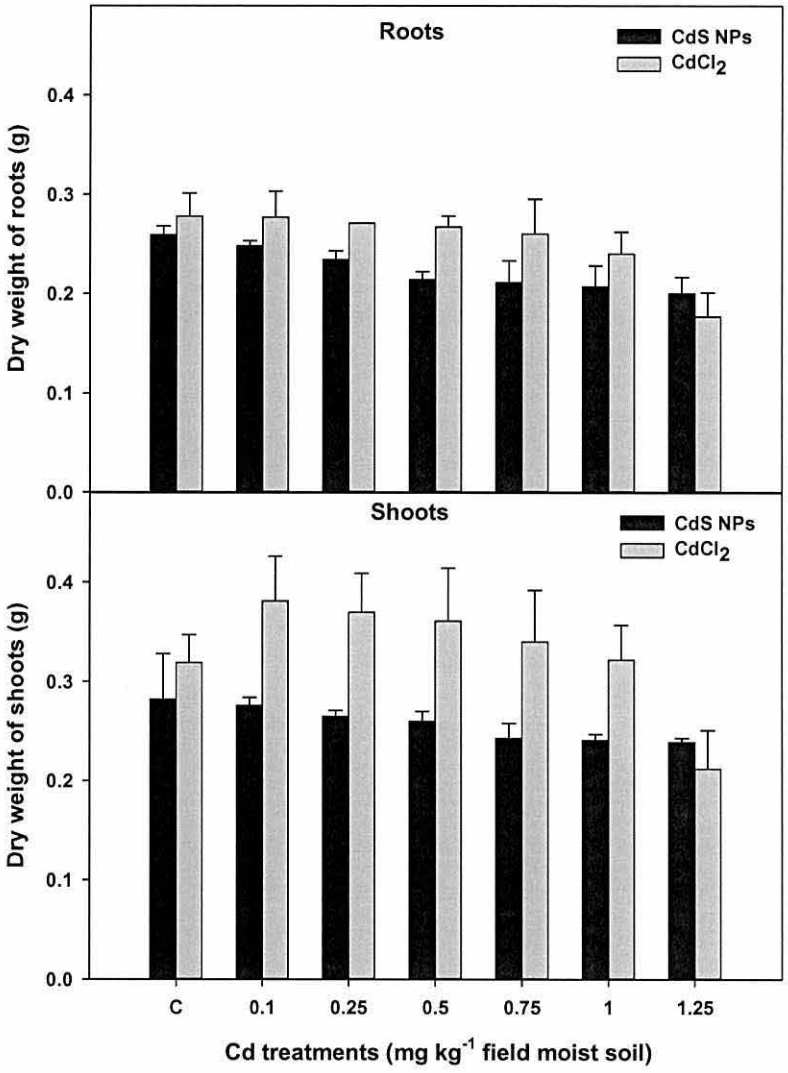

Figure 4. 10. The effects of Cd concentrations on the dry biomass of roots and shoots (g/pot) grown in Eutric Cambisol soil. The soil was treated with 0, 0.1, 0.25, 0.5, 0.75, 1.0 and 1.25 mg of Cd kg^{-1} soil from bulk (CdCl2) or nanomaterial (CdS). C represents control samples. The values are given as mean ±SEM of triplicate samples. Some of the error bars are too small to be visible.

There was no significant difference between length of roots and shoots as regards the concentration of Cd NPs. The mean overall length of roots was (17.30 cm; SE: ± 1.09); that of shoots was 30.79 cm (SE: ± 1.03). The results of ANOVA revealed no significant difference ($p < 0.01$) between length of roots and shoots as regards the concentration of bulk Cd. The mean overall length of roots was 18.74 cm (SE: ± 1.42); that of shoots was 34.47 cm (SE: ± 2.28).

Statistical analysis showed no significant difference between length of roots as regards the concentration of Cd NPs and bulk Cd. There was no significant difference between length of shoots as regards the concentration of Cd NPs and bulk Cd. It has been suggested that low levels of heavy metals together with the soil's organic matter and pH can control their availability and uptake by plants species.[27–29]

Figures 4.9 and 4.10 show that both compounds of Cd gradually decreased the parameters measured for maize with increasing Cd concentrations from each source (CdS and $CdCl_2$) in the Eutric Cambisol soil compared with their control samples. Cd NPs had negative effects on the length of maize roots more than those found at bulk Cd across the majority of Cd concentrations. This can be related to the small size (6–10 nm) of CdS NPs, this could increase CdS NPs reactive phase than that of their bulk counterparts.[25]

Research suggests other NPs have toxic effects on plants. Yang and Watts have suggested that alumina NPs have toxic effects on root longer in five plant species, including maize, when the plants are exposed to high concentrations of the NPs.[30] Similarly, Stampoulis *et al.*[31] have demonstrated significant negative effects of Ag, Cu, ZnO, Si, and MWCNTs NPs on the root elongation and dry biomass of *Cucurbita pepo* (courgette) growing in suspension solutions up to 1000 mg L^{-1}. In this study, bulk Cd decreased the length of roots and shoots at its highest concentration (1.25 mg kg^{-1}). The negative impact of bulk Cd on the dry biomass of maize plants has been discussed in numerous studies at high concentrations (20–60 mg kg^{-1}).[32,33] Similar results as to the effect of bulk Cd on length of maize roots and shoots were obtained by Tantawy,[34] and by El–Kassas *et al.*[35] However, in contrast to results of the present study, these authors found relatively large negative effects of bulk Cd on length of maize roots.

4.10.2. The Bio–concentration ratio of cadmium in different parts of maize plants

The Bio–concentration ratio (BCR) is compares the capacities of different plant parts to absorb metals, their translocation from roots to shoots, and their bio–accumulation. The BCR of Cd from each compound (CdS NPs and $CdCl_2$) in the roots and shoots of maize plants was calculated using the following Equation 4.2.

$$BCR = \text{Element in plant } (\mu g/g \text{ dry weight}) / \text{Element in soil } \mu g/g \text{ soil} \qquad \text{Equation 4.2.}$$

Table 4. 3. Concentrations of Cd and its Bio–concentrations Ratios (BCR) in maize plants (roots and shoots). Plants were cultivated in Eutric Cambisol soil irrigated by different concentrations of CdS NPs and bulk $CdCl_2$ (mg kg^{-1}).

Materials	parameters	Concentrations of Cd used in the soil pots (mg kg^{-1})											
		0.1		0.25		0.5		0.75		1.0		1.25	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
CdS–NPs	Conc. (mg kg^{-1})	0.07 (±0.004)	0.02 (±0.002)	0.11 (±0.002)	0.04 (±0.01)	0.18 (±0.06)	0.11 (±0.002)	0.28 (±0.01)	0.16 (±0.03)	0.40 (±0.05)	0.29 (±0.01)	0.63 (±0.003)	0.39 (±0.02)
	BCR	0.67^{a} (±0.04)	0.23^{a} (±0.05)	0.44^{ab} (±0.01)	0.16^{ab} (±0.03)	0.36^{b} (±0.12)	0.21^{ab} (±0.01)	0.37^{b} (±0.01)	0.22^{ab} (±0.04)	0.40^{b} (±0.05)	0.29^{ab} (±0.01)	0.50^{ab} (±0.002)	0.31^{ab} (±0.01)
$CdCl_2$–Bulk	Conc. (mg kg^{-1})	0.08 (±0.004)	0.06 (±0.004)	0.13 (±0.001)	0.07 (±0.01)	0.32 (±0.03)	0.23 (±0.03)	0.46 (±0.02)	0.34 (±0.02)	0.77 (±0.06)	0.46 (±0.04)	0.96 (±0.05)	0.74 (±0.05)
	BCR	0.85^{a} (±0.04)	0.59^{a} (±0.04)	0.54^{b} (±0.03)	0.29^{b} (±0.02)	0.65^{ab} (±0.06)	0.46^{ab} (±0.06)	0.62^{b} (±0.03)	0.46^{ab} (±0.03)	0.77^{ab} (±0.06)	0.46^{ab} (±0.04)	0.77^{ab} (±0.04)	0.59^{ab} (±0.04)

The Bio–concentration Ratios (BCR) for maize roots and shoots grown in different concentrations of Cd. Conc represents concentration. The values were represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $p \leq 0.05$ level.

Table 4.3 shows that the BCR of Cd in the maize roots and shoots varies according to concentration of Cd in the soil. The mean BCR of bulk Cd in maize roots was 0.70± 0.05; that of shoots was (0.48 ± 0.04). The mean overall BCR of Cd NPs in maize roots was (0.46 ± 0.05); that of shoots was (0.24 ± 0.02). Thus, bulk Cd accumulated in the maize roots and shoots a slightly more than the nanoparticle compound. This is because the solubility of $CdCl_2$ is higher in soil solution compared to CdS NPs.[36]

Table 4.3 also shows that the majority of BCRs for different maize roots and shoots decreased with the increasing concentration of both Cd compounds in the Eutric Cambisol soil compared with the first BCR value at 0.1 mg kg^{-1}. Higher values of BCRs were obtained from roots compared with shoots for all concentrations of Cd compounds. BCRs for bulk Cd were higher than for its nanoparticles for both roots and shoots. The values of the BCRs for the roots and shoots were slightly different compared with each other for all Cd concentrations. This appears to be due to the low levels of added Cd in the tested soil.

The decrease in BCR values with increasing plant age and added concentrations of Cd indicate a decrease in efficiency for each added unit of Cd and its concentration in maize plants. Similar results for bulk Cd were obtained by Tantawy;[34] the results of Tantawy's and the present study also in agreement with those of Chitra *et al.*[37] who reported increasing Cd levels in the soil increases the uptake of the element in the roots and shoots of corn, wheat, and tobacco plants. Similar observations for Cd have been reported in a number of studies including those by: Kacalkova *et al.*[38] in selected plants, including maize (*Z. mays*); Dunbar[39], in two cultivars of potato (*Solanum tuberosum*); Wei *et al.*[40] in French marigold (*Tagetes patula*), and bizzie lizzie (*Impatiens walleriana*); Wangstrand *et al.*[41] in winter wheat (*Triticum aestivum*); Pehlivan *et al.*[39 42] in sugar beet (*Beta vulgaris*); Rascio *et al.*[43] in rice (*Oryza sativa*) and Sun *et al.*[44] in black nightshade (*Solanum nigrum*).

In the case of NPs accumulation in plants grown on soil, no comparable studies have been conducted yet. However, the CB NMs fullerene C_{70} and fullerols are readily accumulated in plants;[45] the majority of MB NPs also appear to be assimilated and to accumulate in plants, although some conflicting data have been reported.[46]

Statistical analysis shows that the BCR of CdS NPs in the maize roots was significantly different ($p < 0.05$) at concentrations of 0.5, 0.75 and 1.0 mg kg^{-1} compared with 0.1 mg kg^{-1}

concentration, but no significant differences was observed in maize shoots for any of Cd concentrations. The BCR for bulk Cd in maize roots was significantly different ($p < 0.05$) at a concentration of 0.75 mg kg^{-1} compared with that of 0.1 mg kg^{-1} level, and there was a significant difference ($p < 0.01$) for maize shoots at the 0.25 mg kg^{-1} level compared with the lower concentration.

Statistical analysis observed a significant difference ($p = 0.05$) between the BCR of roots and shoots as regards the concentration of Cd NPs. The mean overall BCR of roots was 0.46 (SE: ± 0.05); that of shoots was 0.24 (SE: ± 0.02). The results of statistical analysis showed no significant difference between BCR of roots and shoots as regards the concentration of bulk Cd. The mean BCR of roots was 0.70 (SE: ± 0.05); that of shoots was 0.48 (SE: ± 0.04). There was no significant difference between BCR of roots as regards the concentration of Cd NPs and bulk Cd. The results of statistical analysis indicated a significant difference ($p < 0.05$) between BCR of shoots as regards the concentration of Cd NPs that originated from CdS NPs and bulk $CdCl_2$.

In general, the BCR of bulk Cd in maize roots and shoots was higher than that of Cd NPs. This suggests maize plants absorb more bulk Cd than its nanoparticles. The trend in BCRs for Cd, however, was unclear. It varied according to the Cd content of the plant and its availability in the soil. Similar results as regards bulk Cd have been reported by El–Sokkary and Sharaf.[47]

4.10.3. The parameters used to evaluate the effect of cadmium on plant growth

The maize parameters Tolerance Index (TI), Relative Increase (RI %), and Agronomical Efficiency (AE) were used to evaluate the effects of CdS NPs and bulk $CdCl_2$ on the growth of the maize plants and the limitations of the toxic level. Table 4.4 (Part 1 and part 2) shows the DMY, TI, RI, and AE of the maize plants.

Analysis of ANOVA and *Post hoc* test (HSD) showed no significant differences for either Cd compounds on the calculated parameters (TI, RI % and AE) compared with the lowest concentration (0.1 mg kg^{-1}) across any of the applied concentrations or maize parts (roots and shoots). The majority of the calculation parameters decreased with increasing Cd concentrations in the Eutric Cambisol soil.

Table 4. 4. Dry Matter Yield (DMY), Tolerance Index (TI), Relative Increase (RI) and Agronomical Efficiency (AE) of the maize plants as affected by the addition of CdS NPs and bulk $CdCl_2$ (mg kg^{-1}) in Eutric Cambisol soil.

Materials	Plant part	Added concentrations of Cd (mg kg^{-1} soil)												
		Control	0.1				0.25				0.5			
		DMY g/pot	DMY g/pot	TI	RI %	AE	DMY g/pot	TI	RI %	AE	DMY g/pot	TI	RI %	AE
CdS–NPs	Roots	0.26 (±0.01)	0.25 (±0.01)	0.96^{a} (±0.02)	-4.22^{a} (±1.88)	-0.11^{a} (±0.05)	0.23 (±0.01)	0.91^{ab} (±0.06)	-9.21^{ab} (±1.41)	-0.10^{ab} (±0.07)	0.21 (±0.01)	0.83^{ab} (±0.02)	-17.25^{ab} (±1.76)	-0.09^{ab} (±0.01)
	Shoots	0.28 (±0.05)	0.28 (±0.01)	1.04^{a} (±0.18)	3.77^{a} (±0.15)	-0.06^{a} (±0.01)	0.27 (±0.01)	0.99^{ab} (±0.16)	-1.02^{ab} (±0.72)	-0.07^{ab} (±0.01)	0.26 (±0.01)	0.98^{ab} (±0.17)	-2.12^{ab} (±0.05)	-0.04^{ab} (±0.01)
$CdCl_2$– Bulk	Roots	0.28 (±0.02)	0.28 (±0.03)	1.00^{a} (±0.09)	0.26^{a} (±0.18)	-0.01^{a} (±0.01)	0.27 (±0.05)	0.96^{ab} (±0.13)	-4.25^{ab} (±1.09)	-0.03^{ab} (±0.01)	0.27 (±0.01)	0.98^{ab} (±0.10)	-2.28^{ab} (±0.59)	-0.02^{ab} (±0.01)
	Shoots	0.32 (±0.03)	0.38 (±0.05)	1.24^{a} (±0.27)	24.30^{a} (±2.99)	0.62^{a} (±0.03)	0.37 (±0.04)	1.16^{ab} (±0.09)	16.40^{ab} (±2.88)	0.20^{ab} (±0.02)	0.36 (±0.05)	1.17^{ab} (±0.25)	17.05^{ab} (±2.26)	0.08^{ab} (±0.01)

Table Part (2) continues next page

Table 4.4 continuous

Materials	Plant part	Added concentrations of Cd (mg kg^{-1} soil)												
		Control	0.75				1.0				1.25			
		DMY g/pot	DMY g/pot	TI	RI %	AE	DMY g/pot	TI	RI %	AE	DMY g/pot	TI	RI %	AE
CdS–NPs	Roots	0.26 (±0.01)	0.21 (±0.02)	0.81^{ab} (±0.08)	-18.61^{ab} (±0.38)	-0.06^{ab} (±0.01)	0.21 (±0.02)	0.81^{ab} (±0.11)	-19.44^{ab} (±1.37)	-0.05^{ab} (±0.01)	0.20 (±0.02)	0.77^{ab} (±0.04)	-23.19^{ab} (±3.81)	-0.05^{ab} (±0.01)
	Shoots	0.28 (±0.05)	0.24 (±0.01)	0.92^{ab} (±0.18)	-8.33^{ab} (±0.17)	-0.05^{ab} (±0.02)	0.24 (±0.01)	0.90^{ab} (±0.16)	-9.57^{ab} (±0.55)	-0.04^{ab} (±0.02)	0.24 (±0.004)	0.90^{ab} (±0.14)	-10.40^{ab} (±0.49)	-0.03^{ab} (±0.01)
$CdCl_2$– Bulk	Roots	0.28 (±0.02)	0.26 (±0.04)	0.97^{ab} (±0.22)	-2.67^{ab} (±0.68)	-0.02^{ab} (±0.01)	0.24 (±0.02)	0.88^{ab} (±0.12)	-11.85^{ab} (±2.12)	-0.04^{ab} (±0.01)	0.18 (±0.02)	0.66^{ab} (±0.14)	-33.83^{ab} (±0.88)	-0.08^{ab} (±0.01)
	Shoots	0.32 (±0.03)	0.34 (±0.05)	1.06^{ab} (±0.11)	6.12^{ab} (±1.02)	0.03^{ab} (±0.01)	0.32 (±0.04)	1.03^{ab} (±0.17)	3.12^{ab} (±0.65)	0.003^{ab} (±0.001)	0.21 (±0.04)	0.65^{ab} (±0.07)	-34.78^{ab} (±1.36)	-0.09^{ab} (±0.01)

The parameters of maize roots and shoots grown in different concentrations of Cd. The values were represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ levels.

Statistical analysis indicated no significant difference between TI of roots and shoots as regards the concentration of Cd NPs. The mean overall TI of roots was 0.85 (SE: ± 0.04); that of shoots was 0.95 (SE: ± 0.01). There was no significant difference between TI of roots and shoots as regards the concentration of bulk Cd. The mean overall TI of roots was 0.91 (SE: ± 0.09); that of shoots was 1.05 (SE: ± 0.13). There was no significant difference between TI of roots as regards the concentration of Cd NPs and bulk Cd. The results of statistical analysis observed no significant difference between TI of shoots as regards the concentration of Cd NPs and bulk Cd.

The results of statistical analysis showed no significant difference between the RI (%) of roots and shoots as regards the concentration of Cd NPs. There was no significant difference between RI (%) of roots and shoots as regards the concentration of bulk Cd. Results indicated no significant difference between RI (%) of roots as regards the concentration of Cd NPs and bulk Cd. The results of statistical analysis observed no significant difference between RI (%) of shoots as regards the concentration of Cd NPs and bulk Cd. The ANOVA revealed no significant differences between AE of roots and shoots cross all Cd compounds and maize part.

The maize's tolerance of both Cd compounds was determined using the Tolerance index (TI) Equation (see Chapter 3, Section 3.3.1) for the values recorded in Table 4.4. The TI of maize roots and shoots (dry biomass) decreased with increasing treatment concentrations of both Cd compounds in the Euric Cambisol soil for any plant part and any Cd concentration. These results agree with the findings of Bauddh and Singh who tested the Cd TI of five cultivars of mustard (*Brassica juncia L*).[48] They also agree with those of Chen *et al.*[49] who tested pakchoi (*Brassica campestris*) and mustard (*Brassica juncea*), with those of Symeonidis *et al.*[50] who tested three selected plants, and with those of Belimov *et al.*[51] who tested garden peas (*Pisum sativum*).

The TIs of maize roots and shoots for Cd NPs in the present study ranged from 0.77 to 0.96 and 1.04 to 0.90 respectively; the TIs of bulk Cd in maize roots and shoots ranged from 0.66 to 1.0 and 1.24 to 0.65. The TI of maize shoots was higher than that of roots at any Cd concentrations regardless of both Cd compounds compared with 0.1 mg kg^{-1}. The tolerance index of maize roots and shoots for bulk Cd was higher than that of Cd NPs. However, the values of TIs decreased with an increase in Cd levels for the tested soil; this indicated that a high level of Cd can influence the tolerance of maize parts. This agrees with results of previous reseach.[52–54]

The majority of RIs (%) for dry biomass of roots and shoots were negative, RIs (%) decreased for any Cd concentrations regardless of its two compounds. The RI (%) of maize shoots, however, decreased positively at concentrations of 0.25, 0.5, 0.75 and 1.0 mg kg^{-1} compared with 0.1 mg kg^{-1} for bulk Cd. The RI (%) of maize roots and shoots for bulk Cd was higher than those for NPs at any Cd concentration. The AE of maize plant varied according to plant's parts, its growth period, and added levels of the metal. This agrees with results of previous research.[34,55,56]

The majority of AE measurements produced negative values; however, bulk Cd showed that the AE of maize shoots decreased positively with concentrations of 0.25, 0.5, 0.75 and 1.0 mg kg^{-1} compared with 0.1 mg kg^{-1}. The AE values of maize shoots were higher compared with those found for their roots regardless of cadmium's compounds and concentration. This comparison included positive and negative values of AE. A similar finding was obtained for bulk Cd by Tantawy in sorghum (*Sorghum bicolour*), sesame (*Sesamum indicum*), and French beans (*Phaseolus vulgaris*) grown in a sandy soil.[34] The negative values of AE parameters appeared to be because the dry biomass of the treated plants were lower than those of the untreated ones– hence the many negative RI and AE values.

4.10.4. The uptake of cadmium in maize plants

Factors that affect the quantity of heavy metals (bulk materials) absorbed by a plant include their concentration and speciation in the growth media, their movement from the soil's profile to the root's surface, their transportation from the root's surface into the root, and their translocation from the root to the shoot.[57] The uptake of NPs by plants is a very recent field of study. However, the plant uptake may be attributed to factors including specific NP surface area and NP size, chemical composition, NPs concentration and their solubility, NPs physiochemical properties, plant species and age, growth media, and the dilution agent.[58] In the case of NPs uptake by plants the CB (TiO_2, CeO_2, $Fe3O_4$ and ZnO NPs), NMs fullerene C_{70} and fullerols are readily accumulated in plants;[45] the majority of MB NPs also appear to be assimilated and to accumulate in plants, although some conflicting data have been reported.[46] Table 4.5 shows the concentrations of Cd from the two compounds and their uptake by maize roots and shoots (mg kg^{-1}) in Eutric Cambisol soil.

Table 4. 5. The concentrations of Cd and its uptake (μg kg^{-1}) by maize plants (roots and shoots) as affected by the addition of CdS NPs and bulk $CdCl_2$ in Eutric Cambisol soil.

Materials	Plant part	Added concentrations of Cd (mg kg^{-1} soil)													
		control		0.1		0.25		0.5		0.75		1.0		1.25	
		Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot
CdS–NPS	Roots	0.02 (±0.003)	0.01^{a} (±0.001)	0.07 (±0.004)	0.02^{a} (±0.001)	0.11 (±0.002)	0.03^{a} (±0.001)	0.18 (±0.06)	0.04^{a} (±0.01)	0.28 (±0.01)	0.06^{b} (±0.01)	0.40 (±0.05)	0.08^{b} (±0.02)	0.63 (±0.003)	0.13^{b} (±0.01)
	Shoots	0.02 (±0.003)	0.005^{a} (±0.001)	0.02 (±0.002)	0.006^{a} (±0.002)	0.04 (±0.01)	0.01^{a} (±0.002)	0.11 (±0.002)	0.03^{b} (±0.001)	0.16 (±0.03)	0.04^{b} (±0.01)	0.29 (±0.01)	0.07^{b} (±0.002)	0.39 (±0.02)	0.09^{b} (±0.01)
$CdCl_2$_Bulk	Roots	0.04 (±0.001)	0.01^{a} (±0.001)	0.08 ±0.004	0.02^{a} (±0.003)	0.13 (±0.001)	0.04^{a} (±0.01)	0.32 (±0.03)	0.09^{b} (±0.01)	0.46 (±0.02)	0.12^{b} (±0.02)	0.77 (±0.06)	0.18^{b} (±0.01)	0.96 (±0.05)	0.17^{b} (±0.02)
	Shoots	0.02 (±0.003)	0.01^{a} (±0.001)	0.06 (±0.004)	0.02^{a} (±0.003)	0.07 (±0.01)	0.03^{a} (±0.01)	0.23 (±0.03)	0.09^{a} (±0.02)	0.34 (±0.02)	0.12^{b} (±0.02)	0.46 (±0.04)	0.15^{b} (±0.02)	0.74 (±0.05)	0.15^{b} (±0.02)

The uptake of maize roots and shoots grown in different concentrations of Cd. The values were represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ levels.

The present study shows that the concentration of both Cd compounds (CdS NPs and $CdCl_2$) in roots and shoots increased with increasing Cd levels in soil, as discussed in Section 4.10.1. Bulk Cd concentrations were found to be higher in maize roots and shoots compared with Cd NPs.

Table 4.5 indicates that the uptake of both Cd compounds by roots and shoots gradually increases with increasing Cd concentrations to the soil compared with the control samples. In addition, the majority of Cd uptake by the maize roots was higher compared to shoots across any of the used concentrations for both Cd compounds. However, the uptake of both Cd compounds (CdS NPs and $CdCl_2$) by maize roots and shoots was low; this may reflect the low level of Cd that was present in the Eutric Cambisol soil. This accords with conclusions of previous studies.[59,29] Similar results for bulk Cd were obtained by Wang *et al.*[26] who used concentrations of Cd ranging from 10^{-4} to 10^{-6} mM with maize plants. Similarly, Chitra *et al.*[37] found that the uptake and accumulation of Cd was high in maize roots compared with their shoots. In the present study, the uptake of bulk Cd by maize roots and shoots was higher compared with the uptake of Cd NPs.

Statistical analysis of Cd absorption by maize indicated that the uptake of Cd NPs was significantly different ($p < 0.01$) in maize roots at concentrations of 1.0 and 1.25 mg kg^{-1} compared with the control groups, with the exception of Cd NPs uptake in maize roots at 0.75 mg kg^{-1} soil ($p < 0.05$). There was a significant difference ($p < 0.01$) in the uptake of Cd NPs in maize shoots at concentration s of 0.75, 1.0 and 1.25 mg kg^{-1} with the exception of Cd NPs uptake in maize shoots at 0.5 mg kg^{-1} soil ($p < 0.05$). Furthermore, the uptake of bulk Cd revealed significant differences ($p < 0.01$) in maize roots and shoots at the concentrations of 0.5, 0.75, 1 and 1.25 mg kg^{-1} compared to the controls, with the exception of bulk Cd uptake in maize shoots, which was not significantly different at 0.5 mg kg^{-1} soil compared with the control group. There was no significant difference between the uptake of roots and shoots as regards the concentrations of Cd NPs. The mean overall uptake of roots was 0.05 µg/pot (SE: ± 0.01); that of shoots was 0.04 µg/pot (SE: ± 0.01). The results of statistical analysis also showed no significant difference between uptake of roots and shoots as regards the concentrations of bulk Cd. The mean overall uptake of roots was 0.09 µg/pot (SE: ± 0.02); that of shoots was 0.08 µg/pot (SE: ± 0.02). Results also indicated a significant difference ($p = 0.01$) between uptake of roots as regards the concentration of Cd NPs and bulk Cd. The results of statistical analysis showed significant difference ($p < 0.05$) between uptake of shoots as regards the concentration of Cd

NPs and bulk Cd. Table 4.6 shows the uptake ratios and the total uptake of Cd by the roots and shoots. Table shows that the total uptake of bulk Cd was higher compared with its NP form. Statistical analysis of ANOVA and subsequent Tukeys HSD tests indicated the majority of total uptake for both Cd compounds was significantly different ($p < 0.01$) at concentrations of 0.5, 0.75, 1, and 1.25 mg kg^{-1} compared with the control groups. There were no significant differences in the uptake ratios for roots and shoots across any of Cd compounds and the applied concentrations compared with those of the controls. Table 4.6 shows the total uptake and uptake ratios of Cd (CdS NPs and $CdCl_2$) in maize roots and shoots.

Table 4. 6. Total uptake (mg kg^{-1}) and uptake ratio (Uptake R.) of Cd (%) between shoots and roots of maize plants at different concentrations of CdS NPs and bulk $CdCl_2$.

Added concentration (mg kg^{-1})	Materials					
	CdS NPs			$CdCl_2$ Bulk		
	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots
control	0.01^a (±0.001)	55.64^a (±10.24)	44.36^a (±10.24)	0.02^a (±0.001)	58.89^a (±6.27)	41.11^a (±6.27)
0.1	0.02^a (±0.002)	73.63^a (±4.59)	26.37^a (±4.59)	0.05^a (±0.004)	51.10^a (±5.35)	48.90^a (±5.35)
0.25	0.04^a (±0.002)	70.65^a (±3.08)	29.35^a (±3.08)	0.06^a (±0.002)	56.89^a (±8.51)	43.11^a (±8.51)
0.5	0.07^b (±0.01)	55.63^a (±7.39)	44.37^a (±7.39)	0.17^b (±0.03)	51.69^a (±6.05)	48.31^a (±6.05)
0.75	0.10^b (±0.01)	59.85^a (±5.86)	40.15^a (±5.86)	0.24^b (±0.03)	50.94^a (±5.90)	49.06^a (±5.90)
1	0.15^b (±0.02)	53.38^a (±5.33)	46.62^a (±5.33)	0.33^b (±0.02)	55.27^a (±4.69)	44.73^a (±4.69)
1.25	0.22^b (±0.02)	56.96^a (±0.69)	43.04^a (±0.69)	0.32^b (±0.05)	52.70^a (±0.65)	47.30^a (±0.65)

The total uptake of maize roots and shoots grown in different concentrations of Cd. The values are represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $p \leq 0.05$ levels.

The results of statistical analysis (Table 4.6) showed significant difference ($p < 0.01$) between the total uptake of Cd as regards the concentration of Cd NPs and bulk Cd sources. The mean overall total uptake of Cd NPs was 0.09 µg/pot (SE: ± 0.02); that of bulk Cd was 0.17 µg/pot (SE: ± 0.04). Statistical analysis observed a significant difference ($p < 0.01$) between the uptake % of roots and shoots as regards the concentration of Cd NPs. The mean overall uptake % of

roots was 60.8 % (SE: ± 4.73); that of shoots was 39.2 % (SE: ± 4.37). There was no significant difference between uptake % of roots and shoots as regards the concentration of bulk Cd. The mean overall uptake % of roots was 53.9 (SE: ± 3.63); that of shoots was 46.1% (SE: ± 3.36). Results showed no significant difference between uptake % of roots as regards the concentration of Cd NPs and bulk Cd. The results of statistical analysis indicated no significant difference between uptake % of shoots as regards the concentration of Cd NPs and bulk Cd.

4.10.5. Total concentrations of cadmium in the pots soil

An analysis of heavy metals in the Eutric Cambisol soil was performed after 21 days of plants growth as shown in Figure 4.11.

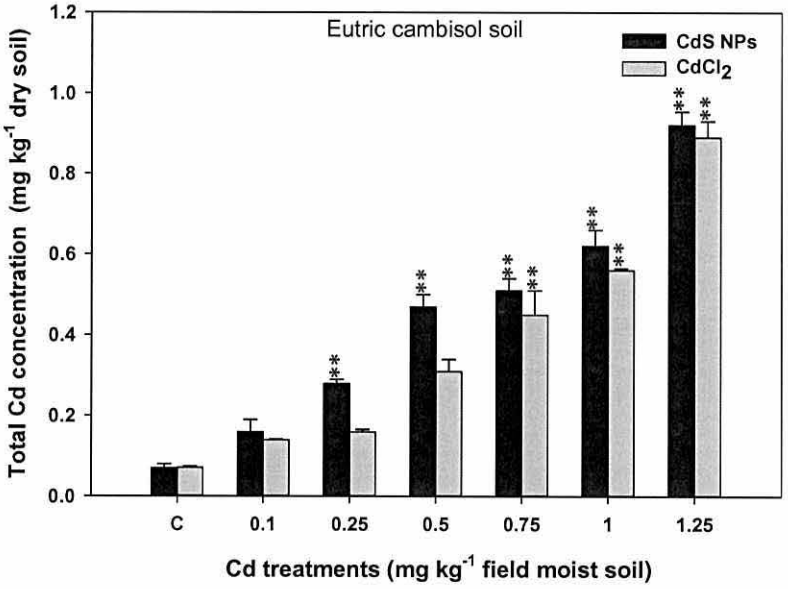

Figure 4. 11. The total concentrations of Cd in Eutric Cambisol soil (mg kg^{-1}), after growing maize plants for 21 days. The soil was treated with 0, 0.1, 0.25, 0.5, 0.75, 1.0 and 1.25 mg of Cd kg^{-1} soil from bulk (CdCl2) or nanomaterial (CdS). C represents control samples. The values represent means ±SEM (n= 3). Asterisks denote degree of statistical difference between the control and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$).

The total concentration of Cd NPs was higher than bulk Cd in the Eutric Cambisol soil as shown in Figure 4.11; this can be attributed to the solubility and precipitation, and the aggregation of CdS NPs compared with those found in $CdCl_2$ (see Chapter 2, Sections 2.5.2, 2.5.2.1, and 2.6.2.1.

Statistical analysis of results observed a significant difference ($p < 0.01$) for CdS at concentrations of 0.25, 0.5, 0.75, 1.0 and 1.25 mg kg^{-1} compared with the controls. Furthermore, $CdCl_2$ showed significant differences ($p < 0.01$) at the concentrations of 0.5, 0.75, 1 and 1.25 mg kg^{-1} compared with their controls. There was no significant difference between the total concentration of Cd as regards the concentrations of Cd NPs and bulk Cd. The mean overall total concentration of Cd NPs was 0.43 mg kg^{-1} (SE: ± 0.10); that of bulk Cd was 0.37 mg kg^{-1} (SE: ± 0.10).

4.11. Assimilated concentrations of zinc in maize plants

The distribution of bulk Zn in plants and soils has been widely studied.[60–66] However, research into Zn NPs has been mainly restricted to examine into germination and cell cultures. Limited investigations have been conducted into the uptake of Zn NPs by plants from their soils.[67]

Results of statistical analysis showed no significant differences ($p > 0.05$) for all concentrations of Zn NPs in maize roots compared with their control samples. However, Zn NPs showed a significant difference ($p < 0.05$) in maize shoots at concentrations of 1.0 and 1.25 mg kg^{-1} compared with their control groups. The concentration of bulk Zn was significantly different ($p < 0.05$) in maize roots at concentrations of 1.0, 1.25 mg kg^{-1} compared with controls. There appeared no significant difference in maize shoots (Figure 4.12).

Statistical analysis observed that was no significant difference between the concentration of Zn NPs in roots and shoots as regards their ZnO NPs concentrations (0–1.25 g kg^{-1}). The concentration of Zn NPs was higher in roots (mean overall = 43.83 mg kg^{-1}; SE: ± 2.49); than that of shoots (21.63 mg kg^{-1}; SE: ± 1.44). There was no significant difference between roots and shoots for the bulk Zn concentration as regards their $ZnCl_2$ concentrations (0–1.25 mg kg^{-1}). The concentration of bulk Zn was higher in roots (mean overall = 51.13 mg kg^{-1}; SE: ± 2.83) than that of shoots (24.86 mg kg^{-1}; SE: ± 1.18).

Statistical analysis showed no significant difference between the concentration of bulk Zn and Zn NPs in maize roots as regards their concentration. Bulk Zn was higher (mean overall = 51.13 mg kg^{-1}; SE: ± 2.83) than that of Zn NPs in roots (mean overall = 43.83 mg kg^{-1}; SE: ± 2.49). There was no significant difference between the concentration of bulk Zn and Zn NPs in maize shoots as regards their concentration (0–1.25 mg kg^{-1}). Bulk Zn was higher (mean overall =

(24.86 mg kg^{-1}; SE: ± 1.18) than that of Zn NPs in roots (mean overall = 21.63 mg kg^{-1}; SE: ± 1.44). Figure 4.12 shows the concentrations of assimilated Zn from the two compounds (ZnO NPs and $ZnCl_2$) in maize roots and shoots grown in the Eutric Cambisol soil.

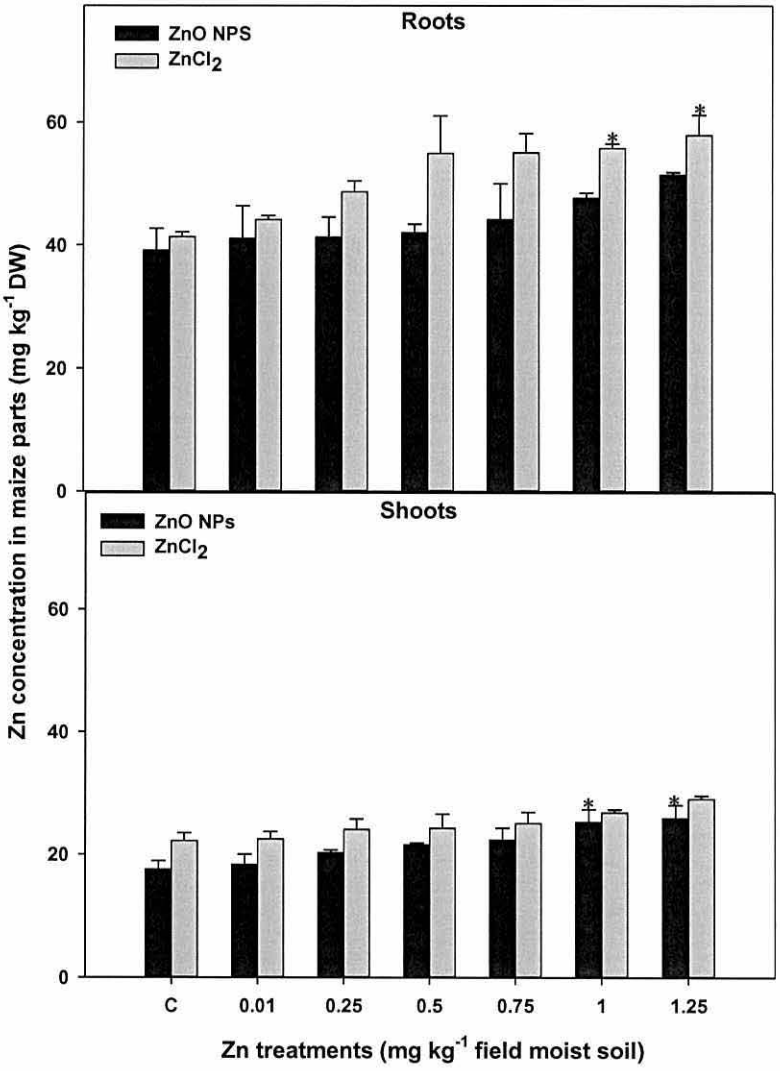

Figure 4. 12. Mean concentrations of Zn in maize roots and shoots grown in Eutric Cambisol soil. The soil was treated with 0, 0.1, 0.25, 0.5, 0.75, 1.0 and 1.25 mg of Zn kg^{-1} soil from bulk ($ZnCl_2$) and nanomaterial (ZnO). C represents control samples. The values represent means ±SEM (n= 3). Asterisks denote degree of statistical difference between the control and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Some of the error bars are too small to be visible.

As indicated (see Section 4.10.4), the amount of adsorbed Zn could be affected by factors including plant species, plant parts and age, the applied concentrations, and the soil's physical

and chemical properties.[60] The concentrations of Zn from the two compounds of Zn in the roots and shoots of maize plants were high levels for any of the applied concentrations compared with initial Zn levels. These results demonstrate that the average accumulation for ZnO and $ZnCl_2$ was approximately 45 and 52 mg kg^{-1} respectively in the maize roots while small amounts about 22 and 25 mg kg^{-1} transferred to the maize shoots across any of Zn concentrations. This could be attributed to the residual effect of the fertilizers applied to the soil during sample collection; fertilizers might increase the Zn concentration in maize parts.[61–63]

These results are in accord with those of Wang *et al.*[64] who reported that soil–applied fertilizers (containing Zn) significantly increase Zn concentration in the ear leaves of spring maize by 15% during its first growing season and by 21% during the second season. The concentrations of metals in plants serve to indicate the metal contamination status of the site also reveal the abilities of various plant species to take up and accumulate metals from polluted soil.[65] Results of the present study suggest the concentration of Zn in the roots and shoots increases as a function of increases in the metal's concentrations in the tested soil across all the Zn's compounds compared with that of controls. This conclusion accords with that of previous studies.[64,66]

A recent study by Zhao *et al.*[67] suggests that the total Zn content in roots and shoots increases with increasing the concentration of ZnO NPs (0–800 mg kg^{-1}) in a sandy loam soil. Zhao *et al.*[67] indicated that a possible explanation for the high concentration of Zn in the plants that the probable formation of Zn-alginates, based on the well-known fact of gel formation by reaction of alginic acid or Na-alginate with divalent cations, could have increased Zn in the soil solution to be assimilated in the plant. Lopez–Moreno *et al.*[10] indicated that the uptake of Zn by soybean seedlings was significantly higher at 500 mg L^{-1} (ZnO NPs). Results suggested that the concentration of Zn in the maize roots was higher than in shoots across any of the Zn's concentrations regardless of the metal's compounds. This result accords with that of previous research.[68,69]

$ZnCl_2$ results indicated that the concentration of bulk Zn in roots and shoots was higher compared to ZnO NPs across all the applied concentrations. This appeared due to the high solubility of $ZnCl_2$ in the soil solution compared with ZnO NPs.[70–72] These results agree with those obtained by Lin and Xing who determined the uptake of Zn NPs in ryegrass using different

treatments of ZnO NPs and Zn ions (bulk material).[6] The researchers found that translocation factors for Zn from roots to shoots were low in the presence of Zn ions and very low for ZnO NPs.

4.11.1. Effect of zinc from bulk and nanoparticulate sources on the growth characteristics of maize plants

The length and dry biomass of maize roots and shoots were recorded (cm and g/pot respectively) to assess the effects of the concentrations of the two Zn compounds on the plant's growth. The majority of the maize's parameters were slightly different across any compound of Zn as shown in Figures 4.13 and 4.14. Figure 4.13 indicates that ZnO NPs has a significant negative effect ($p < 0.01$) on the growth of maize shoots at concentration of 1.25 mg kg^{-1} compared with control group. This may be related to the high concentration of ZnO NPs that was found in maize shoots (25.94 mg kg^{-1} ±2.14) as shown in Figure 4.12.

Figure 4.13 and 4.14; however, this indicates that the length and dry biomass of roots increased as a function of the concentrations of ZnO NPs in the soil compared to those found in $ZnCl_2$. The presence of ZnO NPs reduced the growth of maize shoots compared to maize shoots grown under $ZnCl_2$ treatments. Lin and Xing.[6] showed that due to the small size and large surface energy, ZnO NPs are prone to aggregation in aqueous phase; this may influence its bioavailability and toxicity for ryegrass (*Lolium perenne*). However, ZnO NPs may cause severe damage to the epidermal and cortical cells and even impair the endodermal and vascular cells, and this may be a direct reason for the ryegrass growth inhibition. The phytotoxicity mechanism of ZnO NPs needs further research.

It is difficult to clarify whether the phytotoxicity of ZnO NPs in the tested soil results from their dissolution in the soil solution or from Zn ions in soil fertilizers. ZnO is insoluble in water; however, some research suggests that appreciable dissolution of ZnO starts from 1 mg L^{-1} to several thousand mg L^{-1} in water, and that this is a function of different factors (e.g., the NPs size and the pH of dissolved media).[71]

Generally, both compounds of Zn did not show significant differences with respect to ZnO effects on the length of maize shoots; this agrees with the results for Cd NPs and bulk Cd (see Section 4.10.1). The effects of high concentrations of ZnO was clarified by Ellis who found that its NPs had a significant negative impact on the dry matter of ryegrass (*L. perenne*) at treatments

of 10 and 100 mg kg^{-1}; these effects concerned short and long term plant growth in Eutric Cambisol soil.[13] Stampoulis *et al.*[31] reported that all ZnO treatments reduced courgette (*C. pepo*) biomass by 78–90% relative to controls, but there were no differences when ZnO NPs and bulk Zn were applied.

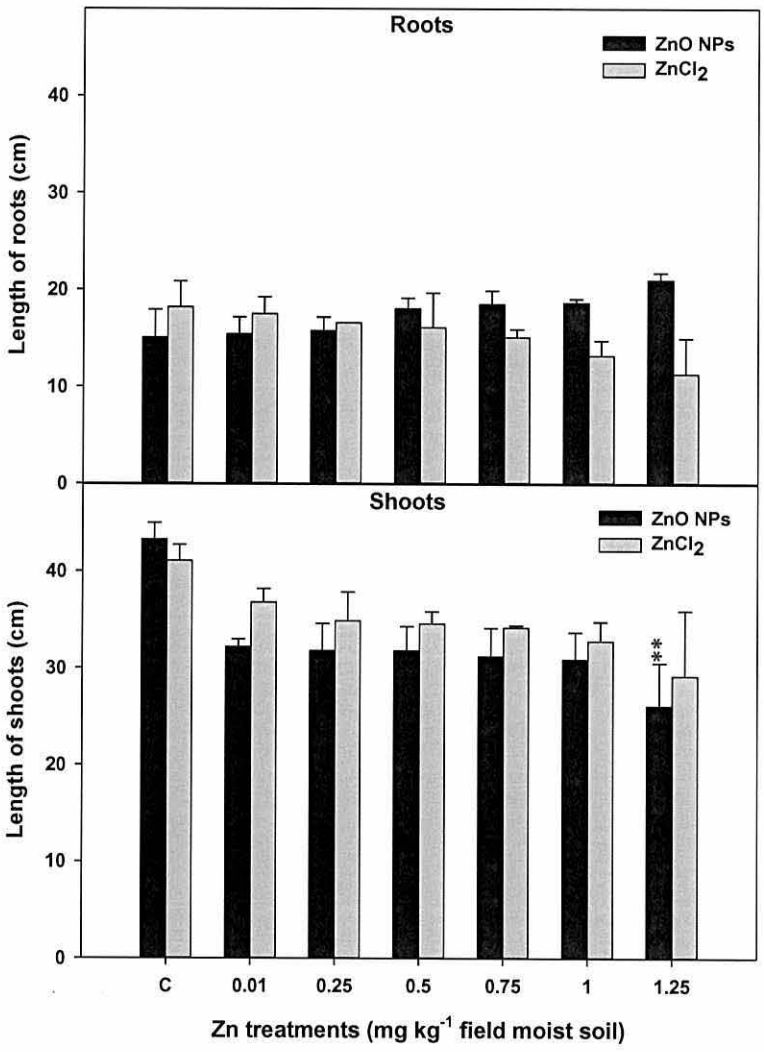

Figure 4. 13. The effects of Zn concentrations on the length of roots and shoots (cm) grown in Eutric Cambisol soil. The soil was treated with 0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg of Zn kg^{-1} soil from bulk ($ZnCl_2$) and nanomaterial (ZnO). C represents control samples. The values were given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Some of the error bars are too small to be visible.

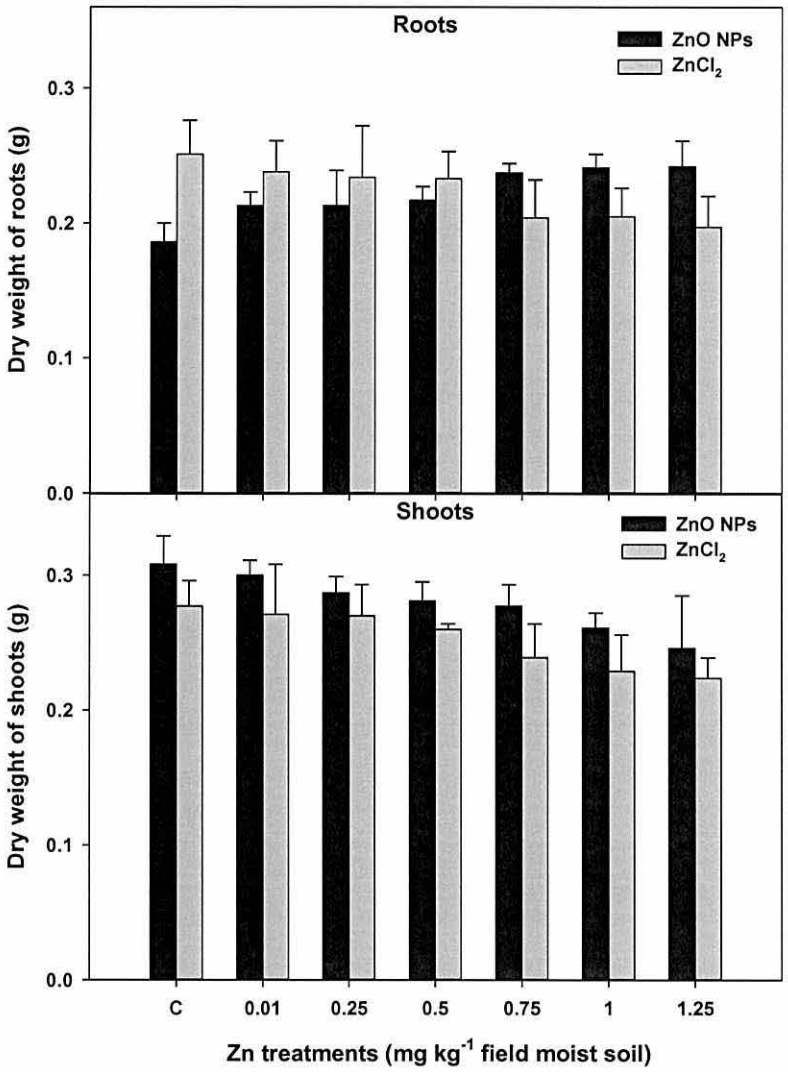

Figure 4. 14. The effects of Zn concentrations on the dry biomass of maize roots and shoots (g/pot) grown in Eutric Cambisol soil. The soil was treated with 0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg of Zn kg^{-1} soil from bulk ($ZnCl_2$) and nanomaterial (ZnO). C represents control samples. The values were given as mean ±SEM of triplicate samples.

Statistical analysis of results showed no significant differences for the ZnO and $ZnCl_2$ concentrations on the maize parameters compared with the control groups save ZnO; here the NPs had a significant negative effect ($p < 0.01$) on the length of maize shoots at 1.25 mg kg^{-1} compared with the control.

Statistical analysis observed a significant difference ($p < 0.05$) between dry biomass of roots and shoots as regards the concentration of Zn NPs applied to the soil. The mean overall dry biomass of roots was (0.22 g/ pot; SE: ± 0.01); that of shoots was 0.28 g/ pot (SE: ± 0.01). There was no

significant difference (no toxic effects) between dry biomass of roots and shoots as regards the concentration of bulk Zn. The mean overall dry biomass of roots was (0.22 g/ pot; SE: ± 0.01); that of shoots was 0.25 g/ pot (SE: ± 0.01). There was no significant difference between dry biomass of roots as regards the concentration of Zn NPs and bulk Zn. The results of statistical analysis showed no significant difference between dry biomass of shoots as regards the concentration of Zn NPs and bulk Zn.

Significant difference ($p < 0.05$) was between length of roots and shoots as regards the concentration of Zn NPs. The mean overall length of roots was (17.48 cm; SE: ± 1.15); that of shoots was 32.46 cm (SE: ± 2.41). The results of ANOVA revealed no significant difference ($p < 0.01$) between length of roots and shoots as regards the concentration of bulk Zn. The mean overall length of roots was 15.42 cm (SE: ± 1.60); that of shoots was 34.77 cm (SE: ± 2.11). There was a significant difference ($p < 0.05$) between length of roots as regards the concentration of ZnO NPs and $ZnCl_2$. The results of statistical analysis showed no significant difference between length of shoots as regards the concentration of Zn NPs and bulk Zn.

Although Zn is not usually phytotoxic as it is an essential element for plant development and growth,[10] and a great number of proteins contain a Zn–binding domain–transcriptional regulatory proteins, for instance–above a certain concentration Zn becomes toxic, causing plants to decrease their biomass or to activate defence mechanisms.[73,74] In this regard, Lin and Xing reported the effects of Zn NPs and Zn ions upon the seedling growth of ryegrass cultured in nutrient solution at concentrations higher than 50 mg L^{-1}; this caused retarded growth.[6] The ryegrass plants suffered shorter roots and shoots compared to their controls. Results of the present study thus broadly agree with the extant literature.

4.11.2. Bio–concentration ratio of zinc in the different parts of a maize plant

The Bio–concentration ratios (BCR) of both Zn compounds (ZnO NPs and $ZnCl_2$) were calculated for maize roots and shoots, as shown in Table 4.7. The data suggest that all Zn BCR values decreased in maize roots and shoots when the metal's concentration increased in the Eutric Cambisol soil across any Zn compounds or Zn concentrations. This result partially agrees with that of Jasiewicz *et al.*[75] who reported that an admixture of weakly–loamy sand soil (pH 6.2) with 5% and 10% of sediment admixture leads to decreased bio–accumulation coefficients of of bulk Zn, Cu, Cd, Cr, and Ni (all doses 5%) in maize aerial biomass.

Table 4. 7. Concentrations of Zn and its Bio–concentration Ratios (BCR) in maize plants (roots and shoots). Plants were cultivated in Eutric Cambisol soil and irrigated by different concentration of ZnO NPs and bulk $ZnCl_2$ (mg kg^{-1}).

Materials	Parameters	Concentrations of Zn used in the soil pots (mg kg^{-1})											
		0.1		0.25		0.5		0.75		1.0		1.25	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
ZnO–NPs	Conc. (mg kg^{-1})	41.08 (±5.28)	18.37 (±1.66)	41.30 (±3.25)	20.25 (±0.48)	42.05 (±1.35)	21.59 (±0.27)	44.15 (±5.88)	22.39 (±1.94)	47.69 (±0.81)	25.30 (±2.01)	51.44 (±0.43)	25.94 (±2.14)
	BCR	410.75^{a} (±12.80)	183.70^{a} (±16.57)	165.20^{b} (±13.01)	81.00^{b} (±1.93)	84.10^{b} (±2.70)	43.19^{b} (±0.54)	58.87^{b} (±7.85)	29.85^{b} (±2.58)	47.69^{b} (±0.81)	25.30^{b} (±2.06)	41.16^{b} (±0.34)	20.75^{b} (±1.72)
$ZnCl_2$_Bulk	Conc (mg kg^{-1})	44.15 (±0.65)	22.51 (±1.11)	48.69 (±1.75)	24.06 (±1.72)	54.95 (±6.12)	24.29 (±2.31)	55.09 (±3.11)	25.08 (±1.83)	55.79 (±0.75)	26.85 (±0.50)	57.93 (±3.24)	29.07 (±0.54)
	BCR	441.45^{a} (±6.49)	225.12^{a} (±11.96)	194.75^{b} (±7.01)	96.25^{b} (±6.87)	109.91^{b} (±12.23)	48.58^{b} (±4.62)	73.45^{b} (±4.15)	33.45^{b} (±2.43)	55.79^{b} (±0.75)	26.85^{b} (±0.50)	46.34^{b} (±2.59)	23.26^{b} (±0.43)

The Bio–Concentration Ratios (BCR) for maize roots and shoots grown in different concentration of Zn. Conc. represents the concentration. The values were represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ levels.

The authors suggest that maize plants more easily accumulate Zn, Cu, Cd, Cr, Ni and Pb within their tissues. Statistical analysis of results observed that there were significant differences ($p < 0.01$) for all Zn BCR values in maize roots and shoots compared to low concentration treatments; this was for both compounds of the Zn.

Statistical analysis also showed a significant difference ($p < 0.01$) between the BCR of roots and shoots as regards the concentration of Zn NPs. The mean overall BCR of roots was 134.62 (SE: ± 15.37); BCR of shoots was 63.96 (SE: ± 10.40). The results of statistical analysis showed a significant difference between BCR of roots and shoots as regards the concentration of bulk Zn. The mean overall BCR of roots was 153.61 (SE: ± 13.61); that of shoots was 75.85 (SE: ± 10.98).

There was no significant difference between BCR of roots as regards the concentration of Zn NPs and bulk Zn. The results of statistical analysis observed a significant difference ($p < 0.05$) between BCR of shoots as regards the concentration of Zn NPs and bulk Zn. The BCR for Zn in maize roots was higher than those found in the shoots across all Zn concentrations and both Zn compounds. The BCRs of bulk Zn in roots and shoots was higher than those found for Zn NPs. This could be due to the solubility of $ZnCl_2$, which, as indicated, is higher than ZnO NPs in the soil solution (Sections 4.11 and 4.11.1).

The BCRs of Zn in maize roots were higher than in maize shoots across any of Zn compounds and the applied concentrations, thus Zn appears to accumulate more in roots than in shoots. The BCR of Zn in maize roots and shoots acquired a high value at low concentrations for both Zn compounds; this suggests that the soil used in the study may have had a high concentration of Zn prior to the soil's treatment. However, the BCR of Zn varied according to the metal's content in the plants and its availability in the soil.

4.11.3. The parameters used to evaluate the effect of zinc on plant growth

The parameters TI, RI, and AE were used to evaluate the effect of the two Zn compounds and their treatment concentrations on the growth of maize plants and the toxicity of Zn to the plants. Table 4.8 (Parts 1 and 2) summarises their values.

The table suggests the maize plant's tolerance for the two Zn compounds were varied considerably across all the applied concentrations. The majority of Zn TIs of roots and shoots

appeared to decrease with increasing concentrations in the soil of both physical types of the metal; however, the Zn TI of roots slightly increased across all of the Zn NP concentration compared with 0.1 mg kg^{-1}. Maize roots showed a high tolerance for Zn NPs at concentrations of 0.5, 0.75, 1.0, and 1.25 mg kg^{-1} compared to those for bulk Zn. Maize shoots appeared more tolerant to Zn NPs compared to bulk Zn across all of the applied concentrations. This may have been due to the high solubility of bulk $ZnCl_2$ in the soil solution compared with ZnO NPs; as indicated, this increases the availability of free Zn ions for maize shoots through the plant's root system (Section 4.11); the majority of maize parameters seem to be decreased by bulk Zn compared to ZnO NPs (Section 4.11.1).

Statistical analysis of results observed no significant differences for any TIs, RIs, and AEs across any of the Zn compounds and concentration with respect to the low concentration of 0.1 mg kg^{-1}, save that the AEs for maize roots were significant ($p < 0.05$) for Zn NPs at concentrations of 0.75, 1.0 and 1.25 mg kg^{-1} compared with 0.1 mg kg^{-1}. ZnO and $ZnCl_2$ followed the same behaviour as Cd compounds (Section 4.10.3).

There was no significant difference between the TI of roots and shoots as regards the concentration of Zn NPs. The mean overall TI of roots was 0.88 (SE: ± 0.03); that of shoots was 1.03 (SE: ± 0.11). The results of statistical analysis showed no significant difference between TI of roots and shoots as regards the concentration of bulk Zn. The mean overall TI of roots was 0.80 (SE: ± 0.09); that of shoots was 0.79 (SE: ± 0.07). There was no significant difference between TI of roots as regards the concentration of Zn NPs and bulk Zn. The results of ANOVA revealed no significant difference between TI of shoots as regards the concentration of Zn NPs and bulk Zn.

Statistical analysis observed no significant difference between the RI (%) of roots and shoots as regards the concentration of Zn NPs. The results of ANOVA also revealed no significant difference between RI (%) of roots and shoots as regards the concentration of bulk Zn. Results indicated no significant difference between RI (%) of roots as regards the concentration of Zn NPs and bulk Zn. There was no significant difference between RI (%) of shoots as regards the concentration of Zn NPs and bulk Zn. There were no significant differences between AE of roots and shoots cross all Zn compounds (ZnO and $ZnCl_2$) and maize part.

Table 4. 8. Dry Matter Yield (DMY), Tolerance index (TI), Relative Increase (RI) and Agronomical Efficiency (AE) of maize plants as affected by the addition of ZnO NPs and bulk $ZnCl_2$ (mg kg^{-1}) in the Eutric Cambisol soil.

Materials	Plant part	Added concentrations of Zn (mg kg^{-1} soil)												
		Control	0.1				0.25				0.5			
		DMY g/pot	DMY g/pot	TI	RI %	AE	DMY g/pot	TI	RI %	AE	DMY g/pot	TI	RI %	AE
ZnO–NPs	Roots	0.19 (±0.01)	0.21 (±0.01)	0.83^{a} (±0.05)	-17.50^{a} (±3.33)	-0.46^{a} (±0.15)	0.21 (±0.03)	0.83^{ab} (±0.11)	-17.22^{ab} (±1.15)	-0.18^{ab} (±0.02)	0.22 (±0.01)	0.84^{ab} (±0.02)	-16.25^{ab} (±2.09)	-0.08^{ab} (±0.01)
	Shoots	0.31 (±0.02)	0.30 (±0.01)	1.11^{a} (±0.16)	10.90^{a} (±1.95)	0.17^{a} (±0.02)	0.29 (±0.01)	1.08^{ab} (±0.20)	7.87^{ab} (±1.82)	0.02^{ab} (±0.001)	0.28 (±0.01)	1.06^{ab} (±0.20)	5.88^{ab} (±1.83)	-0.004^{ab} (±0.001)
Zn –Bulk	Roots	0.25 (±0.03)	0.24 (±0.02)	0.86^{a} (±0.06)	-13.90^{a} (±1.25)	-0.40^{a} (±0.08)	0.23 (±0.04)	0.85^{ab} (±0.15)	-14.67^{ab} (±1.45)	-0.18^{ab} (±0.001)	0.23 (±0.02)	0.86^{ab} (±0.15)	-13.82^{ab} (±1.15)	-0.09^{ab} (±0.01)
	Shoots	0.28 (±0.02)	0.27 (±0.04)	0.85^{a} (±0.10)	-14.74^{a} (±2.61)	-0.48^{a} (±0.04)	0.27 (±0.02)	0.87^{ab} (±0.16)	-12.56^{ab} (±1.10)	-0.20^{ab} (±0.01)	0.26 (±0.004)	0.83^{ab} (±0.08)	-17.13^{ab} (±2.21)	-0.12^{ab} (±0.02)

Table Part (2) continues next page

Table 4.8 continuous

Materials	Plant part	Added concentrations of Zn (mg kg^{-1} soil)												
		Control	0.75				1.0				1.25			
		DMY g/pot	DMY g/pot	TI	RI %	AE	DMY g/pot	TI	RI %	AE	DMY g/pot	TI	RI %	AE
ZnO–NPs	Roots	0.19 (±0.01)	0.24 (±0.01)	0.92^{ab} (±0.04)	-8.39^{ab} (±0.25)	-0.03^{b} (±0.002)	0.24 (±0.01)	0.93^{ab} (±0.02)	-6.95^{ab} (±1.24)	-0.02^{b} (±0.001)	0.24 (±0.02)	0.93^{ab} (±0.04)	-6.79^{ab} (±1.29)	-0.01^{b} (±0.001)
	Shoots	0.31 (±0.02)	0.28 (±0.02)	1.04^{ab} (±0.19)	4.28^{ab} (±0.06)	-0.01^{ab} (±0.001)	0.26 (±0.01)	0.97^{ab} (±0.15)	-3.16^{ab} (±0.78)	-0.02^{ab} (±0.002)	0.25 (±0.04)	0.94^{ab} (±0.26)	-6.43^{ab} (±1.03)	-0.03^{ab} (±0.006)
Zn –Bulk	Roots	0.25 (±0.03)	0.20 (±0.03)	0.76^{ab} (±0.17)	-24.33^{ab} (±1.76)	-0.10^{ab} (±0.01)	0.20 (±0.02)	0.74^{ab} (±0.09)	-25.66^{ab} (±2.05)	-0.07^{ab} (±0.01)	0.20 (±0.02)	0.73^{ab} (±0.15)	-26.85^{ab} (±1.96)	-0.07^{ab} (±0.002)
	Shoots	0.28 (±0.02)	0.24 (±0.03)	0.77^{ab} (±0.14)	-22.95^{ab} (±1.04)	-0.11^{ab} (±0.02)	0.23 (±0.03)	0.72^{ab} (±0.06)	-27.95^{ab} (±4.37)	-0.09^{ab} (±0.02)	0.23 (±0.02)	0.71^{ab} (±0.08)	-28.68^{ab} (±2.53)	-0.08^{ab} (±0.02)

The parameters of maize roots and shoots grown in different concentration of Zn. The values were represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ level.

The majority of RI and AE showed negative values; this may have been because the dry biomass of treated plants was reduced by ZnO NPs and $ZnCl_2$ (Section 4.11.1). Results from Zhao *et al.*[67] indicated that ZnO NPs had reduced the dry weight of maize plants grown in soil treated with 400 to 800 mg kg^{-1} of ZnO NPs. The biomass production (dry weight) was decreased by 30–34% in maize roots and 21–26% in maize shoots.

For ZnO NPs, the RI (%) of maize shoots decreased at concentrations of 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg kg^{-1}; for values in the range 0.1– 0.75 mg kg^{-1}, however, the AE values remained positive; values became negative only for the last two concentrations of ZnO NPs. This could be attributed to the residual effect of Zn ions from soil fertilizers and the dissolution of ZnO NPs into the soil solution, thereby raising the content of Zn in maize roots and shoots with concomitant decrease in the dry biomass of maize parts.[61–63]

4.11.4. The uptake of zinc in maize plants

Table 4.9 summarises the effects of plant parts, plant age, and Zn concentrations (mg kg^{-1}) on the uptake of the two physical types of Zn (ZnO NPs and bulk $ZnCl_2$) and their accumulation in maize plants. Statistical analysis (ANOVA and *Post hoc* tests) showed no significant differences in the uptake of Zn by maize roots and shoots despite the metal's compounds or their concentrations when compared to their respective controls.

There was no significant difference between the uptake of roots and shoots as regards the concentration of Zn NPs. The mean overall uptake of roots was 9.81 µg/pot (SE: ± 0.88); that of shoots was 5.99 µg/pot (SE: ± 0.36). There was no significant difference between uptake of roots and shoots as regards the concentration of bulk Zn. The mean overall uptake of roots was 11.23 µg/pot (SE: ± 0.78); that of shoots was 6.22 µg/pot (SE: ± 0.32). This indicates that Zn^{2+} derived from $ZnCl_2$ may be more toxic to maize plants than ZnO NPs. This may be because that most of ZnO NPs was aggregated on the root surface, and only a few individual particles could move into the roots and were available for transport within maize shoots.

Results indicated no significant difference between uptake of roots as regards the concentration of Zn NPs and bulk Zn. The results of statistical analysis showed no significant difference between uptake of shoots as regards the concentration of Zn NPs and bulk Zn.

Table 4. 9. The concentrations of Zn and its uptake (μg kg^{-1}) by maize plants (roots and shoots) as affected by the addition of ZnO NPs and bulk $ZnCl_2$ in Eutric Cambisol soil.

Materials	Plant part	Added concentrations of Zn (mg kg^{-1} soil)													
		control		0.1		0.25		0.5		0.75		1.0		1.25	
		Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg/kg	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot	Conc. mg kg^{-1}	Uptake μg/pot
ZnO–NPS	Roots	39.13 (±3.58)	7.21[a] (±0.33)	41.08 (±5.28)	8.88[a] (±1.62)	41.30 (±3.25)	8.99[a] (±1.77)	42.05 (±1.35)	9.17[a] (±0.67)	44.15 (±5.88)	10.41[a] (±1.16)	47.69 (±0.81)	11.50[a] (±0.34)	51.44 (±0.43)	12.48[a] (±0.93)
	Shoots	17.58 (±1.38)	5.37[a] (±0.29)	18.37 (±1.66)	5.49[a] (±0.48)	20.25 (±0.48)	5.79[a] (±0.16)	21.59 (±0.27)	6.07[a] (±0.39)	22.39a (±1.94)	6.26[a] (±0.84)	25.30 (±2.01)	6.60[a] (±0.59)	25.94 (±2.14)	6.33[a] (±0.98)
$ZnCl_2$_Bulk	Roots	41.33 (±0.80)	10.36[a] (±0.86)	44.15 (±0.65)	10.54[a] (±0.99)	48.69 (±1.75)	11.40[a] (±1.89)	54.95 (±6.12)	12.79[a] (±1.58)	55.09a (±3.11)	11.07[a] (±0.97)	55.79 (±0.75)	11.42[a] (±1.20)	57.93 (±3.24)	11.44[a] (±1.55)
	Shoots	22.21 (±1.29)	6.14[a] (±0.54)	22.51 (±1.11)	6.08[a] (±0.80)	24.06 (±1.72)	6.42[a] (±0.15)	24.29 (±2.31)	6.30[a] (±0.52)	25.08a (±1.83)	5.99[a] (±0.82)	26.85 (±0.50)	6.12[a] (±0.61)	29.07 (±0.54)	6.50[a] (±0.34)

The uptake of maize roots and shoots grown in different concentrations of Zn. The values were represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ level.

The majority of root and shoot uptakes increased with the increasing Zn concentrations in the test soil. This finding agrees with that of Zhao *et al.*[67] who evaluated the uptake of ZnO NPs in maize plants grown in sandy loam soil.

Table 4. 10. Total uptake (mg kg^{-1}) and uptake ratio (Uptake R.) of Zn (%) between shoots and roots of maize plants at different concentrations of ZnO NPs and bulk $ZnCl_2$.

Added concentration (mg kg^{-1})	Materials					
	ZnO NPs			$ZnCl_2$ Bulk		
	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots
control	12.58[a] (±0.23)	57.30[a] (±2.26)	42.70[a] (±2.26)	16.50[a] (±1.21)	62.74[a] (±2.04)	37.26[a] (±2.04)
0.1	14.37[a] (±1.24)	60.86[a] (±5.76)	39.14[a] (±5.76)	16.61[a] (±1.29)	63.51[a] (±3.56)	36.49[a] (±3.56)
0.25	14.78[a] (±1.71)	59.67[a] (±5.29)	40.33[a] (±5.29)	17.82[a] (±2.02)	63.15[a] (±3.74)	36.85[a] (±3.74)
0.5	15.24[a] (±1.05)	60.12[a] (±0.25)	39.88[a] (±0.25)	19.08[a] (±1.16)	66.49[a] (±4.73)	33.51[a] (±4.73)
0.75	16.67[a] (±1.83)	62.59[a] (±2.61)	37.41[a] (±2.61)	17.06[a] (±1.48)	65.03[a] (±2.77)	34.97[a] (±2.77)
1.0	18.10[a] (±0.85)	63.67[a] (±1.61)	36.33[a] (±1.61)	17.54[a] (±1.36)	64.92[a] (±3.07)	35.08[a] (±3.07)
1.25	18.80[a] (±1.52)	66.57[a] (±3.21)	33.43[a] (±3.21)	17.94[a] (±1.38)	63.25[a] (±3.77)	36.75[a] (±3.77)

The total uptake of maize roots and shoots grown in different concentrations of Zn. The values were represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ level.

The present study's results indicate that the uptake of Zn by the roots was higher than shoots for any of the metal compound or its concentration compared with their controls; this agrees with previous research concerning plant uptake of bulk Zn.[64] The uptake of bulk Zn by maize roots and shoots was higher than that of ZnO NPs. This was plausibly due to the high solubility of bulk $ZnCl_2$ in the soil solution compared with the ZnO NP.

Statistical analysis results of Table 4.10, however, suggest no significant differences as regards the total uptake and the uptake ratios of Zn in roots and shoots for any of the applied concentrations or physical types of the metal compared with their control samples. There was no significant different between the total uptake of Zn as regards the concentration of Zn NPs and

bulk Zn. The mean overall total uptake of Zn NPs was 15.79 μg/pot (SE: ± 1.06); that of bulk Zn was 17.51 μg/pot (SE: ± 0.85).

Statistical analysis observed no significant difference between the uptake % of roots and shoots as regards the concentration of Zn NPs. The mean overall uptake % of roots was 61.54 (SE: ± 2.21); that of shoots was 38.46 (SE: ± 2.20). The results of statistical analysis observed no significant difference between uptake % of roots and shoots as regards the concentration of bulk Zn. The mean overall uptake % of roots was 64.15 (SE: ± 1.96); that of shoots was 35.85 (SE: ± 1.96). Results indicated no significant difference between uptake % of roots as regards the concentration of Zn NPs and bulk Zn. There was no significant difference between uptake % of shoots as regards the concentration of Zn NPs and bulk Zn. Table 4.10, however, suggests that the total uptake and the uptake ratio of maize roots were slightly increased with increasing the Zn concentrations for both physical types of the metal in the tested soil compared with their controls.

The uptake ratios of bulk Zn and Zn NPs in maize shoots gradually decreased compared with their control sample; these ranged from 37.26% to 36.75% and 42.70% to 33.43% respectively. This could have been due to certain increases in the concentration of Zn ions in the maize shoots, which caused the plants to active a different defence mechanism.[74] In line with results reported in Section 4.11, for both compounds the uptake ratio of Zn in roots was than in shoots across any Zn concentrations. Generally, the majority of calculated parameters showed that the accumulation of $ZnCl_2$ in the maize roots and shoots was higher than that of ZnO NPs.

4.11.5. Total concentrations of zinc in the pots soil

The total concentration of Zn in the Eutric Cambisol soil was analysed after 21 days of plant growth, as shown in Figure 4.15. Statistical analysis observed a significant difference ($p < 0.001$) for the concentration of ZnO NPs in the soil at concentrations of 1.0 and 1.25 mg kg^{-1} compared with control groups and significant difference ($p < 0.05$) at the level of 0.75 mg kg^{-1}. Moreover, there was a significant difference ($p < 0.01$) for the concentration of bulk Zn ($ZnCl_2$) at initial concentrations of 0.75, 1.0, and 1.25 mg kg^{-1} compared with control groups, with the exception of bulk Zn concentration at a level of 0.5 mg kg^{-1} ($p < 0.05$).

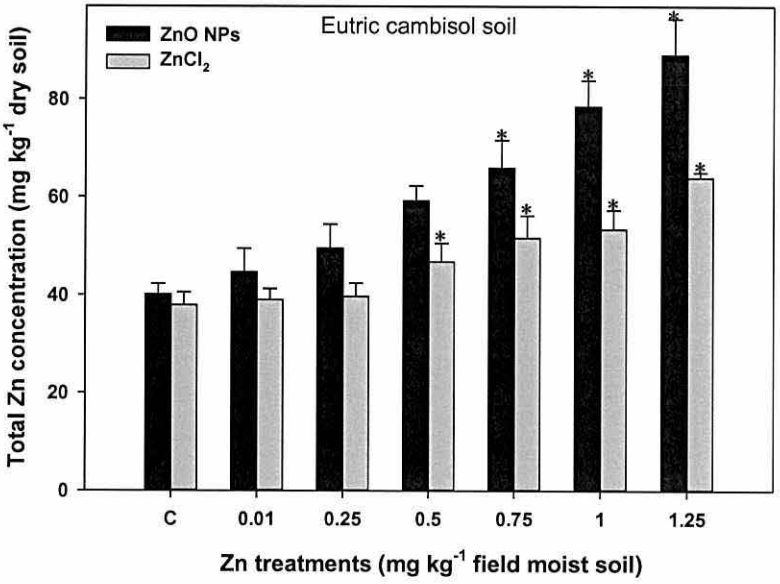

Figure 4. 15. The total concentrations of Zn in Eutric Cambisol soil (mg kg^{-1}), after growing maize plants for 21 days. The soil was treated with 0, 0.1, 0.25, 0.5, 0.75, 1.0, and 1.25 mg of Zn kg^{-1} soil from bulk (ZnCl2) or nanomaterial (ZnO). C represents control samples. The values represent means ±SEM (n= 3). Asterisks denote degree of statistical difference between the control and individual treatments (*** = $p < .001$; ** $p \leq 0.01$; * = $p \leq$ 0.05). Error bars denote one standard error.

The results of statistical analysis observed no significant different between the total concentration of Zn in the soil as regards the concentration of Zn NPs and bulk Zn. The mean overall total concentration of bulk Zn was 61.00 mg kg^{-1} (SE: ± 7.03); that of Zn NPs was 46.22 (SE: ± 4.47).

The concentrations of Zn from the two compounds (ZnO NPs and $ZnCl_2$) in Eutric Cambisol soil were high compared with added levels of Zn. In the present study, the total concentration of ZnO NPs increased 38–fold compared with initial levels of ZnO NPs were added to Eutric Cambisol soil and 43–fold for $ZnCl_2$. This could be attributed to the residual effect of the fertilizers applied to the soil during collection of Eutric Cambisol samples; fertilizers might increase the total concentration of Zn in soil.[61–63]

In general, the presence of ZnO NPs and $ZnCl_2$ in the Eutric Cambisol soil significantly increased the total concentration of Zn NPs in soil solution. The total concentration of Zn NPs was higher than those found in the soil treated with $ZnCl_2$. This could be due to the low solubility, precipitation, and aggregation of ZnO NPs in the soil solution compared with

properties of $ZnCl_2$. These processes can be controlled by factors such as the size of the NPs and the pH of the dissolved media.[71] Furthermore, manufactured nanomaterials (MNMs) adsorbs on the surface of natural organic matter (NOM) and natural colloids, which affect their surface properties, fate, and transport in soil.[76,77]

4.12. Inhibition of maize seeds germination and root growth by nanoparticles

4.12.1. Objectives

The aim of this study was to determine the effect of CdS and ZnO NPs on the germination of maize seeds and to evaluate the development of their primary roots in terms of their length and dry weight by the end of seed germination (8 days). The range of applied concentrations was used to help identify the level at which these NPs become toxic for root development. The investigation into the effects of using different concentrations of CdS and ZnO NPs in maize roots and seeds was also used to help determine their availability and bio–accumulation during the preliminary stage of seed germination.

4.12.2. Materials and methods

4.12.2.1. Seed germination

The maize seeds were obtained from the Environment Centre Wales, Bangor University. Maize seeds (*Zea mays*) were germinated in Petri dishes and according to the procedure used in Chapter 4, Section 4.4.2. The seeds were rinsed three times with deionised water before germination. One piece of filter paper (Whatman® 42 ashless filter paper) was placed in each Petri dish. Five maize seeds were selected for each NP concentration (210 maize seeds in total).

4.12.2.2. Chemical treatments

CdS and ZnO NPs were used to treat the maize seeds in Petri dishes. The characterization of these NPs is described in Table 4.1. The NPs were suspended in deionised water and dispersed using ultrasonic vibration (100 w, 40 kHz) for 30 minutes. The solutions were then stirred before adding them to the seeds to avoid aggregation in the Petri dishes. Different concentrations of CdS and ZnO NPs were prepared as follows: 0, 2, 5, 10, 20, 50 and 100 mg Cd L^{-1}, and 0, 2, 10, 20, 50, 200 and 1000 mg Zn L^{-1}. Each concentration was repeated three times. The controls (0 mg Cd L^{-1} and 0 mg Zn L^{-1}) were watered with deionised water. Five mL of each NP concentration of each metal were added to the maize seeds. A constant distance was maintained between each tested seed. Each Petri dish was covered with a piece of paper and watered twice a day with the prepared solutions. Finally, the dishes were covered with aluminium foil to allow the seeds to germinate in a dark environment and placed on laboratory benches at a room temperature of 25°C for 8 days.[10] The Petri dishes were arranged in a randomised block design,

The Petri dishes used in this experiment (42 Petri dishes) were classified into two main groups (21 Petri dishes for each main group). Maize seedlings shown in Figure 4.16.

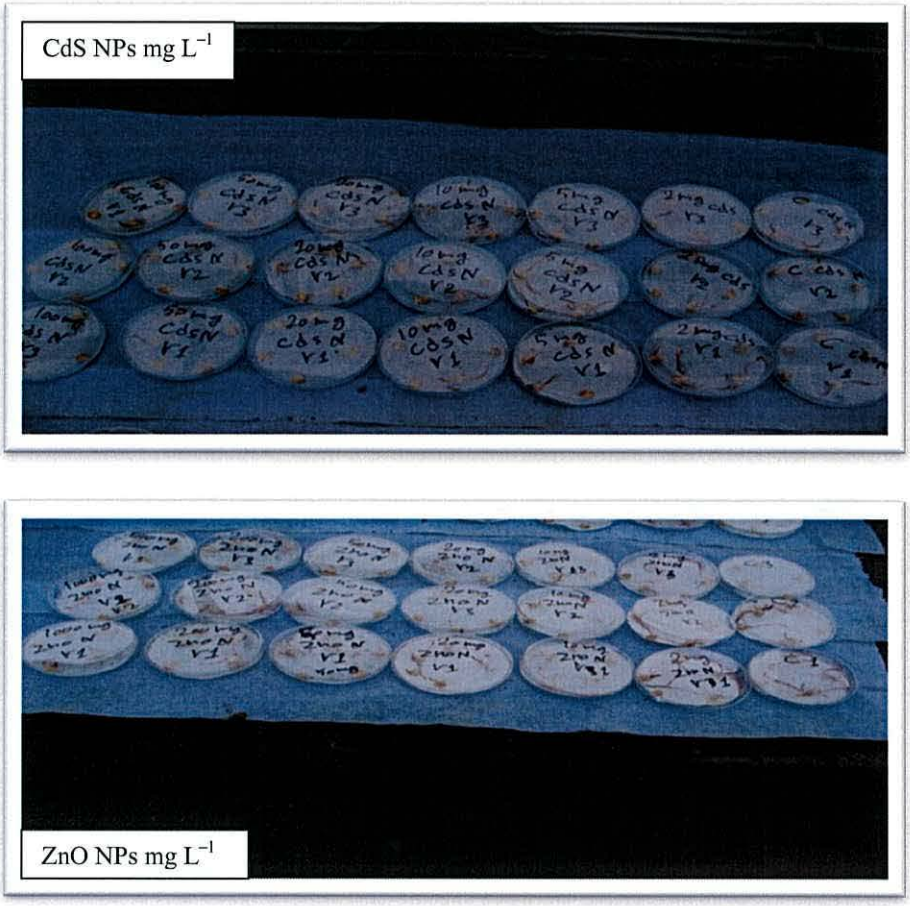

Figure 4. 16. Maize seedlings treated with CdS and ZnO NPs placed in Petri dishes germinating for 8 days.

4.12.2.3. Plant analysis

The method for the analysis of heavy metals in maize plants by inductively coupled plasma spectroscopy (ICP–OES) was as described in Chapter 3, Section 3.2.11.

4.13. Statistical analysis

The concentrations of CdS and $ZnCl_2$ NP in maize roots and seeds and all the maize parameters (the dry weight and the length of maize roots); these were performed with three replicates of each concentration. Means and standard errors were calculated using Microsoft Excel. All the data that pertained to the different concentrations and seedling parameters were subjected to a one way analysis of variance (ANOVA) and differences identified with a Tukey's HSD test using

SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). Significant differences were accepted at the ($p < 0.05$) level.Graphs was plotted using Sigma Plot 12.3 for Windows.

4.14. Results and discussion of Experiment 2

4.14.1. Concentration of cadmium and zinc NPs in the tested seedlings

Statistical analysis of results observed a significant difference ($p < 0.01$) as regards the concentration of Cd NPs in maize seeds at treatment concentrations of 5, 10, 20, 50, and 100 mg L^{-1} compared with the control. The tests also observed a significant difference ($p < 0.01$) as regards the concentration of Cd NPs in maize roots at levels of 20, 50, and 100 mg L^{-1} compared with the control; the results also indicted a significant difference ($p < 0.05$) as regards Cd NPs in roots at a concentration of 10 mg L^{-1}.

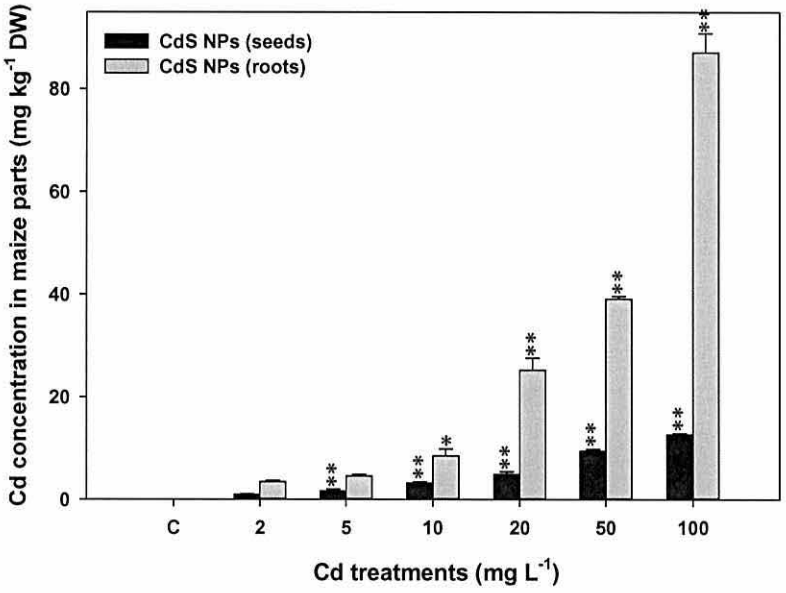

Figure 4. 17. The concentrations of Cd in maize seeds and roots grown for 8 days in suspension. solution. Seeds were treated with 0, 2, 5, 10, 20, 50, and 100 mg of CdS NPs L^{-1}. The values were given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

Results as regards ZnO NPs indicated significant differences ($p < 0.01$) for Zn concentration in maize seeds and roots at concentrations of 20, 50, 200, and 1000 mg L^{-1} compared with their controls, with exception of Zn concentration in maize seed was significant difference ($p < 0.05$) at concentration 10 mg L^{-1}. The concentrations of Cd and Zn NPs increased in maize seeds and roots with increased NPs concentrations in the suspension solution (Figures 4.17 and 4.18).

Lopez–Moreno *et al.*[10] reported that the uptake of Zn by soybean seedlings might be different. The workers found the highest accumulation of Zn (229 mg kg^{-1}) was obtained at 500 mg L^{-1} of ZnO NPs. However, at high concentrations of 1000, 2000, and 4000 mg L^{-1}, the concentrations of Zn in test plant varied from 135 to 150 mg kg^{-1}. In the present study, the highest uptake of Zn NPs by maize roots was at 1000 mg L^{-1} (513.74±1.88 mg Zn kg^{-1}DW). Franklin *et al.*[71] reported that Zn uptakes can be driven by the agglomeration of the NPs in the media and by their dissolution. Also, as indicated, there are many factors that influence the behaviour of NPs in agricultural food crops–their concentration, stability, specific surface area, particle size, physicochemical properties, and the species of the plant, the growth media (soil and hydroponic) and dilution agent.[58] The accumulation of Cd and Zn in maize roots was higher than in its seeds for any of the applied concentrations, as shown in Figures 4.17 and 4.18.

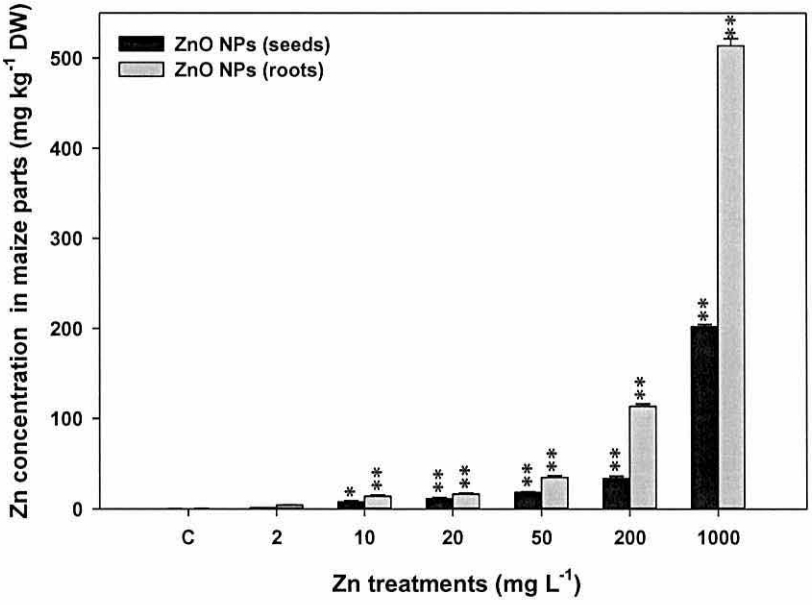

Figure 4. 18. The concentrations of Zn in maize seeds and roots grown for 8 days in suspension solution. Seeds were treated with 0, 2, 10, 20, 50, 200 and 1000 mg of ZnO NPs L^{-1}. The values were given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

In general, the concentration of Zn NPs in maize seeds and roots was higher than that of Cd NPs at any concentration. This could be because Zn is an essential element for root development and

growth.[10] In addition, the solubility of ZnO is greater than that of CdS, which increases the availability of free Zn ion for maize roots.[36,71]

4.14.2. Effect of NP suspensions on seed germination and root growth

Figures 4.19 and 4.20 suggest that CdS has negative effect on the length and dry biomass of maize roots for the majority of Cd concentrations compared with their controls.

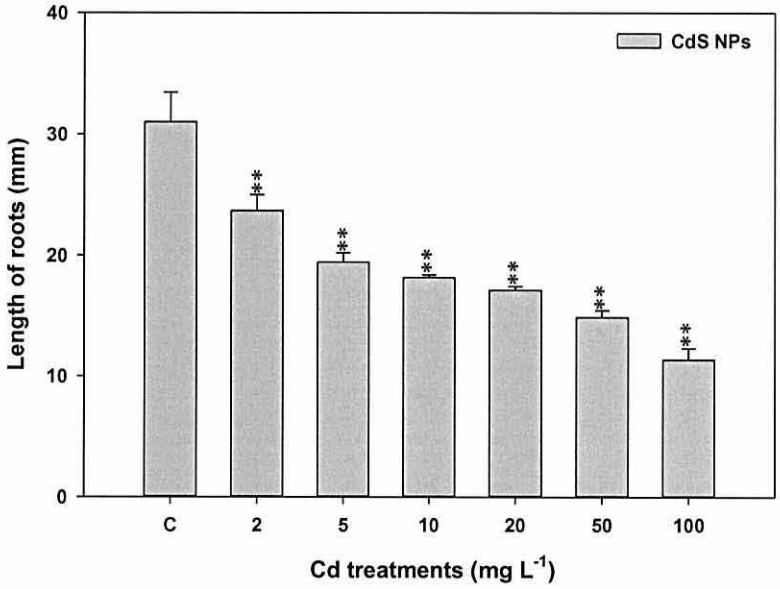

Figure 4. 19. The impact of Cd on the length of maize roots (mm) grown for 8 days in suspension solution. Seeds were treated with 0, 2, 5, 10, 20, 50 and 100 mg of CdS L^{-1}. The values were given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

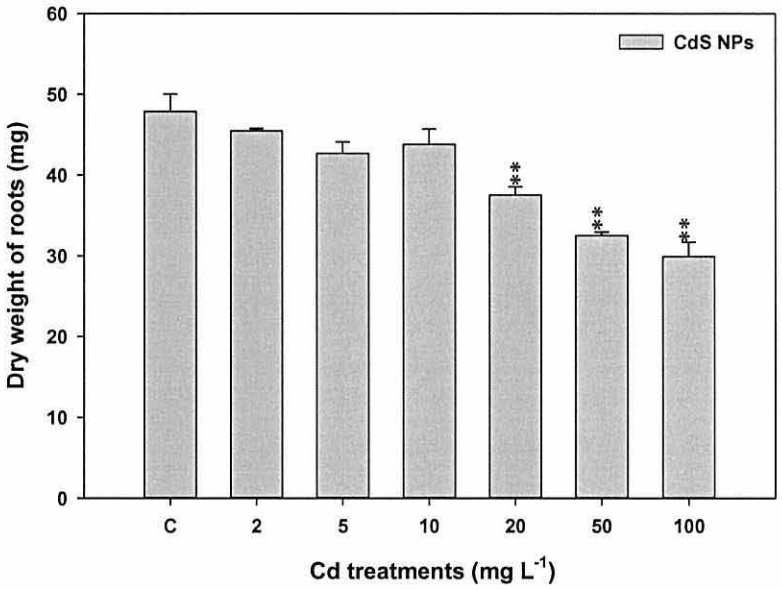

Figure 4. 20. The impact of Cd on the dry biomass of maize roots (mg), grown for 8 days in suspension solution. Seeds were treated with 0, 2, 5, 10, 20, 50 and 100 mg of CdS L^{-1}. The values were given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

Statistical analysis observed significant differences ($p < 0.01$) as regards Cd NPs on the length of maize roots for any of the concentrations compared with their control. Results also indicated a significant difference ($p < 0.01$) as regards Cd NPs on the dry weight of the maize roots at concentrations of 20, 50 and 100 mg L^{-1} compared with their control group. This agrees with results of Tantawy,[34] together with El–Kassas *et al.*[35] on the effect of bulk Cd on maize parameters. Results for the ZnO NPs observed that ZnO concentration has significantly effects ($p < 0.01$) on the length of roots at levels of 200 and 1000 mg L^{-1} compared with their control and a significant difference at concentrations of 50 mg L^{-1} ($p < 0.05$), as shown in Figure 4.21.

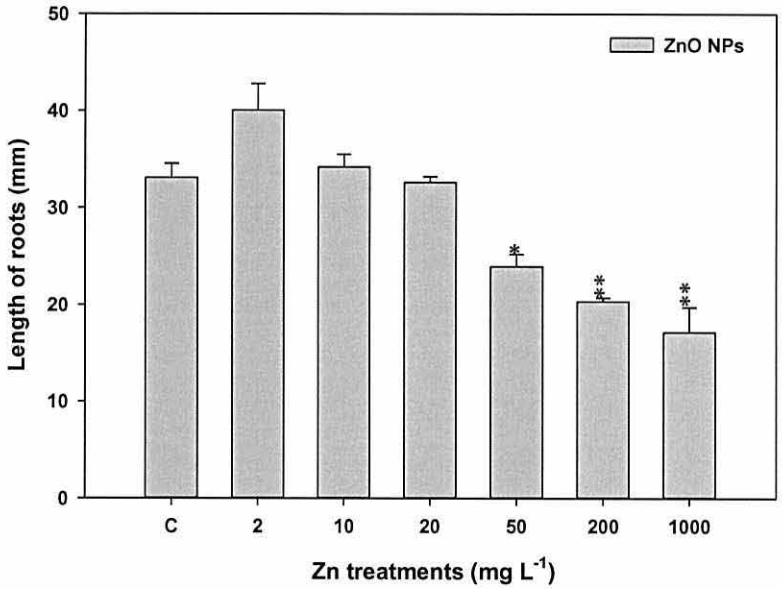

Figure 4. 21. The impact of Zn on the length of maize roots (mm) grown for 8 days in suspension solution. Seeds were treated with 0, 2, 10, 20, 50, 200 and 1000 mg of ZnO NPs L^{-1}. The values were given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p < 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

In addition, results indicated that ZnO has a negative effect on the dry biomass of maize root ($p < 0.01$) at 1000 mg L^{-1} concentration compared with their control, as shown in Figure 4.22. This agrees with results from Lopez–Moreno *et al.*[10] who reported that an inverse U–shape response of root elongation was observed in seedlings treated with ZnO NPs. The NP's maximum size was attained at a concentration of 500 mg L^{-1} (30% over its control) and its minimum size at 4000 mg L^{-1} (40% shorter than control). The results also agree with those found by Lin and Xing who reported a significant decrease in root growth when several plant species were exposed to 2000 mg ZnO NPs L^{-1}.[78] In the present study, the maximum elongation and weight of the roots were observed at 2 mg Zn L^{-1}. Generally, CdS NPs showed a statistically significant inhibition on the elongation and dry weight of roots greater than ZnO NPs at any of the applied concentrations. See Figure 4.23.

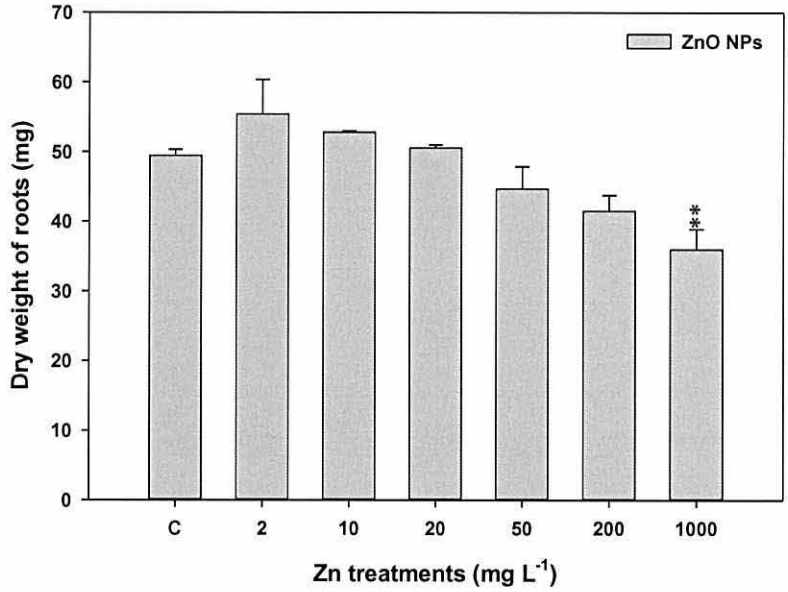

Figure 4. 22. The impact of Zn on the dry biomass of maize roots (mg) grown for 8 days in suspension solution. Seeds were treated with 0, 2, 10, 20, 50, 200 and 1000 mg of ZnO NPs L^{-1}. The values were given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

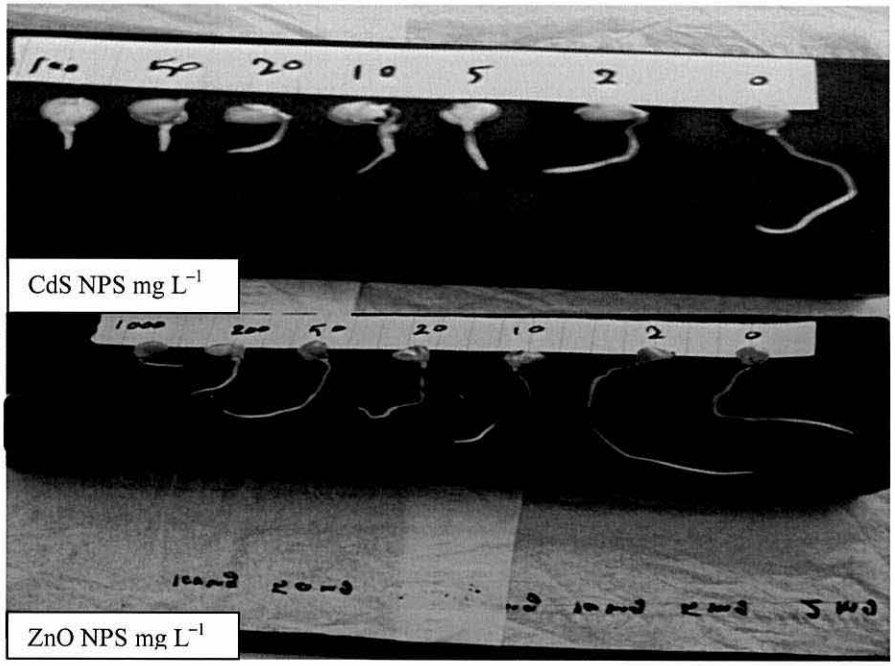

Figure 4. 23. The images of CdS NPs and ZnO NPs impacts on the length of maize roots at different NPs concentrations mg L^{-1}.

Seed germination was inhibited by the NP suspension, which were 69 % (±1.0) for ZnO and 58 % (±0.60) for CdS. This is in contrast to results obtained by Ma *et al.*[79] but this research involved different NPs. Results of the present study, however, agree with those obtained by Lin and Xing.[78]

4.15. Conclusions

- The concentration of all nanoparticles (CdS NPs and ZnO NPs) and their soluble bulk counterparts (bulk $CdCl_2$ and $ZnCl_2$) in maize roots and shoots increased with increasing the level of addition metals to the Eutric Cambisol soil. The concentration of these metals was higher in roots than in shoots. The concentration of bulk counterparts in the maize roots and shoots was higher than those found with nanoparticles. Furthermore, the total concentration of Zn (ZnO NPs and ZnCl2) in the maize roots and shoots were high compared with added levels (0–1.25 mg kg^{-1}) of Zn in the soil. Possible explanation for the high concentration of Zn in the soil solution is that the residual effect of the fertilizers applied to the soil during sample collection; these fertilizers may increase the total concentration of Zn in maize plant. These high levels of Zn in the maize plants appeared to decrease the tolerance index (TI) of maize roots and shoots for the majority of Zn treatments in the Euric Cambisol soil.
- There were no observable negative effects for the majority of nanoparticles and their soluble bulk counterparts on the dry weight of maize roots and shoots compared with controls across all concentrations and compound types. However, bulk Cd had a negative effect on the length of maize shoots at a concentration of 1.25 mg kg^{-1} compared with the control. Both Cd compounds (CdS NPs and bulk $CdCl_2$) had a negative effect on the length of maize roots at concentrations of 1.0 and 1.25 mg kg^{-1} for Cd NPs and 1.25 mg kg^{-1} for bulk Cd. ZnO NPs also showed a negative effect on the length of maize shoots at concentrations of 1.25 mg kg^{-1} compared with the control.
- The BCRs of nanoparticles and their soluble bulk materials in maize roots and shoots slightly varied according to the studied factors including plant part, growth period and concentrations. The majority of BCR increased in the maize roots and shoots with increasing concentration rate for both Cd compounds (CdS and $CdCl_2$) in the Eutric Cambisol soil. However, the BCR of Zn from each compound (ZnO and $ZnCl_2$) decreased in the maize parts as a function of their concentration in the soil. This could be attributed to the residual effect of the fertilizers applied to the soil during sample collection; fertilizers might increase the Zn concentration, and reduce the BCR of Zn in a maize plant.[58,59,60] The BCR of all metals in maize roots was higher than those found in the

shoots across all the applied concentrations and metal types. The BCRs of soluble bulk counterparts in maize roots and shoots were higher than that of nanoparticles.

- The tolerance of maize plant for metals concentrations varied considerably across all metal compounds (nanoparticles and bulk materials) and their concentrations. The tolerance index (TI) of maize roots and shoots appeared to decrease in most treatments with increasing concentration of NPs and their soluble bulk counterparts in the Euric Cambisol soil. However, the TI of maize roots for Zn NPs slightly increased across all of the Zn NP concentrations compared to the first concentration (0.1 mg kg^{-1}). The TI of maize shoots was higher than TI of roots in the most concentrations across all compound types (nanoparticles and bulk materials). The tolerance index of maize roots and shoots for bulk Cd was higher than that of Cd NPs. However, maize roots showed a high tolerance for Zn NPs at concentrations of 0.5, 0.75, 1.0, and 1.25 mg kg^{-1} compared to those for bulk Zn.
- The RI (%) and AE values of dry biomass of shoots and roots decreased in most metal treatments with increases in the concentrations of nanoparticles and their soluble bulk counterparts in the soil. The majority of RI (%) and AE values were negative values. This may have been because the dry biomass of treated plant parts (roots and shoots) was decreased by the different compounds of these metals compared to control samples.
- The calculated uptake and uptake % of nanoparticles and their soluble bulk counterparts by maize roots and shoots gradually increases with increases in their concentrations to the soil in the most of the metal concentrations. In addition, the uptake of these metals by the maize roots was higher than shoots in most metal concentrations across all metal compounds. The uptake of bulk materials by maize roots and shoots was higher than the uptake of nanoparticles. The total concentration of all nanoparticles was higher than their soluble bulk counterparts in the Eutric Cambisol soil. This could have been due to the low solubility, precipitation, and aggregation of nanoparticles in the solution compared to their soluble bulk counterparts. This process can be controlled by factors such as the size of the NPs and the pH of the dissolved media.[68]
- The uptake of CdS and ZnO NPs by maize roots and seeds grown in Petri dishes for 8 days revealed that the concentrations of Cd NPs and Zn NPs increased in maize seeds and roots with increased NPs concentrations in the suspension solution. The accumulation of

Cd and Zn NPs in maize roots was higher than that of seeds for all the applied concentrations. The concentration of Zn NPs in maize seeds and roots was higher than that of Cd NPs at any applied concentration.

- CdS NPs has negative effects on the length of maize roots across all the concentrations compared with their controls. Results also indicated negative effects of CdS NPs on the dry weight of the maize roots at concentrations of 20, 50 and 100 mg L^{-1} compared with their control group.
- Results for the ZnO NPs observed negative effects on the length of roots at concentrations of 50, 200 and 1000 mg L^{-1} compared with the control group. In addition, results revealed ZnO has a negative effect on the dry biomass of maize root at a concentration of 1000 mg L^{-1} compared with the control sample. CdS NPs showed a significant inhibition on the elongation and dry weight of roots greater than ZnO NPs at any of the applied concentrations. Seed germination was inhibited by the NP suspension, which were 69 % for ZnO and 58 % for CdS.

4.16. References

1. Ma, X., Geiser–Lee, J., Deng, Y., and Kolmakov, A. (2010). Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. *Science of the Total Environment.* 408 (16): 3053–3061.

2. Christian, P., Kammer, F., Baalousha, M., Hoffmann, T. (2008). Nanoparticles, structure, properties, preparation and behavior in environmental media. *Ecotoxicology.*17 (5): 326–43.

3. Ju–Nam, Y., and Lead, J. R. (2008). Manufactured nanoparticles: an overview of their chemistry, interactions and their potential environmental implications. *Science of Total Environment.* 400 (1–3): 396–414.

4. Zhu, H., Han, J., Xiao, J.Q., and Jin, Y. (2008). Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. Journal of Environmental Monitoring. 10 (6): 713–717.

5. Lin, S., Reppert, J., Hu, Q., Hudson, J.S., Reid, M.L., Ratnikova, T.A., Rao, A.M., Luo, H., and Ke, P.C. (2009). Uptake, translocation, and transmission of carbon nanomaterials in rice plants. *Small nano micro (weinheim an der bergstrasse, germany).* 5 (10): 1128–1132.

6. Lin D., and Xing, B. (2008). Root uptake and phytotoxicity of ZnO nanoparticles. *Environmental Science and Technology.* 42 (15): 5580–5585.

7. Lee, W.M., An, Y.J., Yoon, H., Kweon, H.S. (2008). Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): plant uptake for water insoluble nanoparticles. *Environmental Toxicology and Chemistry.* 27 (9): 1915–1921.

8. Harris, A., and Bali, R. (2008). On the formation and extent of uptake of silver nanoparticles by live plants. *Journal of Nanoparticle Research.* 10 (4): 691–695.

9. Singh, V., and Chauhan, P. (2009). Synthesis and structural Properties of wurtzite type CdS nanoparticles. *University of Allahabd, India.* 6 (8): 421 – 426.

10. Lopez–Moreno, M.L., De la Rosa, G., Hernandez–Viezcas, J.A., Castillo–Michel, H., Botez, C.E., Peralta–Videa, J.R., and Gardea–Torresdey, J.L. (2010). Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (*Glycine max*) plants. *Environmental Science and Technology.* 44 (19): 7315–7320.

11. Fulcher, B., Tang, B., and Frutnik, D. (2004). Production of zinc oxide with variablemorphology by two novel wet–chemical methods. *Electron microscope Unit*, Faculty of Science, University of Sydney.

12. Karimi, M.A., Roozbahani, S.H., Asadiniya, R., Hatefi–Mehrjardi, A., Behjatmanesh–Ardakani R., Kargar, H., Mashhadizadeh, M.H., Mazloum–Ardakani, M., and Zebarjad, S.M. (2011). Synthesis and

characterization of nanoparticles and nanocomposite of ZnO and MgO by sonochemical method and their application for zinc polycarboxylate dental cement preparation. *International Nano Letters*. 1 (1): 43–51.

13. Ellis, S.C. (2011). Ecotoxicology of metal oxide nanoparticles: Effects on soil process and plant growth. University of Wales, Bangor, *MSc thesis*, pp: 24–40.

14. Perriguey, J., Sterckeman, T., and Morel J. L. (2008). Effect of rhizosphere and plant–related factors on the cadmium uptake by maize (*Zea mays* L.). *Environmental and Experimental Botany*. 63 (1–3): 333–341.

15. Liu, D.H., Wang, M., Zou, J. H., and Jiang, W.S. (2006). Uptake and accumualtion of cadmium and some nutrients ions by roots and shoots of maize (*zea mays*. L). *Pakistan journal of botany*. 38 (3): 701–709.

16. Agarwala, S.c., and Chatterjee, C. (1996). In: Advancement in micronutrients research. (Hemantaranjan. A cd). Scientific Publishers, Jodhpur, India, pp. 401–453.

17. Lindberg, S., Landberg, T., and Greger, M. (2004). A new method to detect cadmium uptake in protoplasts. *Planta*. 219 (3): 526–32.

18. Chaddha, A. (1990). Agricultural Statistics in India, *Commonwealth Publishers*, New Delhi, India.

19. Selvam, A., and Wong, J.W–C. (2009). Cadmium uptake potential of *Brassica napus* cocropped with Brassica parachinensis and *Zea mays*. *Journal of Hazardous Materials*. 167 (1–3): 170–178.

20. Tai, G., Zhou, J., and Guo, W. (2010). Inorganic salt–induced phase control and optical characterization of cadmium sulfide nanoparticles. *Nanotechnology*. 21 (17).

21. Hsieh, C. H. (2007). Spherical zinc oxide nano Particles from zinc acetate in the precipitation method. *Journal of the Chinese Chemical Society*. 54: 31–34.

22. Sabura–begum, P. M., Yusuff., M.K., Rani and Joseph. Studies on the use of nano zinc oxide and modified silica in NR, CR and SBR (2009). *Department of Applied Chemistry*, Cochin University of Science and Technology, chapter (3).

23. Zhang, L., Zhang, L., and Song, F. (2008). Cadmium uptake and distribution by different maize genotypes in maturing stage. *Communications in Soil Science and Plant Analysis*. 39 (9–10): 1517–1531.

24. Chen, F., Wu, F., Dong, J., Vincze, E., Zhang, G., Wang, F., Huang, Y., and Wei, K. (2007). Cadmium translocation and accumulation in developing barley grains. *Planta*. 227 (1): 223–232.

25. Kretzschmar, R., and Schafer, T. (2005). Metal retention and transport on colloidal particles in the environment. *Elements*. 1, 205–210.

26. Wang, M., Zou J., Duan, X., Jiang, W., and Liu, D. (2007). Cadmium accumulation and its effects on metal uptake in maize (*Zea mays* L.). *Bioresource Technology*. 98 (1): 82–88.

27. Aboulroos, S. A., Holah, Sh. Sh., And Badawy, S. H. (1996). Background levels of some heavy metals in soils of Egypt and associated corn plant. Egypt. *Journal of Soil Science*. 36: 83–97.

28. Chaney, R. L., and Hornick, S. B. (1978). Accumulation and effects of cadmium on crops. Cadmium 77: *Proceedings of 1st International Cadmium Conference San Francisco*, Pp 125–140, Metal Bulletin Ltd, London.

29. Lavado, R. S., Porcelli, C. A., and Alvarez, R. (2001). Nutrient and heavy metal concentration and distribution in corn, soybean and wheat as affected by different tillage systems in the Argentine Pampas. *Soil and Tillage Research.* 62 (1–2): 55–60.

30. Yang, L., and Watts, D.J. (2005). Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicology Letters.* 158: 122–132.

31. Stampoulis, D., Sinha, S.K., and White, J.C. (2009). Assay–dependent phytotoxicity of nanoparticles to plants. *Environmental Science and Technology.* 43 (24): 9473–9479.

32. Adiloglu, A. I., Adiloglu, S., Gonulsuz, E ., and Oner, N. (2005). Effect of zinc application on cadmium uptake of maize grown in zinc deficient soil. *Pakistan Journal of Biological Sciences.* 8 (1): 10–12.

33. Artur, N., Kosynets, O., Nosalewicz, M. (2008). Effect of various concentrations of lead and cadmium on early growth of maize. *Acta Agrophysica.* 11 (3): 715–723.

34. Tantawy, M. F. 2004. Monitoring of environmental pollution with heavy metals in some delta soils and its phytoremediation. *PhD thesis.* pp.118–121.

35. El–Kassas, I. H., Bader, A., and Amer, M. N. (2002). phytoremediation of cadmium contaminated soils using different hyperaccumulation plants. *Zagazig Journal of Agricultural Research.* 29 (4): 1199–1213.

36. IPCS. (2005–2007). Cadmium, cadmium chloride, cadmium oxide, cadmium sulphide, cadmium acetate, cadmium sulphate. Geneva, World Health Organization, International Programme on Chemical Safety (International Chemical Safety Cards 0020, 0116, 0117, 0404, 1075 and 1318; http://www.who.int/ipcs/publications/icsc/en/index.html).

37. Chitra, K., Sharavanan, S., and Vijayaragavan, M. (2011). Tobacco, corn and wheat for phytoremediation of cadmium polluted soil. *Science and Technology.* 3 (2): 148–151.

38. Kacalkova, L., Tlustos, P., and Szakova, J. (2009). Phytoextraction of cadmium, copper, zinc and mercury by selected plants. *Plant, Soil and Environment.* 55 (7): 295–304.

39. Dunbar.K.R. (2004). Uptake and partitioning of cadmium in two cultivars of potato (*Solanum tuberosume* L.). *PhD thesis*, University of Adelaide South Australia.

40. Wei, J.L., Lai, H.Y., and Chen, Z, S. (2012). Chelator effects on bioconcentration and translocation of cadmium by hyperaccumulators, *Tagetes* patula and *Impatiens walleriana*. *Ecotoxicology and Environmental Safety.* 84: 173–178.

41. Wangstrand, H., Eriksson, J.,and Oborn, I. (2007). Cadmium concentration in winter wheat as affected by nitrogen fertilization. *European Journal of Agronomy.* 26: 209–214.

42. Pehlivan., E., Yanık, B.H. Ahmetli, G.and Pehlivan, M., 2008. Equilibrium isotherm studies for the uptake of cadmium and lead ions onto sugar beet pulp. *Bioresource Technology.* 99 (9): 352–357.

43. Rascio, N., Vecchia, F. D., Rocca, N.L., Barbato, R., Pagliano, C., Raviolo, M., Gonnelli, C., and Gabbrielli, R. (2008). Metal accumulation and damage in rice (cv. *Vialone nano*) seedlings exposed to cadmium. Environmental and Experimental Botany.62: 267–278.

44. Sun, Y., Zhou, Q., and Diao, C. (2008). Effects of cadmium and arsenic on growth and metal accumulation of Cd–hyperaccumulator *Solanum nigrum* L. *Bioresource Technology.* 99: 1103–1110.

45. Chen, R., Ratnikova, T. A., Stone, M. B., Lin, S., Lard, M., Huang, G., Hudson, J. S., and Ke, P. C. (2010). Differential uptake of carbon nanoparticles by plant and mammalian cells. *Small*, 6: 612–617.

46. Rico, C.M., Majumdar, S., Duarte–Gardea, M., Peralta–Videa, J.R., and Gardea–Torresdey, J.L. (2011). Interaction of nanoparticles with edible plants and their possible implications in the food chain. *Journal of Agricultural and Food Chemistry.* 59 (8): 3485–3498.

47. Elsokkary, I. H., and Sharaf, A. I. (1996). Enrichment of soils and plants irrigated by wastewater by zinc and cadmium. *Egyptian Journal of Soil Science.* 36 (1/4): 209–232.

48. Bauddh, K., and Singh, R.P. (2011). Differential toxicity of cadmium to mustard (*Brassica juncia* L.) genotypes under higher metal levels. *Journal of Environmental Biology / Academy of Environmental Biology*, India. 32 (3): 355–362

49. Chen, X., Wang, J., Shi, Y., Zhao, M.Q., and Chi, G.Y. (2011). Effects of cadmium on growth and photosynthetic activities in pakchoi and mustard. *Botanical Studies.* 52 (1): 41–46.

50. Symeonidis, L., Mcneilly, T., and Bradshaw, A. D. (1985). Differential tolerance of three cultivars of *Agrostis capillaris* L. to cadmium, copper, lead, nickel and zinc. *New Phytologist.* 101 (2): 309–315.

51. Belimov, A.A., Safronova, V.I., Tsyganov, V.E., Borisov, A.Y., Kozhemyakov, A.P., Stepanok, V.V., Martenson, A.M., Gianinazzi–Pearson, V., and Tikhonovich, I.A. (2003). Genetic variability in tolerance to cadmium and accumulation of heavy metals in pea (*Pisum sativum* L.). *Euphytica.* 131 (1): 25–35.

52. Hakmaouia, A., Mohammed, A., Karoly, B., and Matilde, B. (2007). Copper and Cadmium Tolerance, Uptake and Effect on Chloroplast Ultrastructure. Studies on *Salix purpurea* and *Phragmites australis. Journal of Bioscience.* 62: 417–426

53. Kabir, M., Zafar Iqbal, M., and Shafiq, M. (2011). Toxicity and tolerance in *Samanea saman* (JACQ.) Merr. to some metals (Pb, Cd, Cu and Zn). *Pakistan Journal of Botany.* 43 (4): 1909–1914.

54. Odjegba, V., and Fasidi, I. (2004). Accumulation of Trace Elements by *Pistia stratiotes*: Implications for phytoremediation. *Ecotoxicology.* 13 (7): 637–646.

55. Graham, R. D. (1978). Nutrient efficiency objectives in cereal breeding. In Proceedings of the 8th international colloquium on plant analysis and fertilizer problems, 165–170. New Zealand Division of Scientific and Industrial Research, Information Series no. 134. Wellington, New Zealand: Government Printer.

56. Cooke, G.W. (1987). Maximizing fertilizer efficiency by overcoming constraints. *Journal of Plant Nutrient*. 10. 1357–1369.

57. Alloway, B. J. (1995). Heavy metals in soils. London: Blackie Academic and Professional.

58. Keller, A.A., Wang, H., Zhou, D., Lenihan, H.S., Cherr, G., Cardinale, B.J., Miller, R., and Ji Z. (2010). Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environmental Science and Technology*. 44 (6): 1962–1967.

59. Benavides, M.P., Gallego, S.M., Tomaro, M.L. (2005) Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology*. 17:21–34.

60. Holmgren, G. G. S., Meyer, M. W., Chaney, R. L., and Daniels, R. B. (1993). Cadmium, lead, zinc, copper, and nickel in agricultural soils of the United States of America. *Journal of Environmental Quality*. 22 (2): 335.

61. Roberts, P., Roberts J.P., and Jones, D.L. (2006). Behaviour of the endocrine disrupting chemical nonylphenol in soil: Assessing the risk associated with spreading contaminated waste to land. *Soil Biology and Biochemistry*. 38 (7): 1812–1822.

62. Gondek, K. (2009). Zinc content in maize (*Zea mays* L.) and soils fertilized with sewage sludge and sewage sludge mixed with peat. *Polish Journal of Environmental Studies*. 18 (3): 359–368.

63. Martin–Ortiz, D., Hernandez–Apaolaza, L., and Garate, A. (2009). Efficiency of a NPK fertilizer with adhered zinc lignosulfonate as a zinc source for maize (*Zea mays* L.). *Journal of Agricultural and Food Chemistry*. 57 (19): 9071–9078.

64. Wang, J., Mao, H., Zhao, H., Huang, D., and Wang, Z. (2012). Different increases in maize and wheat grain zinc concentrations caused by soil and foliar applications of zinc in Loess Plateau, China. *Field Crops Research*. 135: 89–96.

65. Wang, Q. R., Cui, Y. S., Liu, X. M., Dong, Y. T., and Christine, P. (2003). Soil contamination and plant uptake of heavy metals at polluted sites in China. *Journal of Environmental Science and Health*. 38 (5). P. 823–838.

66. Wang, H., and Jin, J. (2007). Effects of zinc deficiency and drought on plant growth and metabolism of reactive oxygen species in maize (*Zea mays* L). *Agricultural Sciences in China*. 6 (8): 988–995.

67. Zhao, L., Hernandez–Viezcas, J. A., Peralta–Videa, J. R., Bandyopadhyay, S., Peng, B., Munoz, B., Keller, A.A., and Gardea–Torresdey, J.L. (2012). ZnO nanoparticle fate in soil and zinc bioaccumulation

in corn plants (*Zea mays*) influenced by alginate. *Environmental Science: Processes and Impacts.* 15 (1): 260.

68. Vasconcelos, A. C. F.d, Nascimento, C. W. A., and Filho, F. F. d. C. (2011). Distribution of zinc in maize plants as a function of soil and foliar Zn supply. *Journal of Agricultural Science and Soil Science.* 1(1): 001–005.

69. Naylor, L.M., Loehr, R.C., and M. Barmasse. (1987). Uptake of cadmium and zinc by corn on sludge–treated soils. *BioCycle.* 28:4: 37–41.

70. Huang, P, M., Li, Y., and Sumner, M. E. (2012). Handbook of soil sciences: resource management and environmental impacts. *Boca Raton*, CRC Press.

71. Franklin, N.M., Rogers, N.J., Apte, S.C., Batley, G.E., Gadd, G.E., and Casey, P.S. (2007). Comparative toxicity of nanoparticulate ZnO, bulk ZnO and $ZnCl_2$ to a freshwater microalga (*Pseudokirchnerilla subcapitata*): the importance of particle solubility. *Environmental Science and Technology.* 41(24): 8484–8490.

72. Milani, N., McLaughlin, M.J., Hettiaratchchi, G.M., Beak, D.G., Kirby, J.K., and Stacey, S. (2010). Fate of nanoparticulate zinc oxide fertilisers in soil: solubility, diffusion and solid phase speciation. *World Congress of Soil Science, Soil Solutions for a Changing World*, Brisbane, Australia. conference paper. pp:172–174.

73. Kochian, L.V. (2000). Molecular physiology of mineral nutrient acquisition, transport, and utilization. In: Biochemistry and Molecular Biology of Plants (Eds Buchanan, B., Gruissem, W., and Jones, R.).*American Society of Plant Physiologists*, Rockville, MD. pp: 1237–1239.

74. Van de mortel, J.E., Villanueva, A. L., Schat, H., Kwekkeboom, J., Coughlan, S., Moerland, P.D., Themaat, V. L. V. E., Koornneef, M., and Aarts, M.G. (2006). Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of Arabidopsis thaliana and the related metal hyperaccumulator *Thlaspi caerulescens*. *Plant Physiology.* 142 (3): 1127–1147.

75. Jasiewicz.C., Agnieszka, B., Marek, T. (2010). Effect of bottom sediment on content bioaccumulation and translocation of heavy metals in maize biomass. *Journal of Elementology.* 15 (2): 281–290

76. Chen, K. L., Mylon, S. E., and Elimelech, M. (2006). Aggregation kinetics of alginate–coated hematite nanoparticles in monovalent and divalent electrolytes. *Environmental Science and Technology.* 40 (5): 1516–1523.

77. Stankus, D.P., Lohse, S.E., Hutchison, J.E., and Nason, J.A. (2011). Interactions between natural organic matter and gold nanoparticles stabilized with different organic capping agents. *Environmental Science and Technology.* 45 (8): 3238–3244.

78. Lin, D., and Xing, B. (2007). Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environmental Pollution*. 150 (2): 243–250.

79. Ma Y., Kuang L., He X., Bai W., Ding Y., Zhang Z., Zhao Y., and Chai Z. (2010). Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere*. 78 (3): 273–279.

Chapter 5: The uptake of high concentrations of nanoparticles and their partitioning in maize plants

5.1. Introduction

As novel and potentially toxic materials, the eco–toxicity of NPs is now receiving considerable attention from researchers and regulatory authorities.[1,2] Although plants have evolved in the presence of natural NPs, their exposure to MNPs has increased with the increased production and use of the particles in a variety of instruments and goods.[3] MNPs can reach plants by direct application, accidental release, contaminated soil or sediment, or atmospheric fallout.

Little is known about the effects of MNPs on food crops.[4,5] A few studies on the toxicity of MNPs have been performed on crop plants such as rape (*B. napus*), radish (*Raphanus sativus*), lettuce (*L. sativa*), maize (*Z. mays*) and cucumber (*Cucumis sativus*), among others.[6,7] A number of studies have demonstrated NP toxicity in plant species: NPs have been shown to reduce ryegrass (*L. perenne*) and thale cress (*A. thaliana*) seedling growth, and to alter root morphology under hydroponic conditions.[8,9]

ZnO NPs have been shown to enter ryegrass (*L. perenne*) cells and to pass through epidermal and cortical tissues via apoplastic pathways.[8] Magnetization has been detected in the roots and leaves of pumpkin plants (*C. maxima*) exposed to iron oxide NPs in their growth media.[10] Lin and Xing reported C_{70} uptake and accumulation in rice plants and in the seedlings of the next generation.[6] Multi–walled carbon nanotubes have been shown to penetrate directly into periwinkle (*Catharanthus roseus*) protoplasts.[11] Single–walled carbon nanotubes labelled with fluorescein isothiocyanate have been shown to entered Bright Yellow (BY–2) cells through endocytosis.[12] Carbon–coated magnetic NPs have been found to be assimilated by the roots of crops such as peas (*Pisum sativum*), sunflowers (*Helianthus annuus*), tomatoes (*Solanum lycopersicum*), and wheat (*T. aestivum*) and to be distributed throughout their plant tissues.[13] However, the mechanism(s) for their transport between roots and shoots and definitive evidence of their transformation in plant tissues is lacking. CuO NPs are used extensively in commercial applications.[14, 15] The toxicity of CuO NPs in aquatic organisms (e.g., fish and algae) has been observed.[16,17] For terrestrial plants, CuO NPs uptake by wheat (*T. aestivum*) and its toxicity in ryegrass (*L. perenne*), and radish (*R. sativus*) have been reported. However, knowledge about

the NPs' transport and fate in plants remains limited and there is no research focusing on maize plant.[18,19]

5.2. Objectives

The objectives of the present study are as follows:

1. To investigate the toxicity of CdS, ZnO and CuO NPs in maize plants (*Z. mays*) after 21 days of seedling growth in Eutric Cambisol soil and hydroponic culture.
2. To examine the uptake of these NPs by the maize roots and shoots when applied in high concentrations, including their distribution in the maize roots and shoots.
3. To evaluate the effect of the above NPs on maize parameters (dry yield matter and length of roots and shoots g/pot) using the appropriate equations to calculate the Tolerance Index (TI), Agronomical Efficiency (AE), Bio–concentration Ratio (BCR), Relative Increase (RI), and Uptake Ratios of maize roots and shoots.

5.3. Materials and methods

5.3.1. The characterisation of nanoparticulate powders using X–ray Diffraction (XRD) and scanning electron microscopy (SEM)

The sample powder of CuO NPs was prepared for XRD and SEM according to the procedure described in Chapter 4, Sections 4.3.1 and 4.3.2. The particle size for CuO NPs was calculated using image analysis software (Image–J 1.43 for Microscopy, USA).

5.4. Preparation of the soil and nutrient solution

Maize seedlings were grown in two media: Eutric Cambisol soil and hydroponic culture. The seedlings were treated in both cultures with high concentrations of CdS, ZnO, and CuO NPs in concentrations of 0.01, 0.1, and 1.0 g kg^{-1} soil/g L^{-1} nutrient solution. The Eutric Cambisol soil was collected from Bangor University's Henfaes Research Centre, Abergwyngregyn (531140N 41010W). Sample preparation and physical and chemical analysis was as described in Chapter 4, Section 4.4. Table 5.1 summarises soil properties.

Table 5. 1. Chemical composition and physical characteristics of the Eutric Cambisol soil

Measurement	Content
Texture	Clay loam
Moisture content (%)	28.92±0.03
pH	5.50±0.03
EC (ms cm^{-1})	0.64±0.004
Total carbon (g kg^{-1})	49.0±6.1
Total nitrogen (g kg^{-1})	7.7±0.1
NO_3^- (mg N L^{-1})	6.42±1.19
NH_4^+ (mg N L^{-1})	3.18±0.20
Water holding capacity (%)	70.43±0.06
CEC (mmol kg^{-1})	27.0±3.0
Total Zn concentration (mg kg^{-1})	39.06±2.37
Total Cd concentration (mg kg^{-1})	0.071±0.01

Values represent the means of three determinations (Mean ± SEM, n =3).

5.4.1. Nutrient solution

Watering of the maize seedlings was started at the time of planting and ended after 21 days of the growth period. The maize plants were irrigated every day using 33 mL of nutrient solution and de–ionized water, one day with nutrient solution and the alternate day with dH_2O. De–ionised water was used to rinse the excess nutrient solution from the soil. The nutrient solution was diluted (100% to 25 % strength) from macronutrient solution to give final working concentrations as follows: 1 mM KNO_3, 2 mM $Ca(NO_3)_2.4H_2O$, 0.75 mM $MgSO_4.7H_2O$, 0.67 mM $NaH_2PO_4.2H_2O$, and 0.1 mM Fe EDTA. The amount of Zn normally contained as part of the nutrient solution was omitted from this working stock to clarify the impact of added metals on maize plant and Eutric Cambisol soil.

5.4.2. Chemical treatments

The characteristics of the three types of NPs chosen for this study are shown in Table 5.2. Portions of Eutric Cambisol soil were treated with three different concentrations of each type of NP one week before the seedlings were planted. The preparation of the NPs concentrations are shown in detail in Table 5.3 and correspond to a concentration of 0, 0.01, 0.1 and 1.0 g kg^{-1}.

Table 5. 2. The characteristics of the NPs used for the adsorption experiment.

Compound	Size (nm)	Purity (%)	Surface area (m^2/g)	Particular morphology	Molecular weight (g/mol^{-1})
ZnO	90–210	99.9	5–7	irregular	81.39
CuO	40–80	99.9	–	–	79.55
CdS	~ 7.6– 17.7	–	–	–	144.48

The parameters of ZnO and CuO NPs were measured at the IoLitec Nanomaterials Company. The sizes of the CdS NPs were calculated using XRD and SEM (see Chapter 4, Sections 4.9.1 and 4.9.2).

Table 5. 3. The concentrations used to calculate the quantity of metal manufactured NPs applied to de–ionized water and quantity of MNP/ *di*H_2O solution applied to the soil.

NP	0.01 g metal/kg of soil	0.1g/kg of soil	1 g/kg of soil
Cd	1.9 mg MNP to 10 ml diH_2O	19.3 mg MNP to 10 ml diH_2O	192.8 mg MNP to 10 ml diH_2O
Cu	1.9 mg MNP to 10 ml diH_2O	18.8 mg MNP to 10 ml diH_2O	188.2 mg MNP to 10 ml diH_2O
Zn	1.9 mg MNP to 10 ml diH_2O	18.7 mg MNP to 10 ml diH_2O	186.7 mg MNP to 10 ml diH_2O

The solutions of each nanoparticle (CdS, CuO and ZnO NPs) were suspended directly in de–ionized water (DI–water) (10 mL) and dispersed by ultrasonic vibration (100 W, 40 kHz) for 30 minutes before adding them to the Eutric Cambisol soil (see Chapter 4, Section 4.3.2). The hydroponic cultures were prepared by adding the three different types of NPs (CdS, CuO and ZnO NPs) with the same concentrations, (0, 0.01, 0.1 and 1.0 g L^{-1}) to the diluted nutrient solution (25 % strength).[9,20] as described in Table 5.4.

Table 5. 4. The concentration used to calculate the quantity of metal NPs applied to the nutrient solution.

NP	0.01 g/L of soil	0.1g/L of soil	1 g/L of soil
Cd	25.7 mg MNP to 2 L nutrient solution	257.0 mg MNP to 2 L nutrient solution	2570.3 mg MNP to 2 L nutrient solution
Cu	25.1 mg MNP to 2 L nutrient solution	251.0 mg MNP to 2 L nutrient solution	2509.9 mg MNP to 2 L nutrient solution
Zn	24.9 mg MNP to 2 L nutrient solution	248.9 mg MNP to 2 L nutrient solution	2489.4 mg MNP to 2 L nutrient solution

The dosed solutions were agitated by ultrasonic vibration for 30 minutes to attempt dispersal; then their volumes were made up to 2 L with the nutrient solution; 400 mL of nutrient solution with and without NPs (control) were added to each plastic pot. Each concentration was repeated three times to ensure experimental accuracy. The pH of the nutrient solution was adjusted to 6.8.[21] Each pot was oxygenated throughout the 21 days. The soil and hydroponic pots were arranged in a randomised block design. The pots used in this experiment (72 pots) were classified into six main groups (12 pots for each main group). Each NPs concentration was repeated three times. All the seedlings were grown under the same environmental conditions (see below).

5.4.3. Plant material and growth

Maize seeds (*Z. mays*) were germinated according to the procedure described in Chapter 4, Section 4.3.3. Four maize seedlings were selected for each soil and hydroponic pots as shown in Figures 5.1–5.4. For the soil culture, the maize seedlings were transferred into each pot (7.60 cm diameter and 6.7 cm depth) and filled with 150 g of Eutric Cambisol soil. For the hydroponic cultures, four maize seedlings were planted in each plastic pot (9.0 cm diameter, 14.0 cm depth, capacity 570 mL). These pots were painted with black spray to avoid the effect of light on the maize roots. The ends of four centrifuge tubes (1.5 mL) were cut off to allow for the growth of the roots. Four maize seedlings were placed in each centrifuge tube and transferred to fit the four holes in the top of each pot cover.

Figure 5. 1. The growth of maize plants after 21 days from seed germination and the experimental design of the soil pots dosed with CdS NPs (0, 0.01 and 1.0 g kg^{-1}) ($n = 3$).

Figure 5. 2. The growth of maize plants after 21 days from seed germination and the experimental design of the soil pots dosed with CuO NPs (0, 0.01 and 1.0 g kg^{-1}) ($n = 3$).

Figure 5. 3. The growth of maize plants after 21 days from seed germination and the experimental design of the soil pots dosed with ZnO NPs (0, 0.01 and 1.0 g kg^{-1}) ($n = 3$).

Figure 5. 4. The growth of maize plants after 10 days from seed germination and the experimental design of the hydroponic nutrient solution pots dosed with ZnO NPs (0, 0.01 and 1.0 g kg^{-1}) ($n = 3$).

The seedlings were oxygenated to encourage root growth. The seedlings were placed in the growth room (chamber) for 21 days as shown in Figures 5.1–5.4. The environmental conditions were 26.7 °C, relative humidity (RH) 70–80%, and a photoperiod of 16 hours. The plants in the soil culture were watered every day, one day with nutrient solution and other day with distilled water for 21 day as described in Chapter 4, Section 4.4.3. The nutrient solutions of the hydroponic cultures were changed every two days.

5.4.4. Plant harvest

Maize plants were harvested from their pots after 21 days as shown in Figure 5.5 and their parameters recorded.

Figure 5. 5. The harvested maize plant after 21 days from seed germination.

The plant samples from both types of cultures were prepared for chemical analysis as described in Chapter 4, Section 4.4.5.

5.5. Plant and soil analysis

All the plants and soil samples were subjected to the procedure described in Chapter 3, Sections 3.2.11 and 3.2.12.

5.6. Monitoring the effects of NPs on maize plants using assessable parameters

The Tolerance Index (T1), Agronomical Efficiency (AE), Bio–concentration Ratio (BCR) and Relative Increase Percentage (RI) were calculated as described in Chapter 3, Sections 3.3.1–3.3.4.

5.7. Statistical analysis

The concentrations of three NP types and all the maize parameters (dry weight and length of roots and shoots) were performed with three replicates of each concentration in Eutric Cambisol soil. Means and standard errors of each parameter were calculated using Microsoft Excel. Maize plant parameters were evaluated using the appropriate equations. All the data that pertained to the absorption of different NP concentrations (CdS, , ZnO, and CuO) in maize roots and shoots, the impact of these NPs on plant parameters (length and dry weight of maize roots and shoots), the concentration of NPs in test soil and the results of TI, AE, BCR, RI and uptake ratios equations for assessing the effect of heavy metals on maize growth were subjected to a one way analysis of variance (ANOVA) and differences identified with a Tukey (HSD) test using the software package SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). Two–way ANOVA was used to test for significant differences between NP type (CdS, ZnO, and CuO) and concentrations (0, 0.01, 0.1 and 1.0 g kg^{-1}) for all the obtained results. *Post hoc* tests were performed using Tukey's HSD. Significant differences were accepted at the ($p < 0.05$) level for all tests. Graphs were constructed using Sigma Plot 12.3 for Windows. Data normality was tested using Shapiro–Wilk test.

5.8. Results and discussion

5.8.1. X–ray Diffraction peaks and SEM image of CuO NPs

Figure 5.6 shows the presence of interesting nanostructure for CuO powder, from the XRD patterns obtained which are consistent with the JCPDS (5–0661) data of the CuO with monoclinic crystal phase.[21,22] The major reflections were between 30° and 75° (2θ values). The reflection peaks at 2θ values of 32.4°, 35.5°, 38.7°, 48.8°, 53.5°, 58.4°, 61.5°, 66.2° and 68.0° correspond to the planes of (110), (002), (111), (202), (020), (202), (113), (311) and (310) respectively, of the crystalline CuO, this agrees with results obtained by previous studies.[22–24] No other impurities could be detected indicating the high quality of the CuO sample.

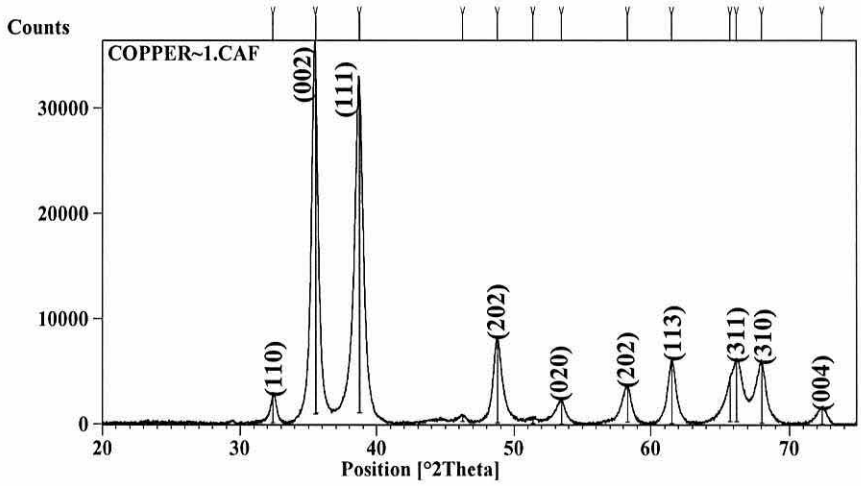

Figure 5. 6. The XRD spectrum of copper oxide nanoparticles (CuO) as a pure compound.

The reflection peaks of CuO were calculated using Bragg's Law (See Chapter 4, Section 4.9.1). The entire reflection of 2θ planes for CuO NPs was used to calculate its size. The size of CuO NPs was found to range from ~ 27.5 nm to ~46.6 nm. The average size of the crystalline CuO was calculated from the half–width of (002), (111), (202), (113) and (311), is ~33.0 nm.

The external shapes and sizes of CuO were studied on an S–4700 scanning electron microscope (SEM). The SEM images of uncoated and coated CuO powder showed spherical particles as shown in figure 5.7, A and B. Figure 5.7 A shows the SEM image of coated CuO sample which indicated that the general morphology of synthesised CuO were observed with large number of CuO agglomerates as nanosphere form with a different particles size. However, there is an

individual spherical particles have nanostructure as shown in coated samples (Figure 5.7, B). This demonstrates that the nanostructure of this compound are composed of non–agglomerated random spherical particles which trend to build or aggregate to form a flower shape structure.[24]

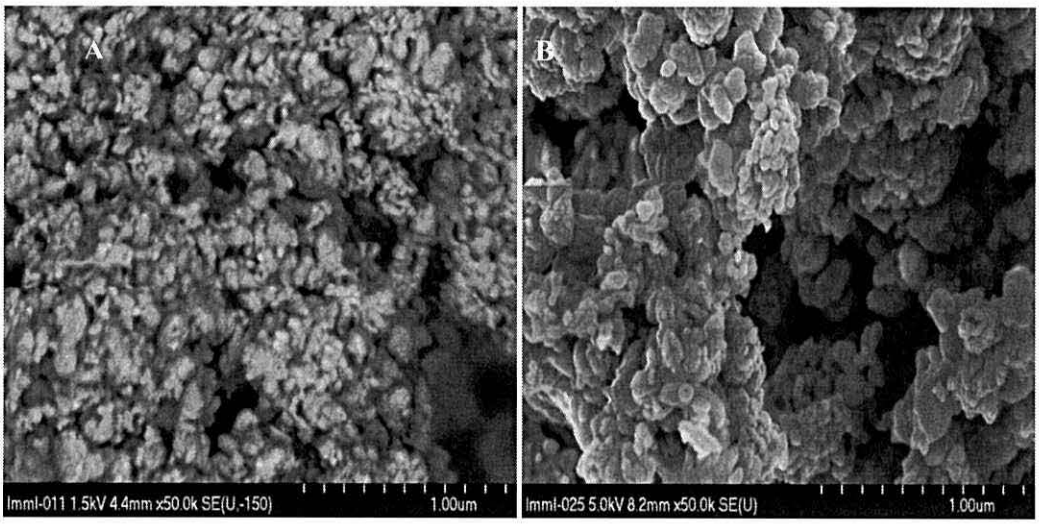

Figure 5. 7. SEM images of CuO nanoparticles as powder: (a) uncoated CuO NPs observed at 1.5 kV, (b) coated CuO NPs observed at 5.0 kV. Scale bar represents 1 μm.

Image analysis software (Image–J 1.43 for Microscopy, USA) was used to calculate the size of CuO NPs. The size of spherical coated CuO was between 1.0 to 99.0 nm and the average size was 64.92 ± 1.67 nm (Figure 5.7, A and B), this classification was performed within nanoparticles range (1–100 nm) with ignoring the sizes above 100 nm, the calculation of CuO sizes were in the range of XRD results which confirm our findings for SEM images.

The fresh maize root and shoots were observed by SEM, plant samples treated with 1.0 g L^{-1} of CdS, CuO and ZnO were selected, and compared with control sample (without NPs). SEM images were taken in order to gain insights into the interaction of NPs with the surface of maize roots and leaves (SEM images presented in Appendix 1). The structure of maize leaves is heterogeneous (Figure A4, b). The leaves surface consists of wax regions that offer a protection for UV radiation or mechanical damage and adaption to moisture. This rough structure of the leaves made it difficult to clarify NPs. In addition, the electron beam caused damage for plant samples (Figure A3, a, b). This image showed that there is an increased chance of beam-induced damage to the sample (beam spot) due to the higher accelerating voltages required for imaging within a gaseous environment. Astigmatism can also cause poor image quality by causing the

beam spot (beam on the sample) to be noncircular, drastically decreasing resolution. However, bigger agglomerates were found nearby closed mesophyll (Figure A2, b) or adhered on the root surface (Figure A2, a). Thus, further studies to confirm the NPs existing in plant tissues are required using transmission electron microscopy (TEM).

5.9. Assimilated concentrations of NPs in maize roots and shoots grown in the soil culture

Plants are a possible pathway for the bioaccumulation of NPs through ecosystems and into the food chain.[25] Figure 5.8 shows the concentrations of Cd, Zn, and Cu NPs.

The accumulation of NPs in the maize roots and shoots varied widely; this can be attributed to a variety of factors, including the NPs' specific surface area, particle size, stability, chemical composition, concentration, and physiochemical properties; the plant's age and growth medium (soil or hydroponic), and the dilution agent are also relevant.[26]

The concentrations of tested NPs increased as a function of their concentration in the Eutric Cambisol soil across all the maize parts. This agrees with the finding of Wang el al.,[25] who reported the Cu concentration of maize roots and shoots after 14 days exposure to CuO NPs in 25% diluted nutrient solution. Wang el al.'s results suggest that the content of CuO NPs in maize tissues increases with increasing concentrations of the particles in the growth medium.[25]

Results of another study suggest total Zn concentration of the ryegrass (*L. perenne*,) roots and shoots under ZnO NP treatments (10–1000 mg L^{-1}) increase in the plant parts after one week grown in nutrient solution.[8] Zhao *et al.*[27] recently reported that the total Zn content in roots and shoots increases with increases in the concentration of ZnO NPs (0–800 mg kg^{-1}) in sandy loam soil. Results of ANOVAs and *Post hoc* tests (Tukey's HSD) showed significant differences ($p < 0.001$) as regards the concentrations of Cd and Cu NPs in maize roots and shoots at concentration leves of 0.1 and 1.0 g kg^{-1} compared with their controls, save the Cu concentration in maize shoots at 0.1 g kg^{-1} soil, but this was still significant ($p < 0.01$). Results also observed that the concentration of Zn NP were significantly different ($p < 0.001$) in maize roots and shoots at concentrations of 0.1 and 1.0 g kg^{-1} compared with their controls, save that Zn concentration in the maize roots at a level of 0.01 g kg^{-1}, but this was still significant ($p < 0.05$).

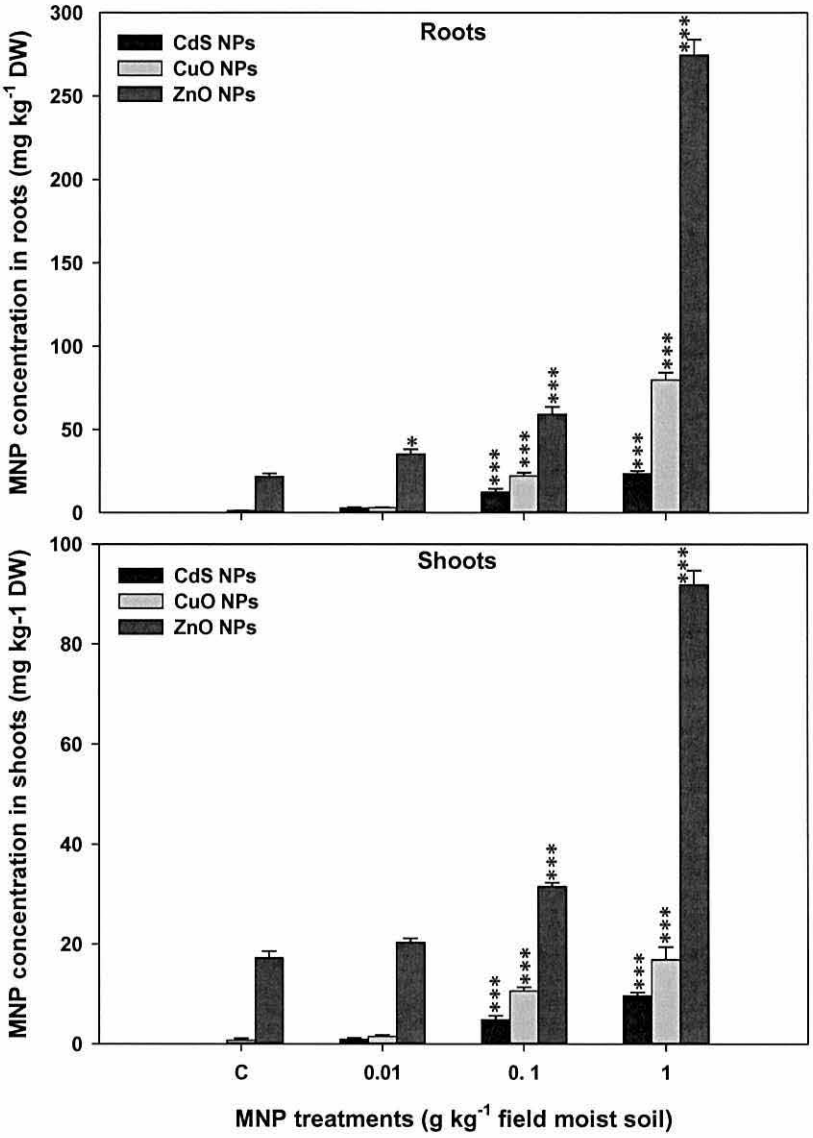

Figure 5. 8. The concentrations of Cd, Cu and Zn NPs in maize roots and shoots. The plants were grown for 21 days in Eutric Cambisol soil treated with 0, 0.01, 0.1 and 1.0 g of CdS, CuO and ZnO NPs kg^{-1} soil. The values are given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). The values of Cd in their control samples were below the detection limit. Error bars denote one standard error. Few of the error bars are too small to be visible.

Statistical analysis showed a significant difference ($p < 0.001$) between the concentrations of Cd, Cu and Zn NPs in maize roots. The concentration of Zn NPs was highest in roots (mean overall = 97.63 mg kg^{-1}; SE: ± 13.07); next highest for Cu NPs (26.57 mg kg^{-1}; SE: ± 9.60); and the lowest for Cd NPs (9.68 mg kg^{-1}; SE: ± 2.80). Moreover, there was a significant difference ($p <$

0.001) between the concentrations of Cd, Cu and Zn NPs in maize shoots. Concentration of Zn NPs in the shoots appeared highest (40.25 mg kg^{-1}; SE: ± 9.14), next highest for Cu (7.43 mg kg^{-1}; SE: ± 2.10), and least high for Cd (3.85 mg kg^{-1}; SE: ± 1.16).

These results suggest that CdS, CuO and ZnO accumulate approximately 15%, 15% and 66% respectively in maize roots and that small amounts (about 5%, 6% and 17% respectively) are transferred to the shoots. In this regard Zhu *et al.*[10] reported that iron oxide NPs accumulates at approximately 45.5 % in pumpkin roots and about 0.6% in the plant's leaves.

Results of the present study and extant research suggest the accumulation of NPs increases within each part of the plant as a function of NP concentrations in soil. The maize roots accumulate more of the NPs than that of shoots. Results of the present study further suggest that ZnO NPS accumulate in plants more than CuO NPs and that CdS NPs accumulate in the plants least of all. The high accumulation of Zn NPs could be related to the significant role that the Zn plays in plant growth and development.[28] Its appreciable dissolution in water (from 1.0 to several thousand mg/L) depends on variety of factors, including NP size and the pH of the dissolved media.[29] The results agree with those obtained by Zhao *et al.*[30] for ZnO NPs. The present study also obtained similar results for CdS and ZnO NPs, as reported in Chapter 4, Sections 4.10 and 4.11. Results also agree with those of Lee *et al.*[31] who found that the presence of higher concentrations of Cu NPs in growth media appears to result in a higher uptake and accumulation of this metal in plant tissues. Lee *et al.*'s study, however, was carried out in agar media so may lack ecological validity.[31]

5.9.1. Effect of CdS, CuO and ZnO NPs on the growth characteristics of maize plants grown in soil culture

The majority of the parameters decreased with increasing concentration of NPs to Eutric Cambisol soil (Figures 5.9 and 5.10). Extant research suggests similar effects in different plants grown in varied growth mediums.[20,32–34]

In the present study, results suggest that the length and dry biomass of maize roots and shoots increase when soil concentrations of ZnO NPs are 0.01 g kg^{-1} compare to control samples. As indicated, Zn is an essential element for plant development and growth, however, above a certain concentration, the element becomes toxic, causing a decrease in plant biomass.[28,35,36]

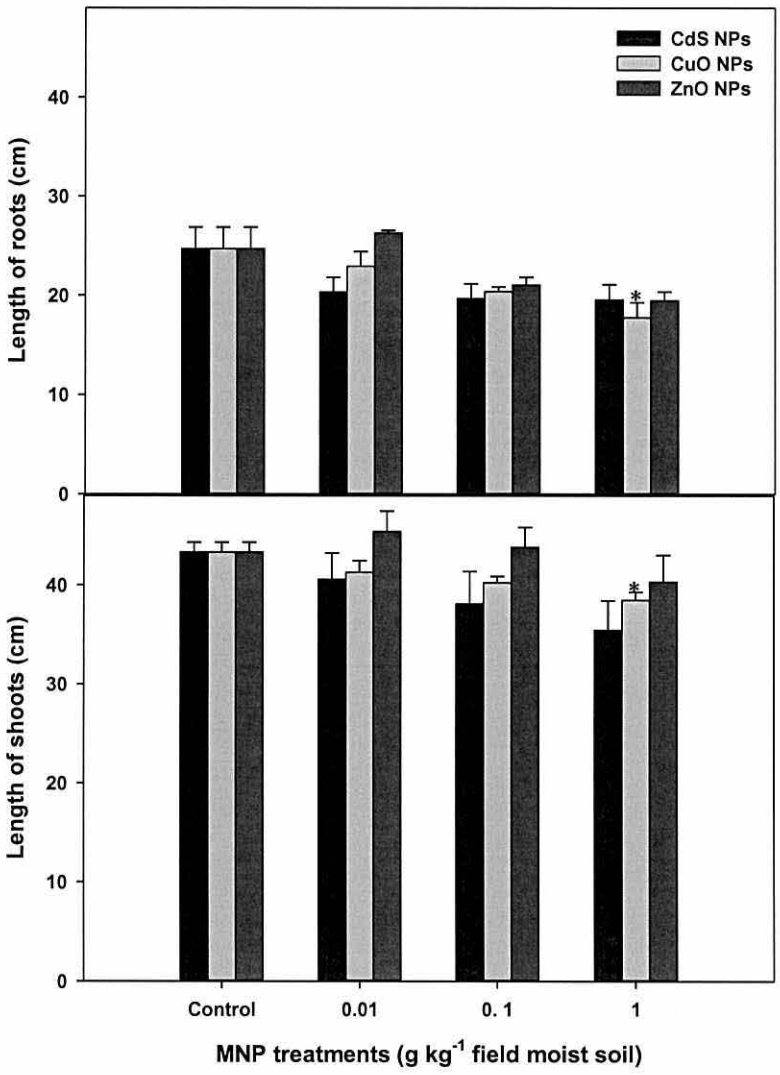

Figure 5. 9. The effects of CdS, Cuo and ZnO NPs concentrations on the length of maize roots and shoots (cm). Plants were grown for 21 days in Eutric Cambisol soil treated with 0, 0.01 and 1.0 g of CdS, CuO and ZnO NPs kg^{-1} soil.The values are given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denots one standard error.

Results of statistical analysis observed no significant effects of NPs on the length and dry biomass of maize roots or shoots, save for CuO, which appeared to decrease the length of maize roots and shoots at a soil concentration of 1.0 g kg^{-1} ($p < 0.05$).

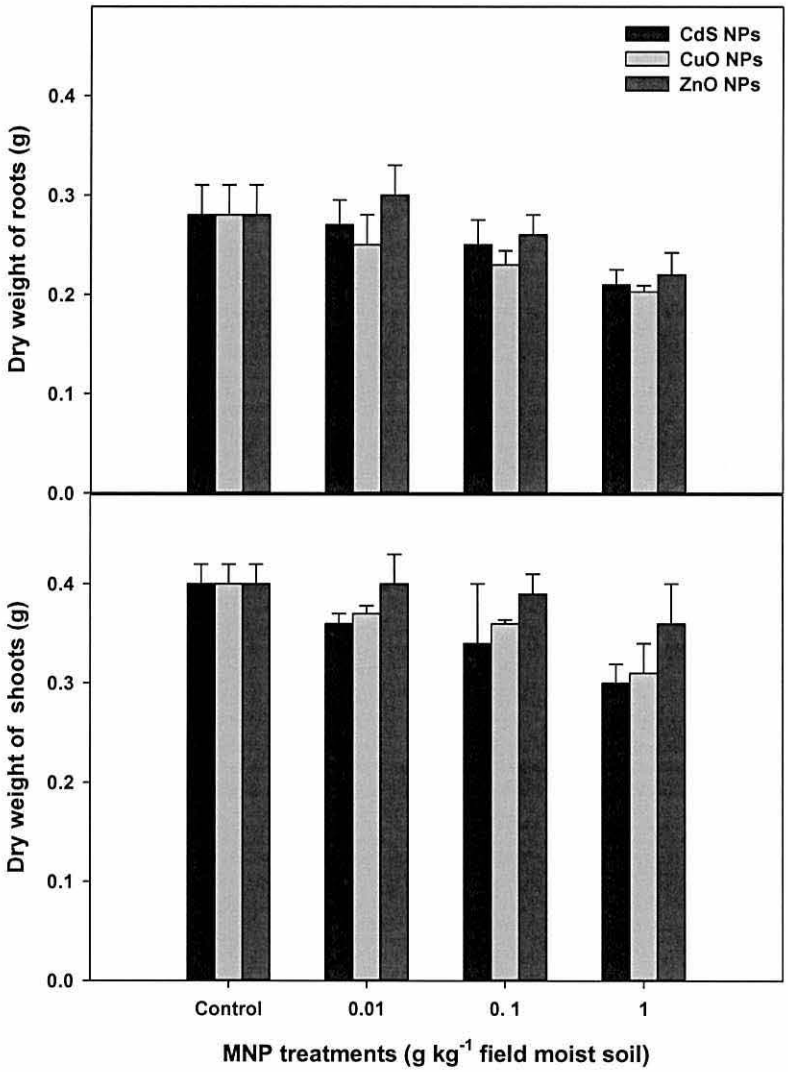

Figure 5. 10. The effects of CdS, Cuo and ZnO NPs concentrations (mg $kg^{-1)}$ on the dry biomass yield of maize roots and shoots (g/pot). The plants were grown for 21 days in Eutric Cambisol soil treated with 0, 0.01 and 1.0 g of CdS, CuO and ZnO NPs kg^{-1} soil.The values are given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

There was no significant difference between dry biomass of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall dry biomass of roots was 0.26 g/ pot (SE: ± 0.01) for Cd NPs; that of Cu NPs was 0.24 g/ pot (SE: ± 0.01); that for Zn was 0.27 g/ pot (SE: ± 0.01). Moreover, there was no significant difference between the dry biomass of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall dry biomass of shoots was 0.35 g/ pot

(SE: ± 0.01) for Cd NPs; that of Cu NPs was 0.36 g/ pot (SE: ± 0.01); that for Zn was (0.39 g/ pot; SE: ± 0.01).

Statistical analysis observed no significant difference between length of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall length of roots was 21.06 cm (SE: ± 1.22) for Cd NPs; that of Cu NPs was 21.43 cm (SE: ± 1.02); that for Zn was 22.88 cm (SE: ± 0.98). Moreover, there was no significant difference between length of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall dry length of shoots was 39.37 cm (SE: ± 1.65) for Cd NPs; that of Cu NPs was 40.843 cm (SE: ± 0.66); that for Zn was (43.23 cm; SE: ± 1.26). The results as regards CdS and ZnO NPs may be due to increased maize plant tolerance for these heavy metals.

Anton *et al.*[37] classified maize as an accumulator and tolerant species of Zn and Cd, and a highly organic soil content would be expected to reduce the toxicity of a given concentration of NPs.[38] The negative impact of CuO can be related to the solubility of CuO NPs; the production of their ions may cause more toxic responses than do the other two NPs.[25,26]

The result as regards CuO agree with those of Ellis who reported that CuO NPs has negative effects on the biomass of *L.perenne* grown in Eutric Cambisol soil (five months) at 1.0 g kg^{-1}.[39] However, Ellis's results also indicated that ZnO has significant negative impact on the plant biomass when at a soil concentration of 0.01 g kg^{-1}; this is at variance with results of the present study, which suggest no such effect. The finding as regards CuO, however, appears sound. Atha *et al.*[19] reported that CuO NPs damage DNA in *Raphanus sativus*, *L. perenne*, and *L. rigidum*, and that CuO seems to promote the accumulation of oxidatively modified compounds that results in mutagenic DNA lesions and led to inhibition of plant growth. Thus, CuO appears to be toxic to a range of plant species, not just maize.

5.9.2. Total concentrations of NPs in the Eutric Cambisol soil

An analysis of NPs in the Eutric Cambisol soil was performed after plant harvest. The total concentrations of the three metal NPs in the Eutric Cambisol soil increased with increasing the level of soil NPs, as shown in Figure 5.11. Inspection of the figure indicates that the total concentration of Zn NPs in the soil solution was higher than the other two metals (CdS NPs and CuO NPs) at added concentrations of 0.01, 0.1 g kg^{-1}. This can be attributed the residual effect of fertilizers when they were applied to the soil during sample collection.[40–42] Inspection also

shows the total concentration of Cu and Cd were slightly different from each other at added concentrations of 0.01 and 0.1 g kg^{-1}. However, the figure also indicates the total concentration of Cd NPs in the soil increased more than those of Cu and Zn at added concentration of 1.0 g kg^{-1}. This can be attributed to the low solubility, precipitation and aggregation of CdS NPs in soil solution compared with the other two metals as discussed in Chapter 4, Sections 4.10 and 4.10.5.

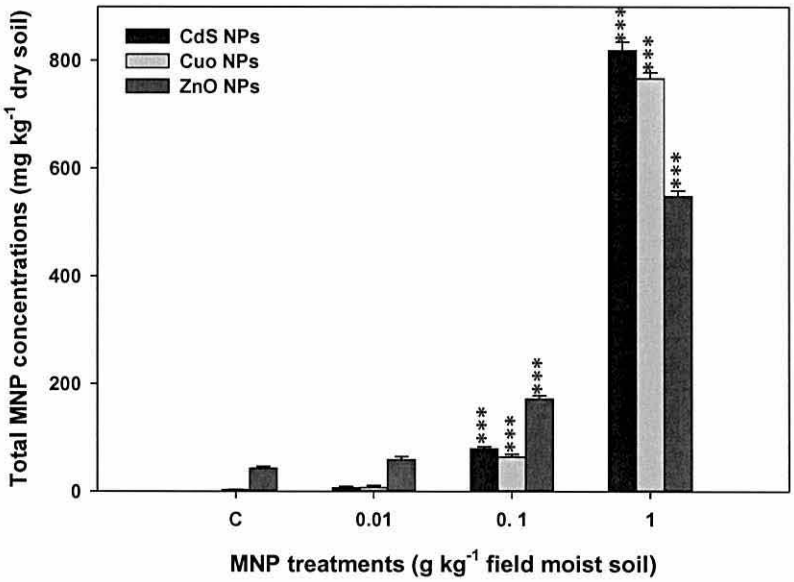

Figure 5. 11. Total concentration of Cd, Cu and Zn NPs in Eutric Cambisol soil after 21 days. The soil was treated with 0, 0.01 and 1.0 g of CdS, CuO and ZnO NPs kg^{-1} soil.The values are given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

Results of statistical analysis as regards all three metal NPS showed significant differences ($p < 0.001$) for the concentrations of three metal NPs at soil concentration of 0.1 and 1.0 g kg^{-1} compared to controls. The results of statistical analysis showed a significant difference ($p < 0.001$) between the total concentrations of Cd, Cu and Zn NPs in the soil. The mean overall total concentration of Cd NPs was 226. 35 (SE: ± 30.64); that of Cu NPs was 210.40 (SE: ± 19.14); that for Zn NPs was (205.60 cm; SE: ± 16.54).

5.10. Assimilated concentrations of NPs in maize roots and shoots grown in hydroponic culture

Although it is difficult to predict the behaviour of NPs in the environment, from the literature discussed in Chapter 2, Sections 2.5.2.1 and 2.5.2.2, two important factors as regards water systems are aggregation and dissolution. Figure 5.12 shows the concentration of three applied NPs and their accumulation within maize roots and shoots in the hydroponic cultures.

Inspection of the figure suggests that concentrations of all three metal NPs increased with increases in their applied concentration in the nutrient solutions compared to the control groups; this agrees with the literature on the NPs and their bulk materials.[25,43–46] The behavior of these NPs was the same as for the soil cultures as discussed in Section 5.5.1.

Results of statistical analysis observed significant differences ($p < 0.001$) for the concentration of Cd NPs in maize roots and shoots at nutrient concentrations of 0.1 and 1.0 g L^{-1} compared with their controls. The concentration of Cu NPs in maize roots was significantly different ($p < 0.01$) at treatments of 0.01 g L^{-1}. Moreover, Cu NPs concentration showed a high significant difference ($p < 0.001$) in maize roots at concentrations of 0.1 and 1.0 g L^{-1} compared to the control.

The concentrations of Cu NP were significantly different ($p < 0.05$) in the maize shoots at treatment of 0.01 g L^{-1} and it was a significant difference ($p < 0.001$) at treatment concentrations of 0.1 and 1.0 g L^{-1} compared with their controls. The majority of Zn NP concentrations showed significant differences ($p < 0.001$) in maize roots and shoots at concentration levels of 0.1 and 1.0 g L^{-1} compared with their controls.

Statistical analysis showed a significant difference ($p < 0.001$) between the concentrations of Cd, Cu and Zn NPs in maize roots. The concentration of Zn NPs was highest in roots (mean overall = 136.43 mg kg^{-1}; SE: ± 15.19); next highest for Cu NPs (43.42 mg kg^{-1}; SE: ± 12.99); and the lowest for Cd NPs (20.41 mg kg^{-1}; SE: ± 5.80). Moreover, there was a significant difference ($p < 0.001$) between the concentrations of Cd, Cu and Zn NPs in maize shoots. Concentration of Zn NPs in the shoots appeared highest (47.80 mg kg^{-1}; SE: ± 11.19), next highest for Cu (19.79 mg kg^{-1}; SE: ± 6.30), and least high for Cd (8.41 mg kg^{-1}; SE: ± 2.48).

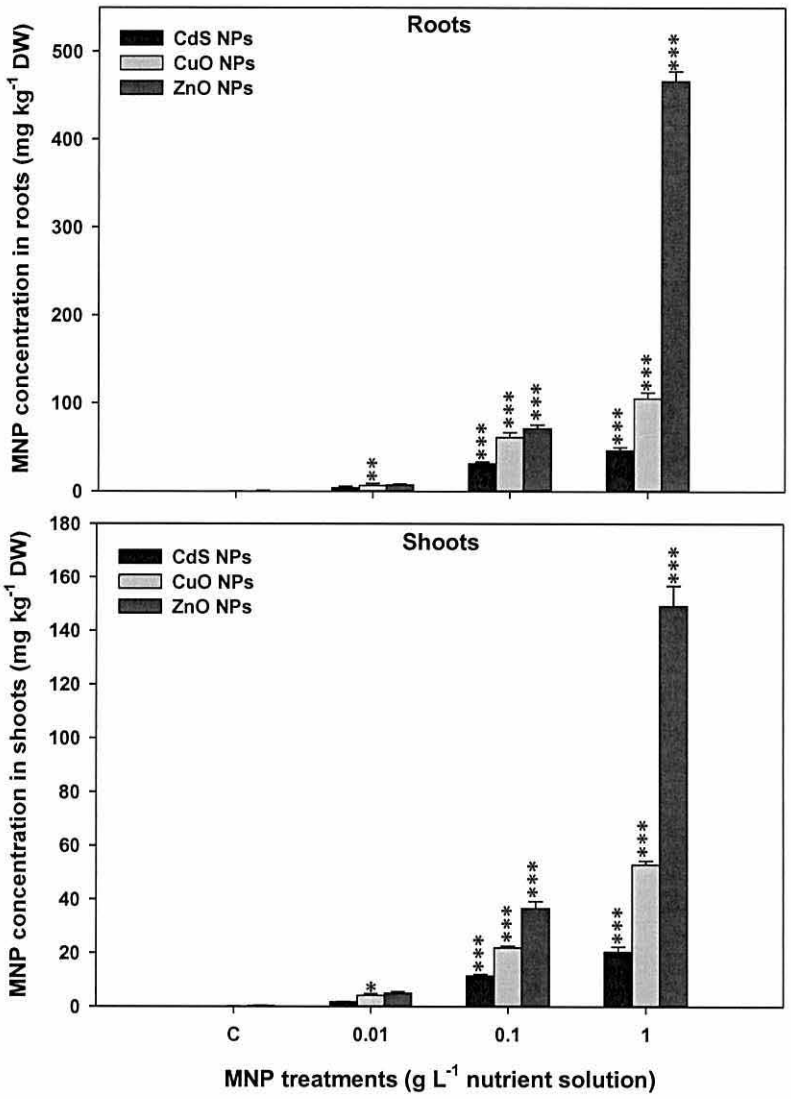

Figure 5. 12. The concentrations of Cd, Cu and Zn NPs in maize roots and shoots. Plants were grown for 21 days in nutrient solution (25 %) with 0, 0.01 and 1.0 g of CdS, CuO and ZnO NPs NPs L^{-1}. The values are given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). The values of Cd were below the detection limit in their control samples. Error bars denote one standard error.

The overall accumulation of Cd, Cu, and Zn NPs in maize roots was about 25%, 46% and 63% respectively. Small amounts of each metal (approximately 10%, 23% and 33% respectively) were transferred to the shoots across all NP concentrations. The accumulation of NPs increased for each plant part with treatments of higher concentrations of these particles to the nutrient. Concentrations of three nano–metals appeared higher in roots than in shoots. Moreover,

concentrations of the NPs in the plants appeared highest for Zn, next highest for Cu, and least high for Cd. These results are agreed with those obtained from soil cultures, as discussed in Section 5.9.

5.10.1. Effect of CdS, CuO and ZnO NPs NPs on the growth characteristics of maize plants grown in hydroponic culture

The length and dry yield of maize roots and shoots were recorded in cm and g/pot respectively to assess the effects of the concentrations of CdS, CuO and ZnO NPs on maize plant grown in hydroponic culture(Figures 5.13 and 5.14).

In general, CdS, CuO and ZnO NPs had reduced the parameters of maize roots and shoots grown in hydroponic culture more than those of the soil culture. This is not surprising given that maize roots interact with NPs directly.[25] Also, increases in NPs solubility for maize plants within nutrient culture may decrease maize parameters. Franklin *et al.*[29] have shown that, although ZnO is insoluble in water, its NPs are rapidly dissolved and this results in 6 mg L^{-1} of Zn being dissolved within 6 hours and 16 mg L^{-1} of Zn being dissolved within 72 hours–this in a buffered algal medium (pH 7.5); this is in excess of the 5 mg Zn L^{-1} that would be toxic to the majority of aquatic biota.[29]

Results of statistical analysis showed that CdS NPs had a negative effect ($p < 0.05$) on the length of roots and shoots at a concentration of 1.0 mg L^{-1} compared with their controls. The influence of bulk Cd on the height of maize shoot and their biomass under hydroponic conditions for 21 days has been discussed by Wang *et al.*[47] their results suggest that the length of maize shoots and their biomass are inhibited at supply levels of over 200 μM (36.6 mg L^{-1}).

On the other hand, there appeared no significant effect of CuO or ZnO NPs on the lengths of maize roots and shoots compared to their control samples across all NP concentrations. However, Wang *et al.*[25] found that 100 mg L^{-1} of CuO NP concentration significantly reduced the fresh and dry weights of maize roots and shoots. The dry weight of maize shoots was significantly ($p < 0.05$) decreased by 1.0 mg L^{-1} CdS NPs compared with the control group.

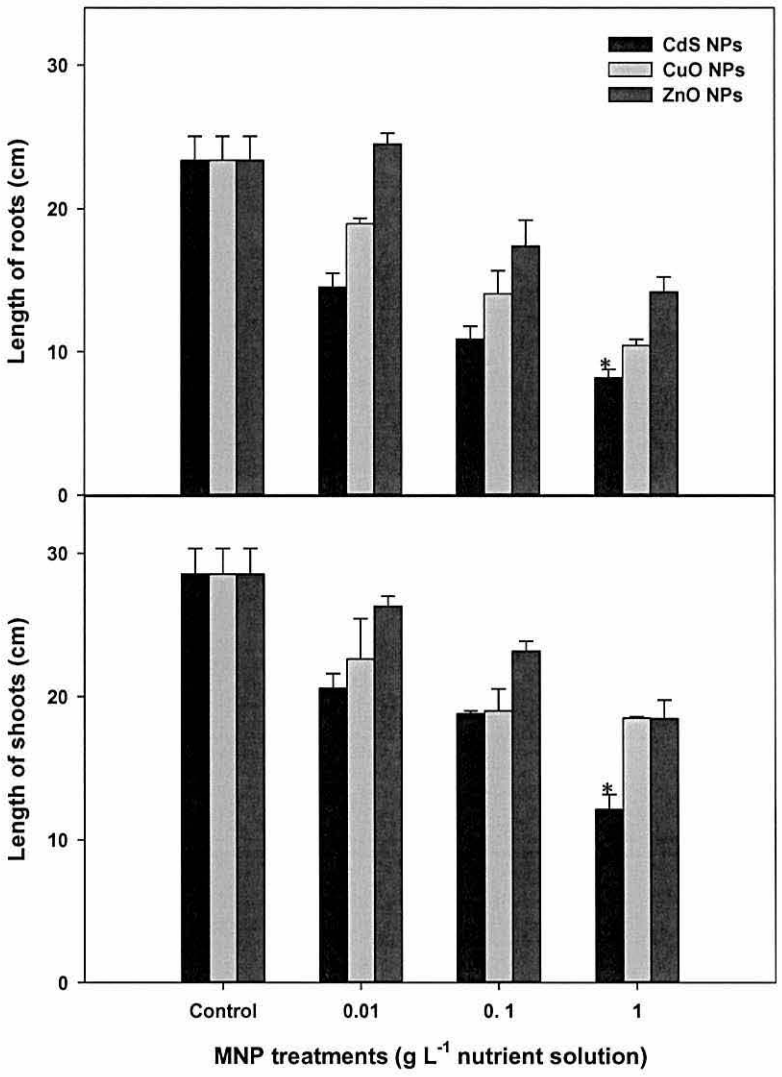

Figure 5. 13. The effects of CdS, CuO and ZnO NPs concentrations on the length of maize roots and shoots (cm). Plants were grown for 21 days in nutrient solution (25 %) treated with 0, 0.01 and 1.0 g of CdS, CuO and ZnO NPs L^{-1}.The values are given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

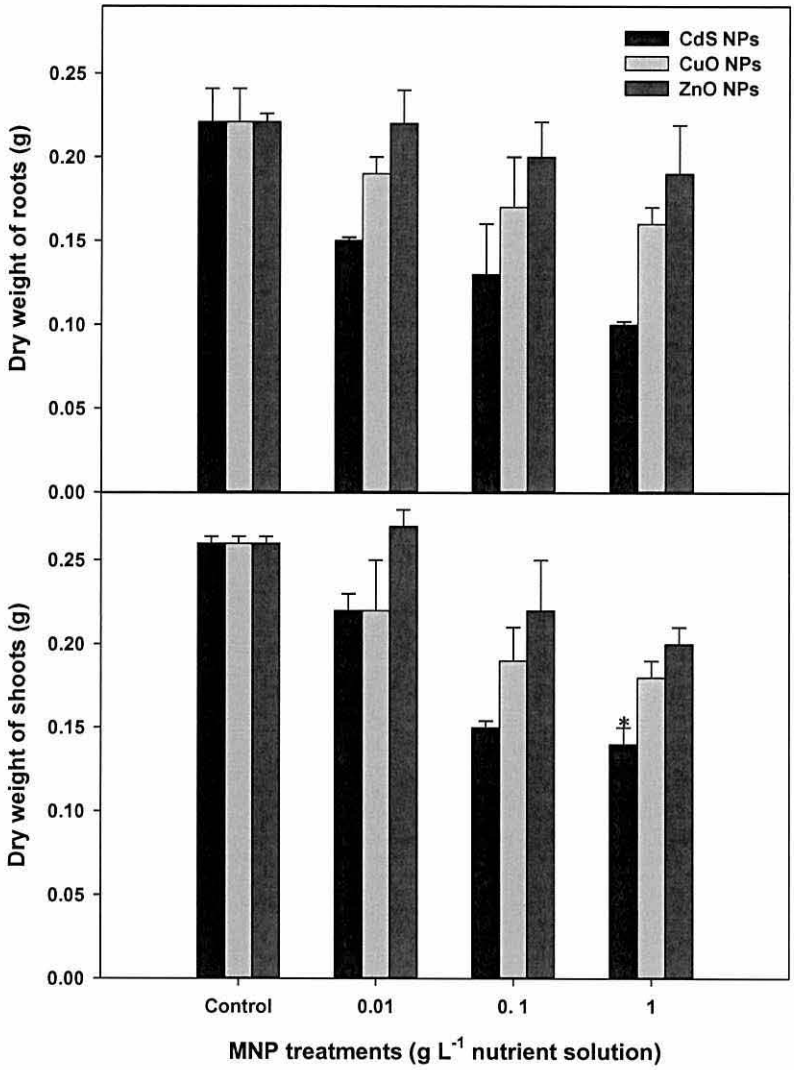

Figure 5. 14. The effects of CdS, CuO and ZnO NPs concentrations (mg $kg^{-1)}$ on the dry biomass yield of maize roots and shoots (g/pot). Plants were grown for 21 days in nutrient solution (25 %) treated with 0, 0.01 and 1.0 g of CdS, CuO and ZnO NPs L^{-1}.The values are given as mean ±SEM of triplicate samples. Asterisks denote degree of statistical difference between the control (C) and individual treatments (*** = $p < 0.001$; ** $p \leq 0.01$; * = $p \leq 0.05$). Error bars denote one standard error.

This agrees with the literature: the negative effect of bulk Cd ions on the dry weight of maize plants grown in different media has been discussed in numerous studies.[33,34,48,49] Cu and Zn NPs appeared to have no significant effects on the dry weights of maize roots and shoots compared with their control groups.

Statistical analysis observed no significant difference between dry biomass of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall dry biomass of roots was 0.15 g/ pot (SE: ± 0.01) for Cd NPs; that of Cu NPs was 0.19 g/ pot (SE: ± 0.02); that for Zn was 0.21 g/ pot (SE: ± 0.01). Moreover, there was no significant difference between the dry biomass of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall dry biomass of shoots was 0.19 g/ pot (SE: ± 0.01) for Cd NPs; that of Cu NPs was 0.21 g/ pot (SE: ± 0.02); that for Zn was 0.23 g/ pot (SE: ± 0.03).

Statistical analysis observed no significant difference between length of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall length of roots was 14.23 cm (SE: ± 2.09) for Cd NPs; that of Cu NPs was 16.70 cm (SE: ± 2.07); that for Zn was 19.85 cm (SE: ± 1.71). Furthermore, there was no significant difference between length of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall length of shoots was 20.01 cm (SE: ± 2.19) for Cd NPs; that of Cu NPs was 20.62 cm (SE: ± 2.50); that for Zn was (24.11 cm; SE: ± 1.69).

Thus CdS NPs appeared the only relevant factor that decrease the length of maize roots and shoots and dry weights of shoots. This effect was clearest at higher NP concentrations. This is slightly at variance with the findings concerning the maize plants grown in soil, which suggested that high levels of CuO NS in soil lead to shorter roots and shoot (see Section 5.9.1).

5.11. Bio–concentration ratios of NPs in the maize roots and shoots grown in soil culture

The BCRs of plant to soil are used to relate chemical concentrations measured in different vegetation tissues to concentrations in the soil supporting that vegetation.[50] The BCR is considered to be an adequate measure of the capacities of plant species or their parts to absorb and translocate metals from their roots to their shoots.[51] The BCRs of CdS, ZnO, and CuO NPs in the roots and shoots of the maize plant was calculated using Equation 5.1. The BCRs of the three metal NPs in the maize roots and shoots are shown in Table 5.5.

BCR = Element in plant (µg/g dry weight) / Element in soil µg/g soil Equation 5.1

The data in the table suggest that the BCRs of maize roots and shoots gradually decreased with treatments of increasing levels of NP concentrations in the Eutric Cambisol soil. This applied to

all three chemicals used in the study and agrees with the results reported in Chapter 4, Sections 4.10.2 and 4.11.2.

Table 5. 5. The concentrations of Cd, Cu and Zn NPs (mg kg^{-1}) and their Bio–concentration ratios (BCR) in the maize roots and shoots cultivated in Eutric Cambisol soil.

Nanoparticles	parameters	Concentrations of nanoparticles used in the soil pots					
		0.01 g kg^{-1}		0.1 g kg^{-1}		1.0 g kg^{-1}	
		Root	Shoot	Root	Shoot	Root	Shoot
CdS NPs	Conc. (mg kg^{-1})	2.88 (±0.39)	0.88 (±0.02)	12.39 (±1.33)	4.83 (±0.80)	23.43 (±1.64)	9.68 (±0.35)
	BCR	0.29^{a} (±0.04)	0.09^{a} (±0.002)	0.12^{b} (±0.01)	0.05^{b} (±0.01)	0.02^{b} (±0.002)	0.01^{b} (±0.0003)
CuO NPs	Conc. (mg kg^{-1})	3.00 (±0.02)	1.47 (±0.31)	22.14 (±0.90)	10.61 (±0.75)	79.88 (±1.31)	16.90 (±2.53)
	BCR	0.30^{a} (±0.002)	0.15^{a} (±0.03)	0.22^{b} (±0.01)	0.11^{ab} (±0.01)	0.08^{b} (±0.001)	0.02^{b} (±0.003)
ZnO NPs	Conc. (mg kg^{-1})	35.22 (±2.12)	20.32 (±0.84)	59.19 (±4.44)	31.55 (±0.77)	274.49 (±1.37)	91.91 (±0.80)
	BCR	3.52^{a} (±0.21)	2.03^{a} (±0.08)	0.59^{b} (±0.04)	0.32^{b} (±0.01)	0.27^{b} (±0.001)	0.09^{b} (±0.001)

The Bio–Concentration Ratios (BCR) of maize roots and shoots grown in different concentrations of NPs. The values are represented as (Mean ± SEM, n =3). Conc. represents concentration. Different letters denote a significant difference at the $p \leq 0.05$ levels.

Results of statistical analysis showed that the BCR of Cd NPs in maize roots were significantly different ($p < 0.01$) at 0.1 and 1.0 g kg^{-1} compared with the BCRs of roots grown in lower soil concentration (0.1 g kg^{-1}). There also appeared a significant difference ($p < 0.01$) for the BCR of maize shoots at 0.1 g kg^{-1} and significant difference ($p < 0.001$) at 1.0 g kg^{-1} when compared with the BCR of shoots grown in the 0.01 g kg^{-1} concentration.

The BCR of Cu NPs in maize roots showed a significant difference ($p < 0.001$) at concentrations of 0.1 and 1.0 kg^{-1} when compared with The BCR of roots grown in the lowest concentration. The BCR for CuO NPs in maize shoots was also significantly different ($p < 0.01$) at a concentration of 1.0 g kg^{-1} compared with the BCR of shoots grown in the lowest concentration. The BCRs of ZnO NPs were significantly different ($p < 0.001$) in maize roots and shoots grown

in 0.1 and 1.0 g kg^{-1} concentrations compared with the BCRs of roots and shoots grown in the 0.01 g kg^{-1} concentration.

Statistical analysis observed a significant difference ($p < 0.001$) between the BCR of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall BCR of Cd NPs was 0.15 (SE: ± 0.04) in roots; that of Cu NPs was 0.20 (SE: ± 0.03); that for Zn was 1.46 (SE: ± 0.50). The results of statistical analysis showed a significant difference ($p < 0.001$) between the BCR of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall BCR of Cd NPs was 0.05 (SE: ± 0.01) in roots; that of Cu NPs was 0.09 (SE: ± 0.02); that for Zn was 0.81 (SE: ± 0.30).

For all three metal NPs, BCRs appeared higher in maize roots than in shoots as shown in Table 5.5. This is plausibly because the NPs accumulated more in roots than in shoots, Section 5.9 indicated that total concentrations of Zn, Cu and Cd NPs appeared higher in roots than in shoots. The BCR of ZnO was the highest, followed by that of CuO; the BCR of CdS was the lowest (Table 5.5). This order is plausibly due to the solubility of ZnO NPs in the soil,[29] that Zn is an essential element for maize development is also a plausible factor.[28,35,36]

5.12. Bio–concentration ratios of NPs in the maize roots and shoots grown in hydroponic culture

As indicated, the BCR of a chemical compounds is defined as the ratio of the concentration of the chemical in an organism (plants or animals) and the concentration of the chemical in an aqueous or soil environment.[52,53] In this study, BCRs were calculated using Equation 5.1, as described in the previous section. The BCRs of Cd, Cu and Zn NPs in the maize's roots and shoots were calculated for hydroponic culture concentrations of 0.01, 0.1 and 1.0 g L^{-1}, as shown in Table 5.6. Inspection of the table suggests that the BCRs decreased with increasing NP concentrations of the nutrient solution across all maize roots and shoots.[52] This in agreement with the soil culture results described in Section 5.11.

Results of statistical analysis observed that the BCRs of tested NPs in maize roots were significantly different ($p < 0.01$) for Cd and Zn NPs and significantly different ($p < 0.001$) for Cu NPs at a concentration of 1.0 g L^{-1} compared with the BCRs of Cd and Zn NPs of maize roots grown in the 0.01 g L^{-1} concentration. Cd ($p < 0.01$), Cu, and Zn NPs ($p < 0.05$) appeared to decrease the BCRs of the maize shoots to significant degree when compared with the BCRs of

shoots grown in the 0.1 g L^{-1} concentration. The BCRs of Cd and Zn NPs in maize shoots appeared significantly different ($p < 0.001$) and significantly different ($p < 0.01$) for Cu NPs in shoots grown in the 1.0 g L^{-1} concentration when compared with the BCRs of shoots grown in the 0.01 g L^{-1} concentration.

Table 5. 6. The concentrations of Cd, Cu and Zn NPs (mg kg^{-1}) and their Bio–concentration ratios (BCRs) in the maize roots and shoots cultivated in hydroponic cultures.

Nanoparticles	parameters	Concentrations of nanoparticles used in the nutrient solution					
		0.01 g L^{-1}		0.1 g L^{-1}		1.0 g L^{-1}	
		Root	Shoot	Root	Shoot	Root	Shoot
CdS NPs	Conc. (mg kg^{-1})	4.06 (±0.59)	1.71 (±0.13)	31.30 (±0.89)	11.47 (±0.54)	46.29 (±1.73)	20.45 (±0.85)
	BCR	0.41^{a} (±0.06)	0.17^{a} (±0.01)	0.31^{ab} (±0.01)	0.11^{b} (±0.01)	0.05^{b} (±0.002)	0.02^{b} (±0.001)
CuO NPs	Conc. (mg kg^{-1})	6.54 (±0.28)	4.12 (±0.56)	61.44 (±0.43)	21.94 (±0.62)	105.59 (±1.55)	53.01 (±1.42)
	BCR	0.65^{a} (±0.03)	0.41^{a} (±0.06)	0.61^{ab} (±0.004)	0.22^{b} (±0.01)	0.11^{b} (±0.002)	0.05^{b} (±0.001)
ZnO NPs	Conc. (mg kg^{-1})	7.21 (±0.44)	4.97 (±0.44)	71.48 (±1.22)	36.66 (±2.54)	466.33 (±20.23)	149.30 (±3.56)
	BCR	0.72^{a} (±0.04)	0.50^{a} (±0.04)	0.71^{ab} (±0.01)	0.37^{b} (±0.03)	0.47^{b} (±0.02)	0.15^{b} (±0.004)

The Bio–concentration ratios (BCR) of maize roots and shoots grown in different concentrations of NPs. The values are represented as (Mean ± SEM, n =3). Conc. represents concentration. Different letters denote significant difference at the $P \leq 0.05$ levels.

Statistical analysis showed a significant difference ($p < 0.001$) between the BCR of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall BCR of Cd NPs was 0.23 (SE: ± 0.05) in roots; that of Cu NPs was 0.46 (SE: ± 0.08); that for Zn was 0.63 (SE: ± 0.04). There was a significant difference ($p < 0.01$) between the BCR of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall BCR of Cd NPs was 0.10 (SE: ± 0.02) in shoots; that of Cu NPs was 0.23 (SE: ± 0.05); that for Zn was 0.34 (SE: ± 0.05). As with the results of the maize roots and shoots grown in soil, for all three metal NPs, BCRs appeared higher in roots than in shoots, and the BCR of ZnO was the highest, followed by that of CuO; the

BCR of CdS was the lowest. The BCRs for three NPs in hydroponic cultures was higher than the BCRs of plants grown in the soil cultures and this was across both maize parts and all soil concentrations. This observation was because the elements were more bioreactive in hydroponic cultures.

5.13. The parameters used to evaluate the effect of NPs on plant growth in soil cultures

The TI, RI, and AE of the maize plants were calculated to evaluate the effect of CdS, CuO, and ZnO NP concentrations on the growth of the plants and their toxicity in Eutric Cambisol soil. Table 5.7 shows summary results.

Results of statistical analysis observed no significant differences as regards the TI, RI (%), and AE across any of the NPs concentrations of maize roots and shoots when compared with the low concentration of 0.01 g kg^{-1}. This agrees with the results for Cd and Zn particles, as described in Chapter 4, Sections 4.10.3 and 4.11.3.

There was no significant difference between TI of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall TI of roots was 0.90 (SE: ± 0.02) for Cd NPs; that of Cu NPs was 0.83 (SE: ± 0.06); that for Zn was 0.95 (SE: ± 0.09). The results of ANOVA also revealed no significant difference between TI of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall TI of shoots was 0.86 (SE: ± 0.06) for Cd NPs; that of Cu NPs was 0.88 (SE: ± 0.04); that for Zn was 0.99 (SE: ± 0.06).

The results of statistical analysis observed no significant differences between RI (%) of roots and the concentrations of Cd, Cu and Zn NPs. Moreover, ANOVA revealed no significant differences between RI (%) of shoots and the concentrations of Cd, Cu and Zn NPs.

The statistical analysis of AE showed the same results as RI (%) across all the NPs compounds and maize parts. The majority of RI (%) and AE indictors were negative; this was plausibly because the dry biomasses of the treated plants were affected by the added NPs compared to control samples. Similar results were obtained by Tantawy for bulk Cd.[48]

The majority of RI (%) and AE values for maize roots and shoots were lower than the values found in plants grown in the 0.01 g kg^{-1} concentration; this suggests that NPs decreased the biomass of maize roots and shoots.[27]

Table 5. 7. Dry matter yield (DMY), Tolerance Index (TI), Relative Increase (RI) and Agronomical Efficiency (AE) of maize plants as affected by adding CdS, CuO and ZnO NPs (g kg^{-1}) to Eutric Cambisol soil.

Nanoparticles	Plant part	Added concentration of nanoparticles in the soil (mg kg^{-1})												
		Control	0.01 g kg^{-1}				0.1 g kg^{-1}				1.0 g kg^{-1}			
		DMY (g/pot)	DMY (g/pot)	TI	RI %	AE	DMY (g/pot)	TI	RI %	AE	DMY (g/pot)	TI	RI %	AE
CdS NPs	Roots	0.28 (±0.03)	0.27 (±0.03)	0.99^{a} (±0.16)	-0.93^{a} (±0.04)	-0.09^{a} (±0.01)	0.25 (±0.03)	0.91^{ab} (±0.17)	-8.77^{ab} (±1.02)	-0.13^{ab} (±0.02)	0.22 (±0.02)	0.79^{ab} (±0.12)	-20.75^{ab} (±2.71)	-0.13^{ab} (±0.001)
	Shoots	0.40 (±0.02)	0.36 (±0.01)	0.92^{a} (±0.07)	-7.54^{a} (±1.72)	-0.33^{a} (±0.04)	0.34 (±0.06)	0.88^{ab} (±0.17)	-0.22^{ab} (±0.03)	-0.002^{ab} (±0.05)	0.30 (±0.02)	0.77^{ab} (±0.10)	-23.31^{ab} (±1.72)	-0.19^{ab} (±0.007)
CuO NPs	Roots	0.28 (±0.03)	0.25 (±0.03)	0.91^{a} (±0.12)	-8.77^{a} (±0.56)	-0.33^{a} (±0.01)	0.23 (±0.01)	0.84^{ab} (±0.10)	-16.02^{ab} (±2.16)	-0.20^{ab} (±0.01)	0.20 (±0.01)	0.73^{ab} (±0.07)	-26.59^{ab} (±1.55)	-0.16^{ab} (±0.01)
	Shoots	0.40 (±0.02)	0.37 (±0.01)	0.93^{a} (±0.04)	-7.01^{a} (±0.93)	-0.29^{a} (±0.001)	0.36 (±0.004)	0.91^{ab} (±0.13)	-8.86^{ab} (±1.49)	-0.16^{ab} (±0.001)	0.31 (±0.03)	-20.63^{ab} (±0.05)	2.65^{ab} (±0.90)	-0.17^{ab} (±0.002)
ZnO NPs	Roots	0.28 (±0.03)	0.30 (±0.03)	1.10^{a} (±0.10)	10.03^{a} (±0.70)	-0.29^{a} (±0.01)	0.26 (±0.02)	0.95^{ab} (±0.06)	-5.41^{ab} (±0.97)	0.21^{ab} (±0.03)	0.22 (±0.02)	0.80^{ab} (±0.06)	-20.14^{ab} (±0.57)	0.02^{ab} (±0.003)
	Shoots	0.40 (±0.02)	0.40 (±0.03)	1.03^{a} (±0.11)	2.80^{a} (±0.45)	-0.22^{a} (±0.03)	0.39 (±0.02)	1.00^{ab} (±0.05)	0.01^{ab} (±0.002)	0.16^{ab} (±0.02)	0.36 (±0.04)	0.93^{ab} (±0.14)	-7.13^{ab} (±1.81)	0.05^{ab} (±0.008)

The parameters of maize roots and shoots grown in different concentrations of NPs. The values are represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $p \leq 0.05$ levels.

Few RI (%) and AE values for ZnO NPs were positive; this was plausibly because the dry weight of the treated plant parts were not influenced by particle additions. That there were more negative than positive RI (%) and AE values suggests that the three NP types influenced the dry matter yield of the maize plants, especially at high levels of NP concentration. In this regard, other authorities have suggested that the AE of added metals can vary as a function of their concentration, plant part, and growth period.[47,53,54]

In the present study, TI values for roots and shoots decreases with increased NP concentration to the soil. These results have reported in previous studies for bulk materials.[55–57] The majority of TI values for maize roots were higher than for maize shoots across all concentrations; this agrees with results reported in Chapter 4, Sections, 4.10.3 and 4.11.3. The TI of ZnO was higher than the TIs of CuO and CdS; this was plausibly because the dry biomass of maize roots and shoots was not affected by additions of Zn but was affected by the other two NPs.

5.14. The parameters used to evaluate the effect of NPs on plant growth in hydroponic cultures

The TI, RI, and AE of the maize plants were calculated for CdS, CuO, and ZnO NPs for maize roots and shoots in hydroponic cultures across all nutrient concentrations. Table 5.8 summarises results.

Results of statistical analysis showed no significant differences for the TI, RI (%), and AE across any NP concentrations for both maize plant parts when compared with the TIs, RIs (%), and AEs of plants grown in the 0.01 g kg^{-1} concentration. A similar result was obtained for maize growing in soil cultures (see above). There was no significant difference between TI of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall TI of roots was 0.67 (SE: ± 0.11) for Cd NPs; that of Cu NPs was 0.97 (SE: ± 0.22); that for Zn was 1.05 (SE: ± 0.16). The results of statistical analysis observed no significant difference between TI of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall TI of shoots was 0.70 (SE: ± 0.08) for Cd NPs; that of Cu NPs was 0.76 (SE: ± 0.12); that for Zn was 0.89 (SE: ± 0.12). There were no significant differences between RI (%) of roots and the concentrations of Cd, Cu and Zn NPs. Moreover, statistical analysis observed no significant differences between RI (%) of shoots and the concentrations of Cd, Cu and Zn NPs.

Table 5. 8. Dry matter yield (DMY), Tolerance Index (TI), Relative Increase (RI), and Agronomical Efficiency (AE) of maize plants as affected by adding CdS, CuO and ZnO NPs (g L^{-1}) to hydroponic cultures.

Nanoparticles	Plant part	Added concentration of nanoparticles in the nutrient solution (mg L^{-1})												
		Control	0.01 g L^{-1}				0.1 g L^{-1}				1.0 g L^{-1}			
		DMY (g/pot)	DMY (g/pot)	TI	RI %	AE	DMY (g/pot)	TI	RI %	AE	DMY (g/pot)	TI	RI %	AE
CdS NPs	Roots	0.22 (±0.005)	0.15 (±0.002)	0.78^{a} (±0.02)	-22.01^{a} (±0.31)	-0.69^{a} (±0.06)	0.13 (±0.003)	0.70^{ab} (±0.02)	-30.44^{ab} (±2.33)	-0.37^{ab} (±0.04)	0.10 (±0.002)	0.53^{ab} (±0.02)	-47.25^{ab} (±1.53)	-0.23^{ab} (±0.01)
	Shoots	0.26 (±0.004)	0.22 (±0.01)	0.92^{a} (±0.12)	-8.11^{a} (±4.81)	-0.32^{a} (±0.01)	0.15 (±0.004)	0.59^{ab} (±0.09)	-40.60^{ab} (±3.51)	-0.42^{ab} (±0.01)	0.14 (±0.01)	0.58^{ab} (±0.05)	-42.17^{ab} (±0.87)	-0.23^{ab} (±0.01)
CuO NPs	Roots	0.22 (±0.005)	0.19 (±0.01)	1.00^{a} (±0.15)	0.04^{a} (±3.83)	-0.30^{a} (±0.08)	0.17 (±0.04)	0.98^{ab} (±0.19)	-1.65^{ab} (±1.95)	-0.19^{ab} (±0.006)	0.16 (±0.01)	0.82^{ab} (±0.05)	-8.44^{ab} (±0.06)	-0.12^{ab} (±0.002)
	Shoots	0.26 (±0.004)	0.22 (±0.03)	0.89^{a} (±0.08)	-11.28^{a} (±4.75)	-0.33^{a} (±0.002)	0.19 (±0.02)	0.78^{ab} (±0.10)	-21.95^{ab} (±4.61)	-0.28^{ab} (±0.003)	0.18 (±0.01)	0.61^{ab} (±0.08)	-39.11^{ab} (±2.15)	-0.16^{ab} (±0.02)
ZnO NPs	Roots	0.22 (±0.005)	0.22 (±0.05)	1.13^{a} (±0.25)	12.89^{a} (±1.52)	0.02^{a} (±0.003)	0.20 (±0.02)	1.03^{ab} (±0.08)	3.27^{ab} (±0.45)	-0.08^{ab} (±0.002)	0.19 (±0.03)	0.99^{ab} (±0.09)	-1.49^{ab} (±0.28)	-0.06^{ab} (±0.001)
	Shoots	0.26 (±0.004)	0.27 (±0.01)	1.05^{a} (±0.17)	5.04^{a} (±1.81)	0.13^{a} (±0.001)	0.22 (±0.03)	0.81^{ab} (±0.11)	-18.77^{ab} (±1.81)	-0.16^{ab} (±0.004)	0.20 (±0.01)	0.81^{ab} (±0.04)	-19.39^{ab} (±1.40)	-0.12^{ab} (±0.005)

The parameters of maize roots and shoots grown in different concentrations of NPs. The values are represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ levels.

The statistical analysis of AE showed the same results as RI (%) across all the NPs compounds and maize parts. Similar to the soil cultures, the majority of RI (%) and AE indictors were negative. The majority of RI (%) and AE values in maize roots and shoots decreased. A few positive RI and AE values were, however, obtained for ZnO NPs. The negative RI (%) and AE values suggest that all three NPs influenced the dry matter yield of the maize plant, especially at high concentration levels.

A similar trend appeared in the TIs of maize plants at different concentrations of NP concentrations. The values of the TI for roots and shoots decreased with increased nano–concentrations of the nutrient solution. The majority of TIs for maize roots were higher than those for shoots across all nano–concentrations and both plant parts. The TI of ZnO was higher than those of CuO and CdS NPs. Similar results were obtained for maize grown in soil cultures described in section 5.13 and Chapter 4, sections 4.10.3 and 4.11.3.

5.15. Uptake of NPs in maize plant parts grown in soil cultures

Table 5.9 shows the distribution of different concentrations (mg kg^{-1}) of the three metal NPs in maize roots and shoots in terms of their uptake and accumulation (μg kg^{-1}).

Results of statistical analysis observed significant differences ($p < 0.01$) in the uptake of Cd and Cu NPs by maize roots and shoots when grown at the concentration of 0.1 and 1.0 g kg^{-1} compared with control groups. The level of significance varied and detailed as follows:

The uptake of Cd by maize roots and shoots was significant ($p < 0.01$) at a concentration of 0.1 g kg^{-1}, but highly significant ($p < 0.001$) at a concentration of 1.0 g kg^{-1} compared with controls (plant samples without added NPs). The uptake of Cu was significant ($p < 0.001$) in maize roots grown in 0.1 and 1.0 g kg^{-1} concentrations but less the significant ($p < 0.01$) in maize shoots grown in 0.1 and 1.0 mg kg^{-1} concentrations compared with controls. The uptake of Zn by maize roots and shoots were each significantly different ($p < 0.001$) at a concentration of 1.0 g kg^{-1} when compared with that of the control samples.

Statistical analysis showed a significant difference ($p < 0.001$ between the uptake of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall uptake of Cd NPs was 2.25 μg/pot (SE: ± 0.63); that of Cu NPs was 5.62 μg /pot (SE: ± 1.93); that for Zn was 23.14 μg/pot (SE: ± 6.89). There was a significant difference ($p < 0.001$) between the uptake of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall uptake of Cd NPs was 1.23 μg/pot (SE: ± 0.36); that of Cu NPs was 2.49 μg/pot (SE: ± 0.681); that for Zn was 15.15 μg/pot (SE: ± 3.31). Nanoparticle uptake by maize roots and shoots for all three metals increased with increasing NP concentrations in Eutric Cambisol soil; this agrees with previous research.[58,59] In addition, the uptake of all types of NP by maize roots was higher than by their shoots. Uptake of Zn NPs was highest, followed by Cu NPs; uptake of Cd NPs was lowest. This result reflected the concentrations of these NPs in maize parts, as discussed in Section 5.9.

Table 5. 9. Concentrations of Cd, Cu and Zn NPs (mg kg^{-1} soil) and their uptake (µg/pot) by maize plant as affected by added nanoparticles in the Eutric Cambisol soil.

Nanparticles	Added concentration of nanoparticles in the soil (mg kg^{-1})							
Concentrations	Control		0.01 g kg^{-1}		0.1 g kg^{-1}		1.0 g kg^{-1}	
Plant part	Conc. mg kg^{-1}	Uptake µg/ pot	Conc. mg kg^{-1}	Uptake µg/ pot	Conc. µg kg^{-1}	Uptake µg/ pot	Conc. mg kg^{-1}	Uptake µg/ pot
CdS NPS Roots	*	*	2.88 (±0.39)	0.77^{ab} (±0.07)	12.39 (±1.33)	3.14^{b} (±0.18)	23.43 (±1.64)	5.09^{b} (±0.48)
CdS NPS Shoots	*	*	0.88 (±0.02)	0.32^{ab} (±0.01)	4.83 (±0.80)	1.71^{b} (±0.28)	9.68 (±0.35)	2.90^{b} (±0.24)
CuO NPS Roots	1.27 (±0.18)	0.31^{a} (±0.04)	3.00 (±0.02)	0.75^{ab} (±0.04)	22.14 (±0.90)	5.14^{b} (±0.29)	79.88 (±1.31)	16.24^{b} (±0.60)
CuO NPS Shoots	0.74 (±0.08)	0.29^{a} (±0.02)	1.47 (±0.31)	0.54^{ab} (±0.11)	10.61 (±0.75)	3.83^{b} (±0.30)	16.90 (±2.53)	5.28^{b} (±0.77)
ZnO NPS Roots	21.63 (±0.75)	6.12^{a} (±0.52)	35.22 (±2.12)	10.72^{ab} (±1.48)	59.19 (±4.44)	15.47^{ab} (±1.25)	274.49 (±1.37)	60.27^{b} (±6.62)
ZnO NPS Shoots	17.21 (±0.34)	6.79^{a} (±0.26)	20.32 (±0.84)	8.21^{ab} (±0.26)	31.55 (±0.77)	12.33^{ab} (±0.94)	91.91 (±0.80)	33.58^{b} (±2.10)

The uptake of maize roots and shoots grown in different concentrations of NPs. The values are represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ levels.

Table 5.10 summarises the total uptake (mg/pot) and uptake ratio of maize roots and shoots at different NP concentrations. Results of statistical analysis showed a significant difference ($p < 0.01$) for the total uptake of Cd at a concentration of 0.1 g kg^{-1} when compared with that of the control sample. Results also observed a significant difference ($p < 0.001$) for the total uptake of Cd in plants grown in the1.0 g kg^{-1} concentration when compared with the control. The uptake ratio of Cd by maize roots and shoots was found to be significant ($p < 0.001$) for plants grown in the 0.01, 0.1 and 1.0 g kg^{-1} concentrations when compared to the controls.

Table 5. 10. Total uptake of Cd, Cu and Zn NPs (µg/pot) and their uptake ratios by maize roots and shoots at different concentrations of nanoparticles in Eutric Cambisol soil.

Added concentration (g kg^{-1})	Nanoparticles								
	CdS NPs			CuO NPs			ZnO NPs		
	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots
Control	*	*	*	0.65^{a} (±0.02	54.64^{a} (±3.33)	45.36^{a} (±3.33)	12.91^{a} (±0.85)	47.13^{a} (±2.58)	52.87^{a} (±2.58)
0.01	1.09^{ab} (±0.05)	70.52^{b} (±2.46)	29.48^{b} (±2.46)	1.29^{ab} (±0.01)	58.36^{ab} (±5.97)	41.64^{ab} (±3.25)	18.93^{ab} (±2.16)	56.39^{ab} (±2.13)	43.61^{ab} (±2.13)
0.1	4.84^{b} (±0.53)	65.71^{b} (±2.35)	34.29^{b} (±2.35)	8.97^{b} (±0.30)	57.83^{ab} (±3.51)	42.17^{ab} (±3.15)	27.80^{ab} (±2.72)	55.13^{ab} (±3.70)	44.87^{ab} (±3.70)
1.0	7.99^{b} (±0.53)	63.58^{b} (±2.54)	36.41^{b} (±2.54)	21.52^{b} (±0.77)	75.62^{ab} (±3.32)	24.38^{ab} (±2.57)	93.56^{b} (±7.53)	64.03^{b} (±2.25)	35.97^{b} (±3.01)

The total uptake of maize roots and shoots grown in different concentrations of NPs. The values are represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ levels.

Results of statistical analysis observed that the total uptake of Cu was significantly different ($p < 0.001$) at concentrations of 0.1 and 1.0 g kg^{-1} when compared with the control. Results observed, however, no significant differences as regards the uptake ratios of Cu in roots and shoots when compared with the control groups for any of the nano–concentrations to the soil.

Results also observed a significant difference ($p < 0.001$) as regards the total uptake of Zn under the 1.0 g kg^{-1} concentration when compared with that of the control. Results also showed significant differences as regards the uptake ratios of Zn in roots and shoots ($p < 0.05$) under the 1.0 g kg^{-1} concentration when compared with that of their controls. There was a significant difference ($p < 0.001$) between the total uptake of NPs as regards the concentration of Cd, Cu

and Zn NPs. The mean overall total uptake of Cd NPs was 3.48 μg/pot (SE: ± 0.90); that of Cu NPs was 8.11 μg/pot (SE: ± 2.54); that for Zn was 38.30 μg/pot (SE: ± 9.11).

There was a significant difference ($p < 0.001$) between the uptake % of roots as regards the concentration of Cd, Cu and Zn NPs. The mean overall uptake % of Cd NPs in roots was 49.96 % (SE: ± 8.78); that of Cu NPs was 61.61 % (SE: ± 3.23); that for Zn was 55.67 % (SE: ± 2.19). The results of ANOVA revealed a significant difference ($p < 0.001$) between the uptake % of shoots as regards the concentration of Cd, Cu and Zn NPs. The mean overall uptake % of Cd NPs in shoots was 25.04 % (SE: ± 4.52); that of Cu NPs was 38.39 % (SE: ± 3.23); that for Zn was 44.33 % (SE: ± 2.19).

NP uptake in maize roots and shoots generally increased as a function of NP concentration in soil–the higher the soil concentration, the higher the uptake. Uptake of all three types of NP was higher in roots than in shoots across all concentrations. The highest uptake was of Zn, followed by Cu; there was least uptake of Cd. This agrees with the results obtained for Zn and Cd as described in Chapter 4, Sections 4.10.4 and 4.11.4.

5.16. Uptake of NPs in maize plant parts grown in hydroponic culture

Table 5.11 summarises the concentrations of the three types of NPs and their uptakes ($\mu g\ kg^{-1)}$ by maize roots and shoots grown in the hydroponic culture.

Inspection of the table suggests that the uptake of three NPs increased in maize roots and shoots as a fuction of their concentration in the nutrient solution–the higher the concentration, the higher the uptake. The uptake of Zn appears higher than that of Cd and Cu; This order is plausibly due to the solubility of ZnO NPs in the nutrient solution,[29] that Zn is an essential element for maize development is also a plausible factor.[28,35,36]

Table 5. 11. Concentrations of Cd, Cu and Zn NPs (mg L^{-1} nutrient solution) and their uptake (µg/pot) by maize plant as affected by added NPs in hydroponic cultures.

Nanparticles	Added concentration of nanoparticles in the nutrient solution (mg L^{-1})							
Concentrations	Control		0.01 g L^{-1}		0.1 g L^{-1}		1.0 g L^{-1}	
	Plant part	Conc. mg kg^{-1}	Uptake µg/ pot	Conc. mg kg^{-1}	Uptake µg/ pot	Conc. mg kg^{-1}	Uptake µg/ pot	Conc. mg kg^{-1}	Uptake µg kg^{-1}
CdS NPS	roots	*	*	4.06 (±0.59)	0.62^{ab} (±0.06)	31.30 (±0.89)	3.97^{b} (±0.46)	46.29 (±1.73)	4.80^{b} (±0.15)
	shoots	*	*	1.71 (±0.13)	0.38^{ab} (±0.01)	11.47 (±0.54)	1.73^{b} (±0.05)	20.45 (±0.85)	2.87^{b} (±0.17)
CuO NPS	Roots	0.13 (±0.04)	0.03^{a} (±0.001)	6.54 (±0.28)	1.25^{ab} (±0.05)	61.44 (±0.43)	10.74^{ab} (±1.09)	105.59 (±1.55)	16.73^{ab} (±1.80)
	Shoots	0.07 (±0.03)	0.02^{a} (±0.001)	4.12 (±0.56)	0.94^{ab} (±0.08)	21.94 (±0.62)	4.11^{ab} (±0.61)	53.01 (±1.42)	9.07^{ab} (±1.90)
ZnO NPS	Roots	0.69 (±0.11)	0.16^{a} (±0.02)	7.21 (±0.44)	1.65^{ab} (±0.07)	71.48 (±1.22)	14.50^{ab} (±1.81)	466.33 (±20.23)	89.94^{b} (±5.72)
	Shoots	0.27 (±0.07)	0.07^{a} (±0.003)	4.97 (±0.44)	1.28^{ab} (±0.04)	36.66 (±2.54)	7.49^{ab} (±1.90)	149.30 (±3.56)	29.08^{b} (±3.62)

The uptake of maize roots and shoots grown in different concentrations of NPs. The values are represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ levels.

Franklin *et al.*[29] have suggested that a buffered solution (pH 7.5), ionic strength, and the solubility of ZnO NPs may play significant roles in their aggregation and availability in aqueous suspensions. A similar result was obtained for maize cultured in Eutric Cambisol soil (see Section 5.15).

Results of statistical analysis observed significant differences (each $p < 0.01$) as regards the uptake of Cd by maize roots and shoots when grown under the 0.1 and 1.0 g kg^{-1} concentrations; however, the appeared no significant differences as regards Cu NP uptake for either maize plant part when grown under any of the applied concentrations. Results observed a significant difference ($p < 0.001$) as regards the uptake of Zn by maize roots when grown under the 1.0 g kg^{-1} concentration when compared with that of the control. The result for shoots grown under this concentration was similar, save that the significance level was lower ($p < 0.05$).

There was a significant difference ($p < 0.001$) between the uptake of roots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall uptake of Cd NPs was 2.35 µg/pot (SE: ± 0.66); that of Cu NPs was 7.19 µg/pot (SE: ± 2.78); that for Zn was 26.57 µg/pot (SE: ± 8.65). The results of statistical analysis observed a significant difference ($p < 0.05$) between the uptake of shoots as regards the concentrations of Cd, Cu and Zn NPs. The mean overall uptake of Cd NPs was 1.25 µg/pot (SE: ± 0.36); that of Cu NPs was 3.53 µg/pot (SE: ± 1.49); that for Zn was 9.48 µg/pot (SE: ± 2.20). As indicated in chapter 2, Section 2.15 various factors such as concentration, specific surface area and particle size, plant species, growth media and stability of NPs affect NPs toxicity in plants. Little is known about the effects of MNPs on plants.[4,5] but a few studies on the toxicity of MNPs have been performed. For instance, Zn NPs have been shown to decrease the root growth of radish, rape, ryegrass, lettuce, corn and cucumber grown in aqueous suspension at level of 2000 mg L^{-1}.[6] In addition, ZnO NPs showed that the biomass of ryegrass was reduced when the plant was grown in Hoagland solution at a concentration of 1000 mg L^{-1}.[8] Cu NPs reduced the seedling growth of mung bean grown in agar culture media at a concentration of < 200 mg L^{-1}.[31] The NPs toxicity in plants has been discussed in Chapter 2, Section 2.11– 2.12.2. Studies indicated that not all plants treated with NPs demonstrated toxicity impacts at threshold concentrations. Studies observed positive or no consequential effects in plants when treated with NPs. Nevertheless, caution must be exercised in making assumptions about the effects of particular NPs. The observed toxicological and Physiological effects in

plants might not be sensitive indicators of NPs toxicity. Thus, studies at genomic, metabolic, and proteomic levels are needed.

The uptake of all NPs in maize roots and shoots generally increased as a function of NP concentration in nutrient solution–the higher the concentration, the higher the uptake. In general, uptake of all three types of NPs was higher in roots than in shoots. The highest uptake was of Zn, followed by Cu; there was least uptake of Cd. Table 5.12 summarises the total uptake and uptake ratio of NPs by maize roots and shoots.

Results of statistical analysis showed a significant difference ($p < 0.01$) as regards the total uptake of Cd at a concentration of 0.1 g L^{-1} when compared with that of the control; and a similar result for the total uptake of Cd at a concentration of 1.0 g L^{-1}, save that the significance was higher ($p < 0.001$). Results observed significant differences ($p < 0.001$) as regards the uptake ratios of Cd in roots and shoots grown under the 0.01, 0.1 and 1.0 g L^{-1} when compared with the controls.

Table 5. 12. Total uptake of Cd, Cu and Zn NPs (µg/pot) and their uptake ratios by maize roots and shoots at different concentrations of nanoparticles in hydroponic cultures.

Added concentration (g L^{-1})	Nanoparticles								
	CdS NPs			CuO NPs			ZnO NPs		
	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots	Total Uptake (µg/pot)	Uptake R. (%) Roots	Uptake R. (%) Shoots
Control	*	*	*	0.05^{a} (±0.001)	57.28^{a} (±2.68)	42.72^{a} (±3.40)	0.23^{a} (±0.02)	65.31^{a} (±3.98)	34.659^{a} (±1.98)
0.01	1.00^{ab} (±0.06)	61.29^{b} (±4.04)	38.71^{b} (±2.15)	2.19^{ab} (±0.03)	58.16^{ab} (±3.92)	41.84^{ab} (±1.45)	2.94^{ab} (±0.02)	54.39^{ab} (±2.34)	45.61^{ab} (±1.34)
0.1	5.70^{b} (±0.47)	68.74^{b} (±3.32)	31.26^{b} (±3.32)	14.84^{ab} (±1.85)	70.69^{ab} (±3.86)	29.31^{ab} (±3.86)	21.99^{ab} (±2.53)	66.59^{ab} (±2.23)	33.41^{ab} (±2.23)
1.0	7.68^{b} (±0.53)	62.92^{b} (±2.58)	37.08^{b} (±2.58)	25.80^{ab} (±2.70)	43.55^{ab} (±3.13)	23.11^{ab} (±1.25)	119.02^{b} (±4.65)	76.68^{ab} (±5.53)	23.32^{ab} (±2.53)

The total uptake of maize roots and shoots grown in different concentrations of NPs. The values are represented as (Mean ± SEM, n =3). Different letters denote significant difference at the $P \leq 0.05$ levels.

Results of statistical analysis showed no significant difference as regards the total uptake and uptake ratios of Cu in maize roots and shoots compared with controls. There was a significant difference ($p < 0.01$) as regards total uptake of Zn in plants grown under the 1.0 g L^{-1} concentration when compared with that of the control. However, results observed no significant

differences as regards the uptake ratios of Zn in maize roots and shoots when compared with those of the controls; this was across all nutrient concentrations.

The results of statistical analysis revealed a significant difference ($p < 0.001$) between the total uptake of NPs as regards the concentration of Cd, Cu and Zn NPs. The total dry biomass of maize roots and shoots was used to calculate the uptake of all NPs in each experimental pot (9.0 cm diameter, 14.0 cm depth, capacity 570 mL). The mean overall total uptake of Cd NPs was 3.59 µg/pot (SE: ± 1.01); that of Cu NPs was 10.72 µg/pot (SE: ± 2.21); that for Zn was 36.04 µg/pot (SE: ± 8.98).

There was a significant difference ($p < 0.01$) between the uptake % of roots as regards the concentration of Cd, Cu and Zn NPs. The mean overall uptake % of Cd NPs in roots was 48.24 % (SE: ± 8.53); that of Cu NPs was 57.42 % (SE: ± 6.91); that for Zn was 65.74 % (SE: ± 4.45). The results of statistical analysis showed no significant difference between the uptake % of shoots as regards the concentration of Cd, Cu and Zn NPs. The mean overall uptake % of Cd NPs in shoots was 26.76 % (SE: ± 4.89); that of Cu NPs was 34.25 % (SE: ± 5.49); that for Zn was 34.26 % (SE: ± 4.48).

The uptake of NPs occurs as follows: ZnO NPs > CuO NPs > CdS NPs and this order correlates with that given for plants grown in soil (Section 5.15): the uptake of all NPs in maize roots and shoots appeared to increase as a function of NP concentrations in nutrient solution–the higher the concentration, the higher the uptake. In general, the calculated uptake of all three types of NP was higher in maize roots than in shoots. The highest uptake was of Zn, followed by that of Cu; there was least uptake of Cd.

5.17. Conclusion

- The results of soil and hydroponic culture suggest that the concentrations of Cd, Cu and Zn NPs increased in the maize roots and shoots as a function of their concentrations in the Eutric Cambisol soil and nutrient solution. The results revealed that the concentration of Cd, Cu and Zn NPs was higher in maize roots than in the shoots. Zn NPs accumulate in maize roots and shoots more than Cu NPs and the accumulation of Cd NPs in the maize parts was the lowest.
- Results indicated no negative effects of Cd, Cu and Zn NPs on the length and dry biomass of maize roots or shoots, save for CuO, which appeared to decrease the length of maize roots and shoots at a soil concentration of 1.0 g kg^{-1}. In addition, hydroponic culture showed that CdS NPs had a negative effect on the length of roots and shoots at a concentration of 1.0 mg L^{-1}. The dry weight of maize shoots was significantly inhibited by 1.0 mg L^{-1} CdS NPs.
- The total concentrations of Cd, Cu and Zn NPs in the Eutric Cambisol soil increased with increases in the level of soil NPs. The results suggest the concentration of Cd NPs in the soil increased more than those of Cu and Zn NPs.
- The BCRs of Cd, Cu and Zn NPs in maize roots and shoots gradually decreased with increases in NPs concentrations in both growth media (soil and nutrient solution). BCRs of Cd, Cu and Zn NPs appeared higher in roots than in shoots. The BCR of ZnO was the highest, followed by that of CuO; the BCR of CdS was the lowest.
- There were no significant differences as regards the TI, RI, and AE across any of the NPs concentrations of maize roots and shoots in both cultures. However, TI values for roots and shoots decreased with increased NP concentrations to the soil and nutrient solution. The TIs of all NPs in maize roots were higher than that of maize shoots in most concentrations under study. The TIs of Zn NPs in maize root and shoots was higher than the TIs of Cu and Cd NPs.
- The majority of RI (%) and AE indictors were decreased with increased NP concentrations to the soil and nutrient solution, these calculated parameters were negative in most concentrations under study; this was plausibly because the dry biomasses of the treated plants were decreased by the added NPs compared to dry biomasses of untreated plants in the controls.

- The uptake of Cd, Cu and Zn NPs by maize roots and shoots increased with increasing NP concentrations in the Eutric Cambisol soil and nutrient solution. The uptake of Cd, Cu and Zn NPs by maize roots was higher than by their shoots. Uptake of Zn NPs was highest, followed by Cu NPs; uptake of Cd NPs was lowest.
- Total uptake of Zn NPs was highest, followed by Cu NPs; total uptake of Cd NPs was lowest across all NP concentrations and culture type (soil and nutrient solution).
- The results of soil and hydroponic culture showed that the uptake % of all NPs in maize root was higher than that of shoots. Uptake % of Cu NPs was highest in maize roots, followed by Zn NPs; uptake % of Cd NPs was lowest in soil culture. However, Uptake % of Zn NPs was highest in maize shoots, followed by Cu NPs; uptake % of Cd NPs was lowest in soil culture. Hydroponic culture showed uptake % of Zn NPs was highest in maize roots and shoots, followed by Cu NPs; uptake % of Cd NPs was lowest.
- The results of comparing between soil and hydroponic culture revealed that the concentration of Cd, Cu and Zn NPs in maize roots and shoots grown in nutrient solution was higher than those grown in the Eutric Cambisol soil. The majority of calculated parameters (BCR, TI, AE, uptake, uptake % and total uptake) for maize roots and shoots in hydroponic culture were higher than those grown in the Eutric Cambisol soil.

5.18. References

1. Moore, M.N. (2006). Do nanoparticles present ecotoxicological risks for the health of the aquatic environment. *Environment International*. 32 (8): 967–976.

2. Nel, A., Xia, T., Madler, L., and Li, N. (2006).Toxic potential of materials at the nanolevel. *Science* (New York, N.Y.). 311 (5761): 622–627.

3. Pan, B., and Xing, B. (2010). Manufactured nanoparticles and their adsorption of organic chemicals. *Advances in Agronomy*. 108 (C): 137–181.

4. Darlington, T.K., Neigh, A.M., Spencer, M.T., Nguyen, O.T., and Oldenburg S.J. (2009). Nanoparticle characteristics affecting environmental fate and transport through soil. *Environmental Toxicology and Chemistry*. 28 (6): 1191–1199.

5. Pidgeon, N., Harthorn, B.H., Bryant, K., and Rogers–Hayden, T. (2009). Deliberating the risks of nanotechnologies for energy and health applications in the United States and United Kingdom. *Nature Nanotechnology*. 4 (2): 95–98.

6. Lin, D., and Xing, B. (2007). Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environmental Pollution*. 150 (2): 243–250.

7. Barrena, R., Casals, E., Colon, J., Font, X., Sanchez,A., and Puntes, V. (2009). Evaluation of the ecotoxicity of model nanoparticles. *Chemosphere*. 75 (7): 850–857.

8. Lin D., and Xing, B. (2008). Root uptake and phytotoxicity of ZnO nanoparticles. *Environmental Science and Technology*. 42 (15): 5580–5585.

9. Kurepa, J., Paunesku, T., Vogt, S., Arora, H., Rabatic, B.M., Lu, J., Wanzer, M.B., Woloschak G.E., and Smalle, J.A., (2010). Uptake and distribution of ultrasmall anatase TiO_2 alizarin red S nanoconjugates in *arabidopsis thaliana*. *Nano Letters*. 10 (7): 2296–2302.

10. Zhu, H., Han, J., Xiao, J.Q., and Jin, Y. (2008). Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. *Journal of Environmental Monitoring*. 10 (6): 713–717.

11. Serag, M. F., Kaji, N., Gaillard, C., Okamoto, Y., Terasaka, K., Jabasini, M., Tokeshi, M., Mizukami, H., Bianco, A., and Baba, Y. (2011). Trafficking and subcellular localization of multiwalled carbon nanotubes in plant cells. *ACS Nano*. 5 (1): 493–499

12. Liu, Q., Chen, B., Wang, Q., Shi, X., Xiao, Z., Lin, J., and Fang, X. (2009). Carbon nanotubes as molecular transporters for walled plant cells. *Nano Letters*. 9 (3): 1007–1010.

13. Cifuentes, Z., Custardoy, L., de la Fuente, JM., Marquina, C., Ibarra, M.R., Rubiales, D., and Perez–de–Luque, A. (2010). Absorption and translocation to the aerial part of magnetic carbon–coated nanoparticles through the root of different crop plants. *Journal of Nanobiotechnology*. 8: 26.

14. Zhu, J., Li, D., Chen, H., Yang, X., Lu, L., and Wang, X. (2004). Highly dispersed CuO nanoparticles prepared by a novel quick–precipitation method. *Materials letters*. 58 (26): 3324–3327.

15. Blinova, I., Ivask A., Heinlaan, M., Mortimer, M., and Kahru, A. (2010). Ecotoxicity of nanoparticles of CuO and ZnO in natural water. *Environmental Pollution*. 158 (1): 41–47.

16. Zhao, J., Wang, Z., Liu, X., Xie, X., Zhang, K., and Xing, B. (2011). Distribution of CuO nanoparticles in juvenile carp (*Cyprinus carpio*) and their potential toxicity. *Journal of Hazardous Materials*. 197: 304–310.

17. Wang Z., Li, J., Zhao, J., and Xing, B. (2011). Toxicity and internalization of CuO nanoparticles to prokaryotic alga microcystis aeruginosa as affected by dissolved organic matter. *Environmental Science and Technology*. 45 (14): 6032–6040.

18. Zhou, D., Jin, S., Li, L., Wang, Y., and Weng, N. (2011). Quantifying the adsorption and uptake of CuO nanoparticles by wheat root based on chemical extractions. *Journal of Environmental Sciences*. 23 (11): 1852–1857.

19. Atha, D. H., Wang, H., Petersen, E. J., Cleveland, D., Holbrook, R. D., Jaruga, P., Dizdaroglu, M., Xing, B., and Nelson, B. C. (2012). Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. *Environmental Science and Technology*. 46 (3): 1819–1827.

20. Yang, L., and Watts, D.J. (2005). Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicology Letters*. 158 (2): 122–132.

21. Joint Committee on Powder Diffraction Standards, Diffraction, Data File, No. 5–661, JCPDS *International Center for Diffraction Data*. (1991). Pennsylvania.

22. Wu, H. Q., Wei, X. W., Shao, M. W., Gu, J. S., and Qu, M. Z. (2002). Synthesis of copper oxide nanoparticles using carbon nanotubes as templates. *Chemical Physics Letters*. 364 (1–2): 152–156.

23. Pendashteh, A., Mousavi, M. F., and Rahmanifar, M. Safi. (2013). Fabrication of anchored copper oxide nanoparticles on graphene oxide nanosheets via an electrostatic coprecipitation and its application as supercapacitor. *Electrochimica Acta*. 88: 347–357.

24. Hassan, K.H., Al–Bayati, T. H., And Abbas, Z. M. A. (2012). Study and characterization of copper oxide nanoparticles prepared by chemical method using X–Ray diffraction and scanning electron microscope. *American Journal of Scientific Research*. 77: 49–53.

25. Wang, Z., Xie, X., Zhao, J., Liu, X., Feng, W., White, J. C., and Xing, B. (2012). Xylem– and phloem–based transport of CuO nanoparticles in maize (*Zea mays* L.). *Environmental Science and Technology*. 46 (8): 4434–4441.

26. Keller, A.A., Wang, H., Zhou, D., Lenihan, H.S., Cherr, G., Cardinale, B.J., Miller, R., and Ji Z. (2010). Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environmental Science and Technology*. 44 (6): 1962–1967.

27. Zhao, L., Hernandez–Viezcas, J. A., Peralta–Videa, J. R., Bandyopadhyay, S., Peng, B., Munoz, B., Keller, A. A., and Gardea–Torresdey, J. L. (2012). ZnO nanoparticle fate in soil and zinc bioaccumulation in corn plants (*Zea mays*) influenced by alginate. *Environmental Science: Processes and Impacts.* 15 (1): 260.

28. Lopez–Moreno, M.L., De la Rosa, G., Hernandez–Viezcas, J.A., Castillo–Michel, H., Botez, C.E., Peralta–Videa, J.R., and Gardea–Torresdey, J.L. (2010). Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (*Glycine max*) plants. *Environmental Science and Technology.* 44 (19): 7315–7320.

29. Franklin, N.M., Rogers, N.J., Apte, S.C., Batley, G.E., Gadd, G.E., and Casey, P.S. (2007). Comparative toxicity of nanoparticulate ZnO, bulk ZnO and $ZnCl_2$ to a freshwater microalga (*Pseudokirchnerilla subcapitata*): the importance of particle solubility. *Environmental Science and Technology.* 41(24): 8484–8490.

30. Zhao, L., Peralta–Videa, J., Ren, M., Varela–Ramirez, A., Li, C., Hernandez–Viezcas, J., Aguilera, R., and Gardea–Torresdey, J. (2012). Transport of Zn in a sandy loam soil treated with ZnO NPs and uptake by corn plants: Electron microprobe and confocal microscopy studies. *Chemical Engineering Journal.* 184: 1–8.

31. Lee, W.M., An, Y.J., Yoon, H., Kweon, H.S. (2008). Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum awstivum*): plant uptake for water insoluble nanoparticles. *Environmental Toxicology and Chemistry.* 27 (9): 1915–1921.

32. Stampoulis, D., Sinha, S.K., and White, J.C. (2009). Assay–dependent phytotoxicity of nanoparticles to plants. *Environmental Science and Technology.* 43 (24): 9473–9479.

33. Adiloglu, A. I., Adiloglu, S., Gonulsuz, E ., and Oner, N. (2005). Effect of zinc application on cadmium uptake of maize grown in zinc deficient soil. *Pakistan Journal of Biological Sciences.* 8 (1): 10–12.

34. Artur, N., Kosynets, O., Nosalewicz, M. (2008). Effect of various concentrations of lead and cadmium on early growth of maize. *Acta Agrophysica.* 11 (3): 715–723.

35. Van de mortel, J.E., Villanueva, A. L., Schat, H., Kwekkeboom, J., Coughlan, S., Moerland, P.D., Themaat, V. L. V. E., Koornneef, M., and Aarts, M.G. (2006). Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of Arabidopsis thaliana and the related metal hyperaccumulator *Thlaspi caerulescens*. *Plant Physiology.* 142 (3): 1127–1147.

36. Kochian, L.V. (2000). Molecular physiology of mineral nutrient acquisition, transport, and utilization. In: Biochemistry and molecular biology of plants (Eds Buchanan, B., Gruissem, W., and Jones, R.). *American Society of Plant Physiologists*. Rockville, MD. pp: 1237–1239.

37. Anton, A., and Mathe–Gaspar, G. (2005). Factors affecting heavy metal uptake in plant selection for phytoremediation. *Zeitschrift Fur Naturforschung. C. Journal of Biosciences.* 60 (3–4): 244–246.

38. Wilkins, D. A. (1978). The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist.* 80 (3): 623–633.

39. Ellis, S.C. (2011). Ecotoxicology of metal Oxide nanoparticles: Effects on soil Process and plant growth. University of Wales, Bangor, *MSc thesis*, pp: 24–40.

40. Roberts, P., Roberts J.P., and Jones, D.L. (2006). Behaviour of the endocrine disrupting chemical nonylphenol in soil: Assessing the risk associated with spreading contaminated waste to land. *Soil Biology and Biochemistry.* 38 (7): 1812–1822.

41. Gondek, K. (2009). Zinc content in maize (*Zea mays* L.) and soils fertilized with sewage sludge and sewage sludge mixed with peat. *Polish Journal of Environmental Studies.* 18 (3): 359–368.

42. Martin–Ortiz, D., Hernandez–Apaolaza, L., and Garate, A. (2009). Efficiency of a NPK fertilizer with adhered zinc lignosulfonate as a zinc source for maize (*Zea mays* L.). *Journal of Agricultural and Food Chemistry.* 57 (19): 9071–9078.

43. Liu, D.H., Wang, M., Zou, J. H., and Jiang, W.S. (2006). Uptake and accumualtion of cadmium and some nutrients ions by roots and shoots of maize (*zea mays*. L). *Pakistan Journal of Botany*. 38 (3): 701–709.

44. Zhang, L., Zhang, L., and Song, F. (2008). Cadmium uptake and distribution by different maize genotypes in maturing stage. *Communications in Soil Science and Plant Analysis.* 39 (9–10): 1517–1531.

45. Wang, J., Mao, H., Zhao, H., Huang, D., and Wang, Z. (2012). Different increases in maize and wheat grain zinc concentrations caused by soil and foliar applications of zinc in Loess Plateau, China. *Field Crops Research.* 135: 89–96.

46. Wang, H., and Jin, J. (2007). Effects of zinc deficiency and drought on plant growth and metabolism of reactive oxygen species in maize (*Zea mays* L). *Agricultural Sciences in China.* 6 (8): 988–995.

47. Wang, C.X., Tao, L., and Ren, J. (2013). The response of maize seedlings to cadmium stress under hydroponic conditions. *Russian Journal of Plant Physiology.* 60 (2): 295–299.

48.Tantawy, M. F. 2004. Monitoring of environmental pollution with heavy metals in some delta soils and its phytoremediation. *phD thesis*. pp.118–121.

49. El–Kassas, I. H., Bader, A., and Amer, M. N. (2002). phytoremediation of cadmium contaminated soils using different hyperaccumulation plants. *Zagazig Journal of Agricultural research*. 29 (4): 1199–1213.

50. McKone, T. E., and Maddalena, R. L. (2007). Plant uptake of organic pollutants from soil: bioconcentration estimates based on models and experiments. *Environmental Toxicology and Chemistry.* 26 (12): 2494–504.

51. Alloway, B. J. (1995). Heavy metals in soils. London: Blackie Academic and Professional.

52. Jasiewicz.C., Agnieszka, B., Marek, T. (2010). Effect of bottom sediment on content bioaccumulation and translocation of heavy metals in maize biomass. *Journal of Elementol.* 15 (2): 281–290

53. Graham, R. D. (1978). Nutrient efficiency objectives in cereal breeding. In Proceedings of the 8th international colloquium on plant analysis and fertilizer problems, 165–170. New Zealand Division of Scientific and Industrial Research, Information Series no. 134. *Wellington*, New Zealand: Government Printer.

54. Cooke, G.W. (1987). Maximizing fertilizer efficiency by overcoming constraints. *Journal of Plant Nutrient.* 10: 1357–1369.

55. Bauddh, K., and Singh, R. P. (2011). Differential toxicity of cadmium to mustard (*Brassica juncia* L.) genotypes under higher metal levels. *Journal of Environmental Biology / Academy of Environmental Biology, India.* 32 (3): 355–362.

56. Chen, X., Wang, J., Shi, Y., Zhao, M.Q., and Chi, G.Y. (2011). Effects of cadmium on growth and photosynthetic activities in pakchoi and mustard. *Botanical Studies.* 52 (1): 41–46.

57. Symeonidis, L., Mcneilly, T., and Bradshaw, A. D. (1985). Differential tolerance of three cultivars of agrostis capillaris L. to cadmium, copper, lead, nickel and zinc. *New Phytologist.* 101 (2): 309–315.

58. Wang, M., Zou J., Duan, X., Jiang, W., and Liu, D. (2007). Cadmium accumulation and its effects on metal uptake in maize (*Zea mays* L.). *Bioresource Technology.* 98 (1): 82–88.

59. Chitra, K., Sharavanan, S., and Vijayaragavan, M. (2011). Tobacco, corn and wheat for phytoremediation of cadmium polluted soil. *Science and Technology.* 3 (2): 148–151.

Chapter 6: Adsorption and desorption of nanoparticles in different soils cultivated for various years in Wales (UK) and the Gefara Plain (Libya)

6.1. Introduction

Metal oxide NPs have applications in the manufacture of commercial and industrial products.[1,2] Metal oxide NPs have potentially toxic, and it is inevitable that, with increasing use, they will be released into the environment. To date little research has been conducted into the ecological effects of these manufactured NPs (MNPs). CuO and ZnO NPs are used in a large variety of applications.[3–7] The use of NPs as semi–conductors has attracted interest in recent years; this is because of their properties are different from those of their analogous bulk materials and from those of the single atoms of which they are comprised.[8,9] CdS NPs has unique photochemical and photo–physical properties.[10–12]

Adsorption and desorption reactions on the surfaces of soils are factors that control the concentration of bulk Zn, Cu, and Cd in soil solutions.[13–15] Differences in adsorption affinities of heavy metals for soil surfaces has been attributed to the hydrolysis constant, electronegative scale, Lewis acidity, charge density, and the solubility of precipitates (including hydroxides and carbonates) of a given metal.[16–18] Thus, the potential toxicity of heavy metals in soils may mostly depend on the composition of the soil's solids, particularly the amounts and types of clay minerals and organic matter.[19,20] The bio–availability and mobility of heavy metals in soils appear mainly to be a function of their physicochemical forms (i.e. their chemical fraction or speciation).[21]

The interactions of NPs with soil minerals and organic matter do not appear to have been evaluated but are likely to be a function of particle size, shape, and surface properties–their specific surface area and surface charge for instance.[22] To ensure ecological validity, it is important that studies be carried out in different soils – the NPs may have different effects in different soil types. However, there is little information on the behaviour and toxicity of NPs in terrestrial systems; this appears to be due to difficulties in assessing dose against a background of natural NPs in the soil matrix. Heterogeneity and incorporation of NPs into soil is also an issue for ecotoxicological testing. There are a few reports concerning the adverse effects of certain NPs on terrestrial species cultured *in vitro*, but to date there is little evidence that they have any significant adverse effects on terrestrial species in soil exposures. Further studies are needed that

include a wide range of terrestrial species, nanoparticulate materials, and soil environments to determine if results from preliminary data are sound.[22]

6.2. Objectives

1. To investigate the adsorption of CdS, ZnO and CuO NPs for first–contact times on the surfaces of dry Eutric Cambisol, a Haplic podzol, a Sandy and a Libyan sandy soil with solutions have different concentration levels of each nanomaterial.
2. To assess the ease of desorption of CdS, ZnO and CuO NPs in these soils using three solutions of varying pH.
3. To evaluate adsorption isotherms, descriptive constants of CdS, ZnO and CuO NPs in the above soils using the Langmuir and Freundlich equation.

6.3. Materials and methods

6.3.1. Site description of the soils

The characteristics and site description of the tested soils were described in Chapter 2, Section 2.16.

6.3.2. Preparation of soil samples

The preparation of soils was described in Chapter 3, Section 3.2.2. The soils were sterilized by autoclave at 100 °C to stop microbial activity affecting the adsorption results. Their total carbon and nitrogen content was estimated, as described in Chapter 3, Section 3.2.10, as was their nitrate and ammonium content, as described in Chapter 3, Section 3.2.8. The soluble N was extracted by the centrifugal drainage technique for soil solution. Their pH and electrical conductivity were measured, as described Chapter 3, Section 3.2.5. The soils' Cation Exchange Capacities (CEC) and particle sizes distribution were tested as described in Chapter 3, Sections 3.2.6 and 3.2.7 respectively. Their moisture content and organic matter (OM) content (%) were determined as described in Chapter 3, Section 3.2.4 together with their water holding capacity (WHC), as described in Chapter 3, Section 3.2.3. Table 6.1 summarises the properties of the four soil types.

Table 6. 1. Selected properties of Eutric Cambisol, Haplic podzol, Sandy and Libyan sandy soils.

Properties of soils	Soil type			
	Eutric Cambisol	Haplic podzol	Sandy	Libyan Sandy
pH	5.50±0.03	4.70±0.05	5.74±0.01	7.77±0.09
EC (ms/ cm^{-1})	0.64±0.004	0.11±0.001	0.28±0.001	0.20±0.002
Organic matter (%)	9.27±0.01	3.86±0.03	3.57±0.01	1.07±0.01
Water holding capacity (%)	70.43±0.06	38.82±0.16	50.26±0.98	23.90±0.61
Moisture content %	28.92±0.03	7.33±0.03	7.52±0.06	5.81±0.07
Total C (mg kg^{-1})	49.0±6.1	16.0±2.9	38.0±2.3	3.50±0.96
Total N (mg kg^{-1})	7.70±0.1	1.6±0.3	3.9±0.3	0.50±0.12
NO_3^- (mg N L^{-1})	6.42±1.19	5.68±1.41	12.01±1.00	15.5±1.67
NH_4+ (mg N L^{-1})	3.18±0.20	5.37±0.80	2.53±0.19	1.5±0.19
CEC (mmol kg^{-1})	27.0±3.1	20.0±2.0	23.60±4.0	19.90±2.0
Clay (%)	4.0±0.95	2.0±0.641	4.0±0.19	7.50±0.39
Silt (%)	21.0±1.03	21.0±0.74	3.0±0.02	2.50±0.25
Sand (%)	75.0±1.59	77.0±0.99	93.0±2.9	90.0±3.06
Texture	clay loam	loamy sand	sand	sand

Values represent the means of three determinations (Mean ± SEM, n =3). CEC is cation exchange capacity, EC characterizes the electrical conductivity of the soil.

6.3.3. Adsorption and kinetic experiment

The properties of the three metal NPs are summarized in table 6.2.

Table 6. 2. The characteristic of NPs used for the adsorption experiment

Compound	Size (nm)	Purity (%)	Surface area (m^2/g)	Particular morphology	Molecular weight (g/mol^{-1})
ZnO	90–210	99.9	5–7	Irregular	81.39
CuO	40–80	99.9	–	–	79.55
CdS	~ 7.6– 17.7	–	–	–	144.48

The parameters of ZnO and CuO NPs provided from the IoLitec Nanomaterials Company. The CdS NPs was synthesized at Bangor University; their size was calculated using XRD and SEM. (see Chapter 4, Sections 4.9.1 and 4.9.2).

Solutions with various concentrations of CdS, ZnO, and CuO NPs were prepared in one litre of deionised water. Each solution was agitated for 30 minutes (ultrasonic vibration, 100 W, 40 kHz)–this to attempt even dispersal of the NPs within the water. Metal adsorption and kinetic

studies were performed using batch experiment. Eight different concentrations of each NP solution were used: 0, 25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1}. Each concentration was repeated three times to help ensure accuracy.[23] 60.0 mL of each tested solution was permitted to equilibrate with 1.0 g of each soil in a 125 mL plastic bottle. Each solution was shaken at room temperature (≈22°C) for 168 hours at 250 rev. min^{-1} (Gallenkamp Incubator Shaker. England) to create a slurry. The slurry was then centrifuged for 30 minutes at 4020 rcf (Hettich–Zentrifugen, Rotanta 460 R. 2002). 5.0 mL of each supernatant was removed at different times; these intervals were: 0, 3, 6, 24, 48, 72, 96, 120, 144, and 168 hours. Each solution was passed through Whatman® 42 ashless filter paper. Each sample was stored at 4°C prior to soil analysis. The amount of sorbed metals was calculated as the difference between the intial concentration (amount added) and concentration remaining in solution after equilibrium.

6.3.4. Leachability experiment

Three solutions of varying pH were prepared to test the leachability of the test NPs. The pHs were as follows:

0.1 M Acetic acid pH 3.00 ± 0.01

Rainwater pH 5.30 ± 0.09

0.1 M Sodium hydroxide pH 10.0 ±1.23

The solid residues remaining in the plastic bottles after the adsorption experiments were mixed with 60.0 mL of acetic acid solution for extraction. The slurries were then shaken for 24 hours at room temperature (≈22°C) in the Gallenkamp Shaker Incubator and centrifuged at 4020 rcf for 30 minutes using the Hettich–Zentrifugen Centrifuge. This procedure was repeated with the rainwater and sodium hydroxide solution. Finally, the supernatant of the four soils were separated and digested for metal analysis using 60.0 ml of 6 M HCl followed by same method of pH leachability.[23]

6.3.5. Soil analysis

The method used to analyze the heavy metals in the tested soils was described in Section 6.3.4 using 6 M HCl. The nano–metal solutions were analyzed using an Atomic Absorption Spectrometer (Model Varian–220 FS).

6.4. Statistical analysis

The adsorption and desorption of CdS, CuO and ZnO NPs were performed with three replicates of each concentration (n = 2754 in total). Experimental means and standard errors were calculated using Microsoft Excel. The adsorption of each NP type and concentrations, in addition the desorption % of each NP type and concentrations in four soils were subjected to an one way analysis of variance (ANOVA) and differences identified with a Tukey test using the software package SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). Two–way ANOVA used to test for significant differences between NP type (CdS, CuO, and ZnO) and concentrations (25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1}) on adsorption. Two–way ANOVA also was tested the significant differences between soil type (Libyan sandy, Eutric Campisol, Sandy, and Haplic podzol) and concentrations on adsorption. Data normality was tested using Shapiro–Wilk test.

Two–way ANOVA tested the significant differences between NP type and concentrations on desorption %, it is also used to test the significant differences between soil type and concentrations on desorption %. *Post hoc* tests for between measures were performed using Tukey's HSD. All statistical analyses were conducted using SPSS for Windows, Version 20 (Chicago, IL). Significant differences were accepted at the ($p < 0.05$) level. Graphs were constructed using Sigma Plot 12.3 for Windows using means and standard errors.

6.5. Results and discussion

6.5.1. Sorption isotherms

The mean adsorption of Cd, Cu and Zn on Libyan sandy, Eutric compisol, Sandy, and Haplic podzol soil are shown in figures 6.1 and 6.2. The amount of adsorbed NPs in four soils was calculated using Equation 6.1.

$$Sorbed\ metals = (C_0 - C_e)\frac{V}{M} \qquad \text{Equation 6.1}$$

Where: C_e is the equilibrium concentration (mg L^{-1}); C_0 is the initial concentration of metal ions (mg L^{-1}); V is the total volume of the solution (mL); and M is the mass of test soil (g).[23] The overall amount of each adsorbed NPs was plotted against the initial concentration of added NPs. Figure 6.1 showed the overall adsorption of all NPs compared to each other.

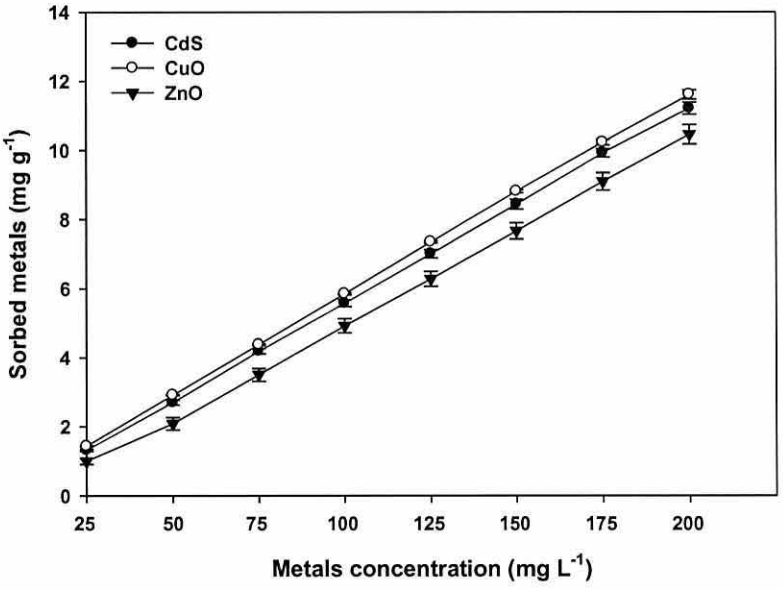

Figure 6. 1. Mean adsorption of CdS, CuO and ZnO NPs in four soils. The values were given as a mean overall ±SEM. Some of the error bars are too small to be visible.

Statistical analysis observed a significant difference between NP type and concentration ($p < 0.001$) as shown in Figure 6.1. Inspection of this figure revealed that adsorption of all types of NP increased with increasing their concentrations in all soils. The highest adsorption was of CuO NPs, followed by CdS NPs, and the adsorption of ZnO NPs was the lowest.

Figure 6.2 showed the overall adsorption of all soils compared to each other. The results of statistical analysis showed a significant between soil type and concentrations ($p < 0.001$) as shown in figure 6.2. Inspection of this figure suggests also that adsorption in all types of soil increased as a function of concentration, with higher levels of soil inoculation leading to progressively higher levels of NPs adsorption. The highest adsorption was of Libyan sandy soil, followed by Eutric Cambisol soil, Sandy soil and Haplic podzol soil respectively.

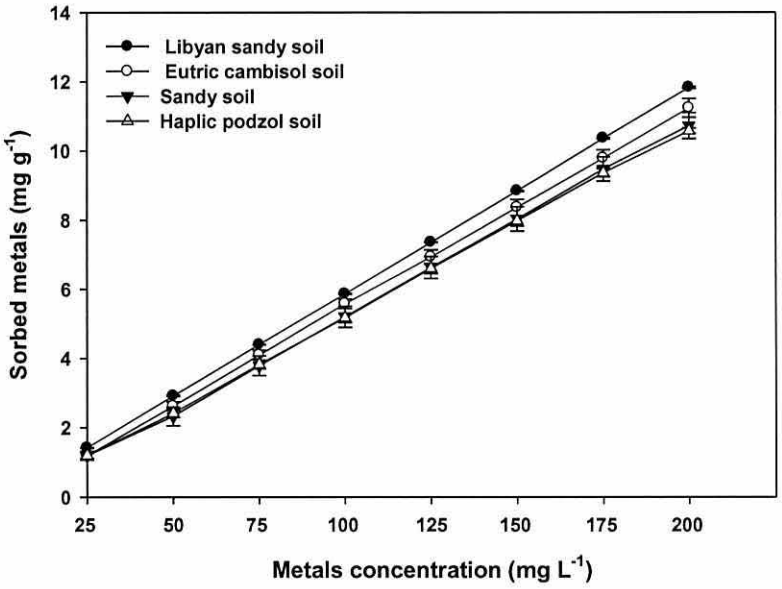

Figure 6. 2. Mean adsorption of four soils across all applied NPs. The values were given as a mean overall ±SEM. Some of the error bars are too small to be visible.

The nano–metal sorption isotherms of Cd, Cu and Zn are shown in Figures 6.3–6.5. These isotherms characterize the behaviour of these NPs in all four soils as a function of their increasing concentration in aqueous solution after equilibrium.

Results of ANOVA and *Post hoc* tests (Tukey's HSD) observed high significant differences ($p < 0.01$) for the adsorption of Cd NPs compared to their control groups across all soil types and concentrations (25–200 mg L^{-1}). The mean overall of CdS NPs adsorption in the Libyan sandy soil was 6.60 mg g^{-1} (SE: ± 0.14); that in Eutric Cambisol soil was 6.57 mg g^{-1} (SE: ± 0.14); that in Sandy was 6.26 mg g^{-1} (SE: ± 0.14); that in Haplic podzol was 5.75 mg g^{-1} (SE: ± 0.13). Adsorption of CdS NPs appeared highest in the Libyan sandy soil (Figure 6.3).

Results of statistical analysis showed significant differences ($p < 0.01$) for the adsorption of Cu NPs compared to their control groups across all soil types and concentrations (25–200 mg L^{-1}). The mean overall of CuO NPs adsorption in the Libyan sandy soil was 6.63 mg g^{-1} of soil (SE: ± 0.71); that in Eutric Cambisol soil was 6.60 mg g^{-1} of soil (SE: ± 0.71); that in Sandy was 6.72 mg g^{-1} of soil (SE: ± 0.71); that in Haplic podzol was 6.34 mg g^{-1} of soil (SE: ± 0.65). Adsorption of CuO NPs appeared highest in the Sandy soil (Figure 6.4).

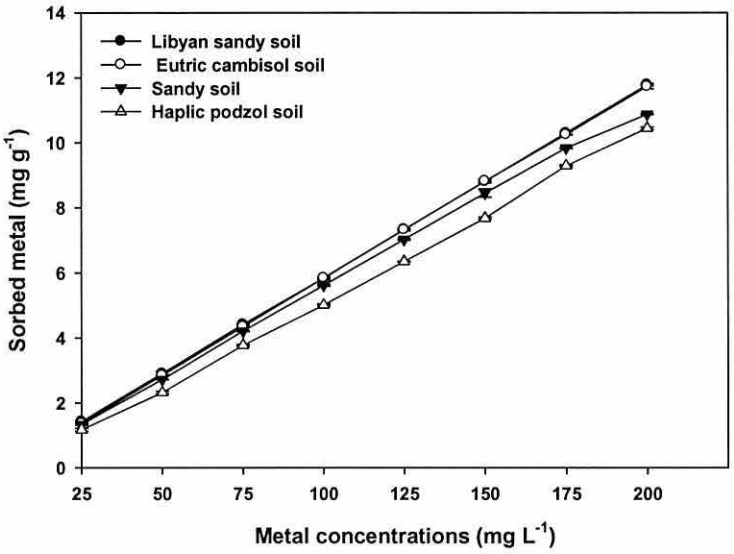

Figure 6. 3. Sorption isotherms for Cd on Libyan Sandy, Eutric Cambisol, Sandy and Haplic podzol soils treated with 25, 50, 75, 100, 125, 150, 175 and 200 mg L^{-1} of CdS NPs for 168 hours. Some of the error bars are too small to be visible. The values were given as a mean ±SEM of triplicate samples. Comparisons were made between each concentration. Some of the error bars are too small to be visible.

Results of statistical analysis indicated significant differences ($p < 0.01$) for the adsorption of Zn NPs compared to their control groups across all soil types and concentrations (25–200 mg L^{-1}). The mean overall of ZnO NPs adsorption in the Libyan sandy soil was 6.64 mg g^{-1} of soil (SE: ± 0.71); that in Eutric Cambisol soil was 5.51 mg g^{-1} of soil (SE: ± 0.64); that in Sandy was 4.78 mg g^{-1} of soil (SE: ± 0.62); that in Haplic podzol was 5.58 mg g^{-1} of soil (SE: ± 0.66). Adsorption of ZnO NPs appeared highest in the Libyan sandy soil (Figure 6.5).

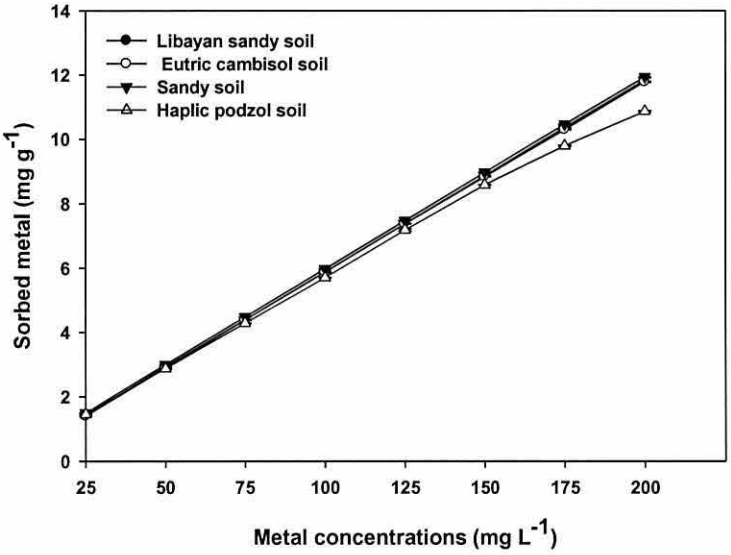

Figure 6. 4. Sorption isotherms for Cu on Libyan Sandy, Eutric Cambisol, Sandy and Haplic podzol soils treated with 25, 50, 75, 100, 125, 150, 175 and 200 mg L^{-1} of CuO NPs for 168 hours. The values were given as a mean ±SEM of triplicate samples. Comparisons were made between each concentration. Some of the error bars are too small to be visible.

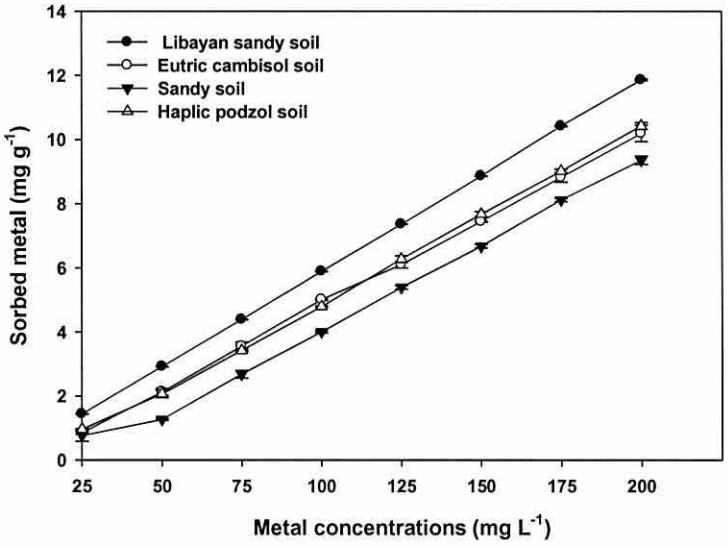

Figure 6. 5. Sorption isotherms for Zn on Libyan Sandy, Eutric Cambisol, Sandy and Haplic podzol soils treated with 25, 50, 75, 100, 125, 150, 175 and 200 mg L^{-1} of ZnO NPs for 168 hours. The values were given as a mean ±SEM of triplicate samples. Comparisons were made between each concentration. Some of the error bars are too small to be visible.

Inspection of the figures 6.3–6.5 suggests that adsorption of all types of soil increased as a function of concentrations, with higher levels of soil concentration leading to progressively higher levels of adsorption. The Figures also show that the relationship between concentration and adsorption appears almost perfectly linear ($R^2 > 0.99$).

Figure 6.3 shows that adsorption of Cd, for example, was highest in Libyan sandy soil, followed by that of Eutric Cambisol soil, Sandy soil and Haplic podzol soil respectively. Moreover adsorption of Zn was highest in Libyan sandy soil, followed by that of Haplic podzol soil, Eutric Cambisol soil and Sandy soil respectively (see Figure 6.5). Adsorption of Cu was highest in Sandy soil followed by that of Libyan sandy soil, Eutric Cambisol soil and Haplic podzol soil respectively. Adsorption of Cu, however, appeared virtually identical for all soil types as shown in figure 6.2. Finally, Figures suggest that the adsorption of Cd and Cu at the lowest level of soil NP inoculation (25 g mL^{-1}) were practically identical in all soil types, but the adsorption of Zn at this level in Libyan sandy soil differed–the adsorption of Zn and Cd was higher in Libyan sandy soil than was the adsorption of the other types of soil; this suggests that, plausibly, the higher clay content of the Libyan soil (7.50±0.39) may have facilitated adsorption.

6.5.2. Effects of test nanoparticles on adsorption

There was a significant effect of concentrations on all NPs adsorption ($p < 0.001$). Tests of measures contrasts suggested the relationship was almost perfectly linear. Statistical analysis observed a significant difference of NP type ($p < 0.001$). *Post hoc* tests showed significant differences were between ZnO, CdS and CuO compared to each other ($p < 0.001$). The mean overall adsorption of CdS was 6.30 mg g^{-1} of soil (SE: ± 0.337); that of CuO was 6.58 mg g^{-1} of soil (SE: ± 0.34); that of ZnO was 5.63 mg g^{-1} of soil (SE: ± 0.330). Adsorption of CuO appeared higher than that of the other types of NP. The statistical analysis also observed no significant differences between NP types and concentration of NPs applied.

6.5.3. Effects of soil type on adsorption

The results of statistical analysis showed that there was a significant effect of concentrations on all NPs adsorption ($p < 0.001$). Tests of between measures indicated the relationship was almost perfectly linear. The results of statistical analysis observed a significant difference of soil type ($p < 0.001$). There were significant differences between all soils compared to each other ($p < 0.001$), save that no significant difference was revealed between Sandy soil and Haplic podzol

soil. The mean overall adsorption in the Libyan sandy soil was 6.63 mg g^{-1} (SE: ± 0.40); that in Eutric Cambisol soil was 6.23 mg g^{-1} (SE: ± 0.39); that in Sandy soil was 5.92 mg g^{-1} (SE: ± 0.39); that in Haplic podzol soil was 5.89 mg g^{-1} (SE: ± 0.37). Adsorption of NPs appeared highest in the Libyan sandy soil. Statistical analysis showed no significant differences between soil type and concentrations.

The results of NPs adsorption showed that all types of NP, at least as regards levels of adsorption, behaved in broadly similar ways in all soil types as shown in Figures 6.3 – 6.5. This is reflected in the highly linear relationship between level of adsorption and level of soil concentration found for all types of NP and for all soils. It is also reflected in the low overall level of adsorption for all types of NP in all types of soil–at the highest level (max. adsorption 12 mg g^{-1} out of a possible 200 mg mL^{-1}) this was only 6%. Relevant factors that might cause low adsorption include preservation, co–precipitation, precipitation, diffusion of metals, and surface adsorption of the soils, all of which may affect adsorption levels within the soils.[23] Nonetheless, that there was some adsorption of all types of NP in all soil types indicates an affinity of the four soil types for Cd, Cu, and Zn.

Adsorption of ZnO, as indicated, was highest in Libyan sandy soil, and, of the four soil types, Libyan sandy soil had the lowest level of organic matter (about 1% organic matter as opposed to over 9% for the Eutric Cambisol and over 3% for the other soil types)–this despite the Clay % was higher (7.50±0.39) than that of three soils.

As indicated, the adsorption of CuO NPs was significantly higher than that of CdS and ZnO across all soil types. The difference as regards CuO could be attributed to the Cu ion's smaller hydrated radius (Cu^{2+} = 0.412 nm, Cd^{2+} = 0.426 nm, Zn^{2+} = 0.430 nm) and hydration energy (Cu^{2+} = – 2105 kJ/mol, Cd^{2+} = –1807 kJ/ mol, Zn^{2+} = –2046 kJ/mol).[24,25]

Another relevant factor is that the groups present on the surface of the soil's humus may be carboxylic and phenolic; these are strong Lewis bases. The Cu^{2+} ion is a stronger (borderline) Lewis acid; by contrast, Cd^{2+} and Zn^{2+} ions are relatively weak Lewis acids.[26] This could help explain the greater affinity of Cu compared with that of Cd, and Zn. Another plausible reason for the Cu's greater affinity could be the higher electronegativity of Cu compared with that of Cd and Zn (Cu^{2+} = 1.90, Cd^{2+} = 1.7 and Zn^{2+} = 1.6) for electrostatic and inner sphere surface complexation reactions.[27]

Some studies suggest that Cr, Pb, and Cu are usually more active in adsorption; conversely, studies indicate Ni, Zn, and Cd are suppressed in a competitive adsorption system regardless of the nature of the adsorbents.[28,29] Gomes *et al.*[30] have shown that heavy metal selectivity sequences is varied among soils but most commonly adsorption of Cr is highest, followed by decreasing levels of Pb, Cu, Cd, Zn, and Ni. Arias *et al.*[13] have suggested that Cu and Zn could be introduced into the soil by the application of inorganic fertilizers, organic manure, or pesticides. Several inquiries into competitive adsorption have concurred that Pb, Cu, and Cr are more strongly retained by synthetic minerals and soil samples than are Zn, Ni, and Cd.[31] Jalali and Moharrami studied the competitive adsorption of Cd, Cu, Zn, Ni and Mn on surface of ten calcareous soil (Soil contains $CaCO_3$ and $MgCO_3$) in western Iran. Their findings suggest that most adsorption isotherms of these trace elements are well described by the Langmuir equation.[32]

In the present study, all four soils showed a different adsorption capacity and binding strength for CuO NPs compared to other NP types. The adsorption capacity of Libyan sandy soil was greatest for Cd, next for Zn, and lowest for Cu when using the Langmuir equation.

Covelo *et al.*[29] reported that the selectivity sequence for the adsorption by four Humic umbrisol soils was highest for Cr, followed by decreasing levels of Cu, Cd, and Ni and Zn–these last two elements being broadly equal.[29] This result agrees with results of the present study. Selective adsorptions of Cr, Cu, and Zn might be related to their susceptibility to hydration, the charge–radius ratio, and electronegativity.[33]

The Cu^{2+} ion in the present study's batch experiment had the highest valence and smallest hydrated radius; therefore, it can bind tightly to negatively charged functional groups. The difference of ionic radius between Cu^{2+} (0.73 Å) and Zn^{2+} ions (0.74 Å) is very small. The radii of their hydrated ions increase 10 times–2.06 Å for Cu^{2+} and 2.16 Å for Zn^{2+} ions.[28] It is physically more difficult for these larger hydrated ions to approach the adsorption sites. The electron configuration of Cu^{2+}, Cd^{2+}, and Zn^{2+} ions can be represented as [Ar] $3d^9 4s^0$, [Kr] $4d^{10}$, and [Ar] $3d^{10}4s^0$ respectively. The $3d^{10}$ orbitals of Zn^{2+} and Cd^{2+} ions are full, and there is no empty orbital for them to form strong bonding or an induced dipole moment with negatively charged functional groups. That the affinity for Zn and Cd in the four soils tested in the present study appears weak suggests that Zn adsorption amounts are lower than those of Cu and Cd.[28]

Transition metals with smaller ionic radii and empty orbitals are easier to complex with organic materials. Important organic metabolites abundant in soil include oxalic, citric, acetic, and malic acids. The carbonyl groups of carboxylic acids found in clay soils can form complexes with metals. The p*Ka* values of these organic acids are a measure of their bonding strength with metals. Bonding strength increases with increasing p*Ka* values.

Echeverria *et al.*[34] and Morera *et al.*[35] have suggested that, in addition to cationic exchange, surface complexity is another retention mechanism. The authors' results suggest that loam, clay loam, loam, and silty clay soils have a greater number of surface binding sites and a higher affinity for Pb and Cu than have Cd, Ni and Zn. Results of present study agree with this–they suggest greatest adsorption of CuO.

Work by LeGeroes,[36] and by Zhou *et al.*[37] corroborated Echeverria *et al.*'s results.[34] and suggested that Cu complexes formed by surface adsorption are more stable than those of Zn and Cd, but cations with ionic radii smaller than Ca^{2+} ions (0.099 nm) have less opportunity to be incorporated into a soil minerals (apatite structure) than have cations with larger ionic radii. Therefore, precipitation of Zn^{2+} (0.069 nm) with Ca^{2+} ions should be less likely than the precipitation of larger Cu^{2+} (0.073nm) and Cd^{2+} (0.097 nm) cations.[37] Again, results of the present study agree with this, with greatest adsorption of CuO.

6.5.4. The Langmuir and Freundlich equations

The Langmuir and Freundlich equations quantify the adsorption behaviour of the metal NPs in the different experimental soils in terms of parameters associated with their threshold concentrations. Equation 6.2 shows the Langmuir adsorption equation.

$$q = \frac{KCb}{(1+Kc)} \qquad \text{Equation 6.2}$$

Where: q is the amount of sorbed NPs by different soils (mg g^{-1}), C is the equilibrium concentration in (mg L^{-1}), k is the Langmuir constant (L mg^{-1}), and b is the maximum adsorption capacity (mg g^{-1}).[23]

Rearranging Equation 6.2 in linear form provides Equation 6.3.

$$\frac{C}{q} = \frac{1}{kb} + \frac{C}{b}$$ Equation 6.3

Plotting C/q vs C, the slope is $1/b$ and the intercept is $1/kb$.

Equation 6.4 shows the Freundlich adsorption equation.

$$Log\ q_e = log\ K + \frac{1}{n} log\ C_e$$ Equation 6.4

Where, k and $1/n$ are the Freundlich constants related to the adsorption capacity and intensity respectively or as Kf, which represents the Freundlich adsorption coefficient and gives an estimate of the adsorptive capacity. $1/n$ describes the isotherm curvature and gives an estimate of the adsorptive intensity.[15] The NP adsorbed of NPs in four soils was calculated using Equation 6.1. The calculated parameters of Langmuir and Freundlich equations for the adsorption of Cd, Cu, and Zn NPs in the four experimental soil types are shown in Table 6.3.

Table 6. 3. The parameters of Langmuir and Freundlich equations for the adsorption of Cd, Cu, and Zn NPs by the experimental soils

Soil type	metals	Langmuir constant			Freundlich constant		
		b (mg g^{-1})	k (1 mg^{-1})	R^2	K_f	$1/n$	R^2
Libyan sandy soils	Cd	434.78	0.002	0.53	17.64	1.00	1
	Cu	250.00	0.003	0.61	17.60	0.99	1
	Zn	322.58	0.003	0.83	17.63	0.99	1
Eutric Cambisol Soil	Cd	161.29	0.01	0.62	17.52	0.99	1
	Cu	232.56	0.004	0.61	17.58	0.99	1
	Zn	37.45	0.04	0.68	16.84	0.99	1
Sandy soil	Cd	196.49	0.01	0.94	17.55	0.99	1
	Cu	333.33	0.0002	0.78	17.73	1.00	1
	Zn	23.75	0.09	0.87	16.53	0.99	1
Haplic podzol soil	Cd	80.00	0.02	0.81	17.28	0.99	1
	Cu	169.49	0.01	0.79	17.78	0.99	1
	Zn	37.88	0.01	0.89	16.90	0.99	1

Inspection of the table suggests that all b values (mg g^{-1}) were higher for Cu^{2+} ions than for Cd^{2+} and Zn^{2+} ions. Also, the explained variance (R^2) provided by the Langmuir equation ranges from

0.53 to 0.94, but the explained variance provided by the Freundlich equation is 1 in all cases. The sorption data was fitted to the linear form of the Langmuir equation as shown in Figures 6.6 – 6.8. The sorption data was fitted to the linear form of the Freundlich equation as shown in Figures 6.9.

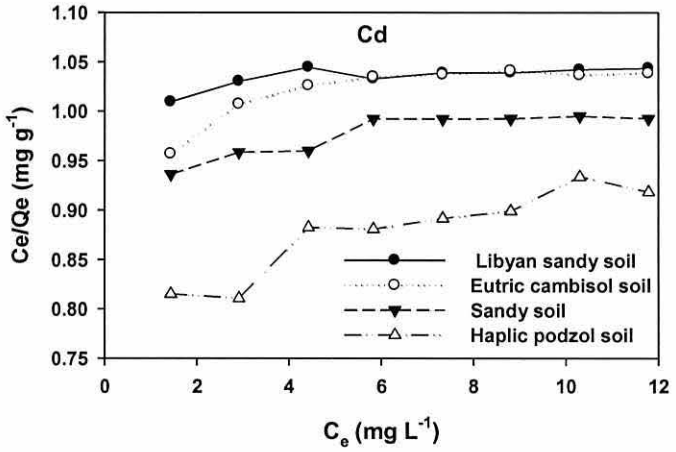

Figure 6. 6. Equilibrium isotherms for the adsorption of Cd by Libyan sandy, Eutric Cambisol, Sandy, and Haplic podzol soils as a function of adsorbed metal concentration.

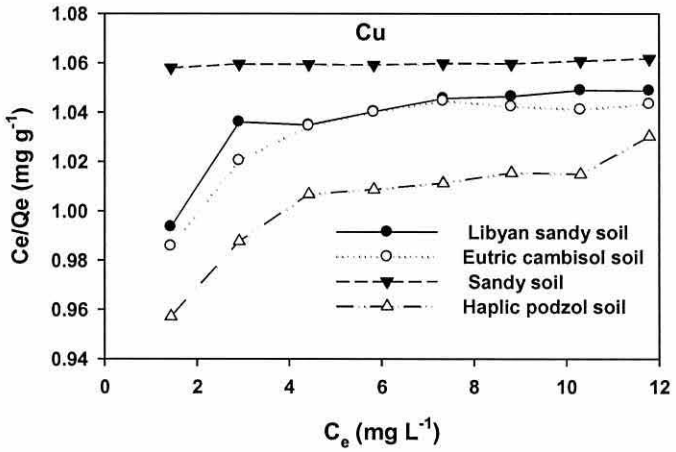

Figure 6. 7. Equilibrium isotherms for the adsorption of Cu by Libyan sandy, Eutric Cambisol, Sandy, and Haplic podzol soils as a function of adsorbed metal concentration.

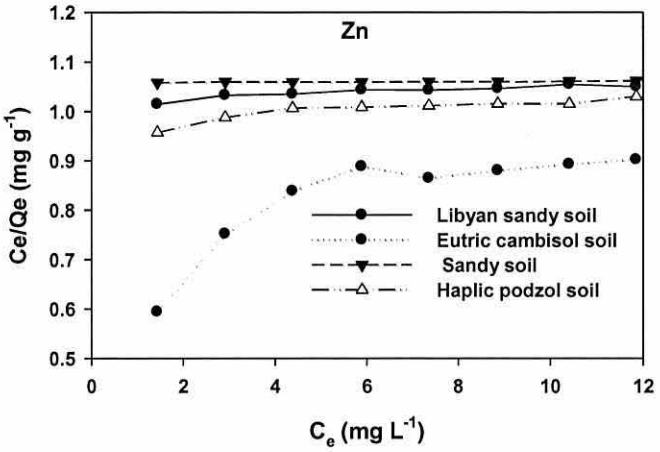

Figure 6. 8. Equilibrium isotherms for the adsorption of Zn by Libyan sandy, Eutric Cambisol, Sandy, and Haplic podzol soils as a function of adsorbed metal concentration.

The results obtained from Langmuir equation do not fit adsorption data (R^2 ranges from 0.53 to 0.94), but Freundlich equation fit all the adsorption data ($R^2 = 1$) as shown in table 6.5. Because the Freundlich equation explains all the correlation coefficients ($R^2 = 1$) in variables, it can be used to describe the adsorption of Cu, Zn, and Cd by the experimental soils better than the Langmuir equation. The parameters for the three soils varied between the three metals as shown by adsorption data along the Langmuir isotherm, using the Langmuir equation, results suggest that maximum adsorption capacity (b) for Eurtic Cambisol, Sandy, and Haplic podzol soils is higher for Cu than for Zn and Cd. The maximum adsorption (b mg g^{-1}) of Libyan sandy, Eutric Cambisol, and sandy soil was relatively stronger than Haplic podzol soil. The b values of Eutric Cambisol, and Sandy and Haplic podzol soils were ordered as follows highest for Cu, next highest for Cd, and lowest for Zn across for all adsorption parameters; this agrees with results of NPs adsorption of the present study.

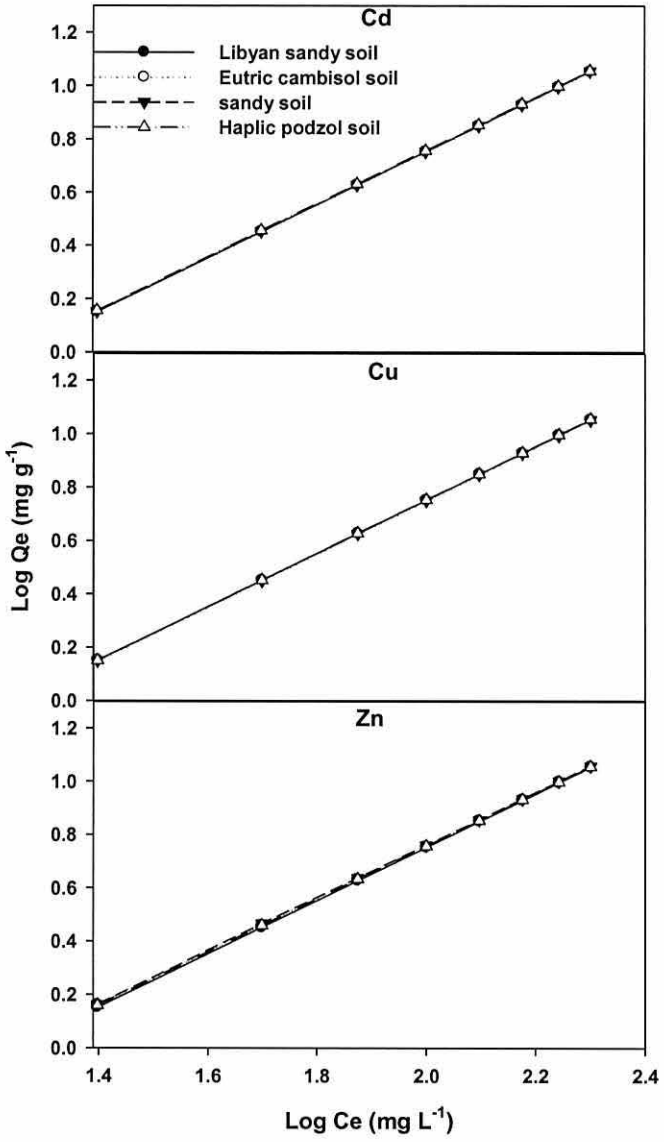

Figure 6. 9. The linear fitting of equilibrium isotherms for the adsorption of Cd, Cu, and Zn for different soil types. The soils are Libyan sandy, Eutric Cambisol, Sandy and Haplic podzol soils as a function of the adsorbed metal concentration using the Freundlich equation.

The Libyan sandy soil showed highest adsorption of Cd, next highest of Zn, and least of Cu; however, when using the Freundlish equation, the adsorption capacities of the three types of NP were small across all soil types compared with that of Langmuir parameters. The K_f values arranged form 16.90 to 17.78 across all soil types (see Table 6.3). A Langmuir graphical model closely approximated to Cu (the maximum adsorption) but less successfully for Zn and Cd. This

suggests that the removal of Cu from these soils is mostly achieved through an adsorption process but that other mechanisms may be involved in the removal of Zn and Cd.

6.5.5. Sorption kinetics

Adsorption kinetic studies were carried out in order to understand the behaviour of Libyan sandy, Eutric Cambisol, Sandy, and Haplic podzol soils towards metal NPs. The adsorption kinetics included two phases: a rapid metal adsorption stage before equilibrium and slow one before equilibrium. Previous research suggests mass transfer is important for metal adsorption.[38] Adsorption kinetics describe a metal's adsorption rate; this governs the residence time of adsorption reactions and the efficiency of the process. Out of the several kinetic models available to examine the mechanism of adsorption, kinetics process, and test the experimental data, the present study used the Lagrangian or pseudo–first–order equation and the pseudo–second–order equation to study metal adsorption kinetics of the four test soils.

The linear form of the pseudo–first–order is provided by Equation 6.5.

$$Log\ (q_e - q) = log\ q_e - \frac{K1}{2.303} t \qquad \text{Equation 6.5}$$

Where q_e is the metal sorbed at equilibrium (mg g^{-1}); q is the amount of the metal adsorbed (mg g^{-1}) at any time t; $k1$ is the first–order rate constant.

The first–order rate constant $k1$ and q were determined from the slope and the intercept of plots of log $(q_e - q)$ vs t at different metal concentrations.[23]

The linear form of the pseudo–second–order equation for the kinetics of absorption as described by Ho and Chiang is provided by Equation 6.6.[39]

$$\frac{t}{q} = \frac{1}{k2q_e^2} + \frac{t}{q_e} \qquad \text{Equation 6.6}$$

The second–order rate constants ($k2$) and q were determined from the slope and intercept of the plot obtained by plotting t/q vs t. The pseudo–second–order kinetic model obtained for all three NPs across all soil types at different concentrations provided better correlations with adsorption data used in the present study than did the pseudo–first–order equation model (Tables 6.4–6.7).

Table 6. 4. The correlations of first and second–order reactions for Libyan sandy soil.

Metals	Conc. (mg L^{-1})	q (exp) (mg g^{-1})	First–order constant			Second–order constant		
			$k1$ (min^{-1})	q (cal) (mg g^{-1})	R^2	$k2$ (g mg^{-1} min^{-1})	q (cal) (mg g^{-1})	R^2
Cd	25	1.43±0.03	0.06	0.02	0.83	1.54	1.47	0.99
	50	2.91±0.06	0.03	0.07	0.69	1.41	2.92	0.99
	75	4.42±0.01	0.02	0.33	0.79	1.49	4.40	0.99
	100	5.84±0.05	0.10	0.01	0.76	14.21	5.93	1
	125	7.34±0.01	0.06	0.002	0.88	0.80	7.39	0.99
	150	8.80±0.03	0.08	0.09	0.60	0.51	8.94	1
	175	10.30±0.04	0.04	0.14	0.86	0.85	10.42	1
	200	11.78±0.04	0.09	0.01	0.86	1.62	11.86	1
Cu	25	1.42±0.001	0.05	0.01	0.71	1.89	1.45	0.99
	50	2.92±0.01	0.03	0.003	0.43	1.69	2.95	1
	75	4.41±0.02	0.06	0.04	0.68	1.74	4.45	1
	100	5.89±0.01	0.05	0.02	0.71	10.89	5.94	1
	125	7.39±0.02	0.04	0.01	0.61	1.74	7.44	1
	150	8.84±0.04	0.04	0.01	0.57	1.14	8.91	1
	175	10.36±0.05	0.04	0.01	0.64	1.73	10.44	1
	200	11.88±0.004	0.03	0.01	0.55	1.14	11.90	1
Zn	25	1.43±0.01	0.03	0.01	0.54	2.06	1.49	0.99
	50	2.92±0.01	0.03	0.003	0.49	1.73	2.97	0.99
	75	4.39±0.01	0.04	0.003	0.56	1.59	4.43	1
	100	5.89±0.01	0.03	0.01	0.56	0.94	5.93	1
	125	7.36±0.003	0.05	0.002	0.64	2.61	7.40	1
	150	8.86±0.003	0.05	0.002	0.68	10.57	8.88	1
	175	10.41±0.02	0.04	0.002	0.60	3.57	10.39	1
	200	11.86±0.03	0.04	0.002	0.59	3.72	11.89	1

Values of metal adsorbed by Libyan sandy soil are represented as a mean of triplicates ± SE; q(exp) represents the amount of metals adsorbed (mg g^{-1}) at any time during the experiment; q (cal) represents the calculation of adsorbed metals using first and second order reaction equations.

Table 6. 5. The correlation of first and second–order reactions for Eutric Cambisol soil.

Metals	Conc. (mg L^{-1})	q (exp) (mg g^{-1})	First–order constant			Second–order constant		
			$k1$ (min^{-1})	q (cal) (mg g^{-1})	R^2	$k2$ (g mg^{-1} min^{-1})	q (cal) (mg g^{-1})	R^2
Cd	25	1.36±0.01	0.00	0.04	0.61	10.00	1.33	0.99
	50	2.85±0.03	1.11	0.05	0.54	79.30	2.81	1
	75	4.35±0.03	0.01	0.03	0.60	1.46	4.27	0.99
	100	5.85±0.01	2.61	0.02	0.42	0.75	5.72	0.99
	125	7.32±0.04	2.62	0.02	0.42	0.49	7.18	0.99
	150	8.82±0.06	3.01	0.02	0.48	0.51	8.66	1
	175	10.25±0.01	2.53	0.02	0.40	0.65	10.13	1
	200	11.73±0.07	2.73	0.02	0.44	0.61	11.61	1
Cu	25	1.40±0.001	0.05	0.001	0.59	4.38	1.46	0.99
	50	2.89±0.001	0.05	0.002	0.70	1.80	2.93	0.99
	75	4.38±0.003	0.04	0.002	0.59	2.21	4.42	1
	100	5.87±0.001	0.04	0.003	0.54	1.57	5.91	1
	125	7.37±0.0004	0.05	0.01	0.69	2.16	7.39	1
	150	8.83±0.001	0.04	0.01	0.70	3.97	8.87	1
	175	10.29±0.001	0.06	0.03	0.84	8.50	10.34	1
	200	11.78±0.02	0.07	0.02	0.89	35.96	11.79	1
Zn	25	0.84±0.04	0.03	0.03	0.70	1.19	0.82	0.98
	50	2.12±0.11	0.04	0.05	0.85	0.25	2.28	0.99
	75	3.55±0.13	0.02	0.08	0.69	0.16	3.73	0.99
	100	5.02±0.03	0.05	0.29	0.85	0.14	5.17	0.99
	125	6.10±0.10	0.04	0.36	0.92	0.14	6.56	0.99
	150	7.45±0.02	0.02	0.33	0.84	0.22	7.75	0.99
	175	8.82±0.15	0.01	0.25	0.75	0.24	9.14	0.99
	200	10.19±0.26	0.004	0.97	0.89	0.79	10.38	0.99

Values of metal adsorbed by Eutric Cambisol soil are represented as a mean of triplicates ± SE; q(exp) represents the amount of metals adsorbed (mg g^{-1}) at any time during the experiment; q (cal) represents the calculation of adsorbed metals using first and second order reaction equations.

Table 6. 6. The correlation of first and second–order reactions for Sandy soil.

Metals	Conc. (mg L^{-1})	q (exp) (mg g^{-1})	First–order constant			Second–order constant		
			$k1$ (min^{-1})	q (cal) (mg g^{-1})	R^2	$k2$ (g mg^{-1} min^{-1})	q (cal) (mg g^{-1})	R^2
Cd	25	1.33±0.02	0.05	0.02	0.71	1.36	1.33	0.98
	50	2.72±0.01	0.05	0.04	0.65	0.59	2.74	0.99
	75	4.21±0.01	0.03	0.03	0.65	0.67	4.47	0.99
	100	5.62±0.03	0.05	0.25	0.49	0.26	5.54	0.99
	125	7.03±0.02	0.03	0.08	0.74	0.36	6.97	0.99
	150	8.45±0.13	0.04	0.09	0.74	0.33	8.26	0.99
	175	9.84±0.02	0.02	0.18	0.69	0.57	9.54	0.99
	200	10.88±0.02	0.04	0.06	0.70	0.20	10.73	0.99
Cu	25	1.50±0.001	0.02	0.01	0.25	1.16	1.46	0.98
	50	2.99±0.0002	0.02	0.01	0.34	0.65	2.93	0.99
	75	4.48±0.0003	0.01	0.03	0.30	0.48	4.40	0.99
	100	5.98±0.0003	0.01	0.04	0.35	0.39	5.88	0.99
	125	7.47±0.0001	0.01	0.04	0.34	0.41	7.38	0.99
	150	8.97±0.0001	0.002	0.07	0.64	0.48	8.84	0.99
	175	10.46±0.001	0.04	0.09	0.80	0.63	10.30	0.99
	200	11.94±0.001	0.03	0.12	0.80	0.58	11.67	0.99
Zn	25	0.76±0.17	0.05	0.08	0.90	0.19	0.69	0.90
	50	1.26±0.03	0.06	1.00	0.96	0.18	1.86	0.98
	75	2.68±0.12	0.05	1.28	0.92	0.13	3.33	0.99
	100	4.00±0.04	0.03	0.99	0.98	0.14	4.68	0.99
	125	5.39±0.05	0.04	1.41	0.97	0.12	6.15	0.99
	150	6.68±0.06	0.05	1.71	0.94	0.13	7.53	0.99
	175	8.12±0.05	0.03	1.71	0.91	0.10	8.91	0.99
	200	9.36±0.13	0.03	3.02	0.89	0.07	10.38	0.99

Values of metal adsorbed by Sandy soil are represented as a mean of triplicates ± SE; q(exp) represents the amount of metals adsorbed (mg g^{-1}) at any time during the experiment; q (cal) represents the calculation of adsorbed metals using first and second order reaction equations.

Table 6. 7. The correlation of first and second–order reactions for Haplic podzol soil.

Metals	Conc. (mg L^{-1})	q (exp) (mg g^{-1})	First–order constant			Second–order constant		
			$K1$ (min^{-1})	q(cal) (mg g^{-1})	R^2	$k2$ (g mg^{-1} min^{-1})	q(cal) (mg g^{-1})	R^2
Cd	25	1.17±0.045	0.04	0.01	0.61	1.55	1.13	0.99
	50	2.32±0.037	0.03	0.01	0.38	4.45	2.33	0.99
	75	3.77±0.028	0.06	0.01	0.67	1.42	3.75	0.99
	100	5.02±0.027	0.05	0.01	0.77	1.88	5.04	0.99
	125	6.35±0.009	0.03	0.07	0.71	0.40	6.20	0.99
	150	7.68±0.036	0.03	0.08	0.84	0.43	7.56	0.99
	175	9.29±0.026	0.03	0.11	0.73	0.61	8.98	0.99
	200	10.44±0.043	0.004	0.07	0.81	0.57	10.37	1
Cu	25	1.46±0.003	0.05	0.01	0.75	8.97	1.48	0.99
	50	2.87±0.014	0.07	0.10	0.87	0.76	2.96	0.99
	75	4.28±0.007	0.05	0.09	0.76	0.33	4.42	0.99
	100	5.70±0.011	0.02	0.07	0.55	0.77	5.77	0.99
	125	7.18±0.034	0.05	0.10	0.78	0.46	7.17	0.99
	150	8.58±0.004	0.03	0.13	0.65	0.21	8.67	0.99
	175	9.79±0.011	0.03	0.18	0.89	0.20	10.09	0.99
	200	10.86±0.018	0.04	0.01	0.66	0.11	11.53	0.99
Zn	25	0.96±0.014	0.01	0.15	0.64	0.28	1.18	0.97
	50	2.06±0.119	0.02	0.22	0.76	0.17	2.34	0.99
	75	3.42±0.048	0.04	0.21	0.87	0.21	3.68	0.99
	100	4.80±0.026	0.04	0.11	0.88	0.27	5.00	0.99
	125	6.28±0.101	0.04	0.07	0.80	0.31	6.40	0.99
	150	7.68±0.085	0.03	0.16	0.62	0.25	7.76	0.99
	175	9.02±0.062	0.05	0.03	0.80	2.44	8.97	0.99
	200	10.42±0.101	0.06	0.04	0.86	1.12	10.35	0.99

Values of metal adsorbed by Haplic podzol soil are represented as a mean of triplicates ± SE; q(exp) represents the amount of metals adsorbed (mg g^{-1}) at any time during the experiment; q (cal) represents the calculation of adsorbed metals using first and second order reaction equations.

The amount of the ions adsorbed against time is shown in Figures 6.10 – 6.13. As indicated the amount of adsorbed NPs in four soils was calculated using Equation 6.1. The plots show that a rapid rate of metal adsorption was observed at the beginning of the experiment. This could be due to the presence of active sites in the different soils that are available for metal adsorption.

Once the sorptive sites are exhausted, the adsorption rate may be controlled by the rate of intra–particle diffusion. This indicates that increasing the contact time above the equilibrium time has no significant increased adsorption by the biomass. The plots further showed that, increasing the initial concentration of NP ions resulted in a decrease or stability in the initial rate especially for low concentrations of NPs ion.

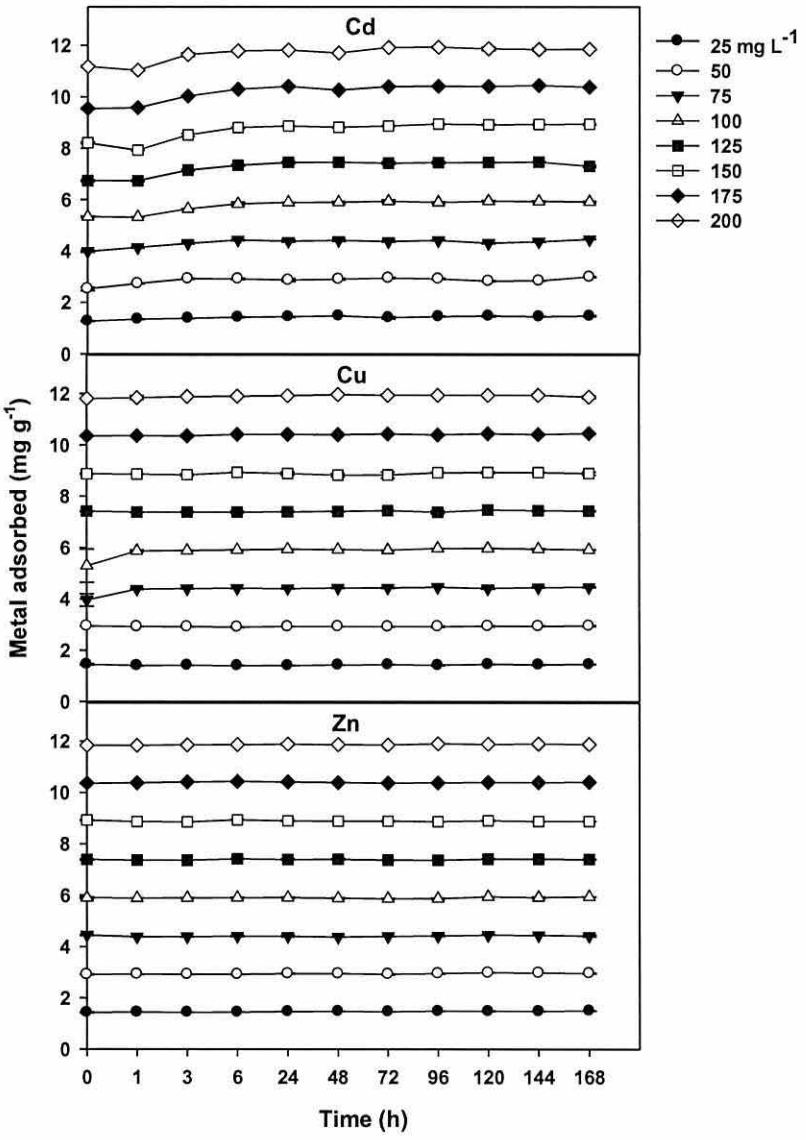

Figure 6. 10. The adsorption of Cd, Cu, and Zn by Libyan sandy soil treated with 25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1} of Cds, CuO, and ZnO NPs for 168 hours. The values are given as a mean ±SEM of triplicate samples. The values of adsorption were equilibrated for 6 hours for all NPs.

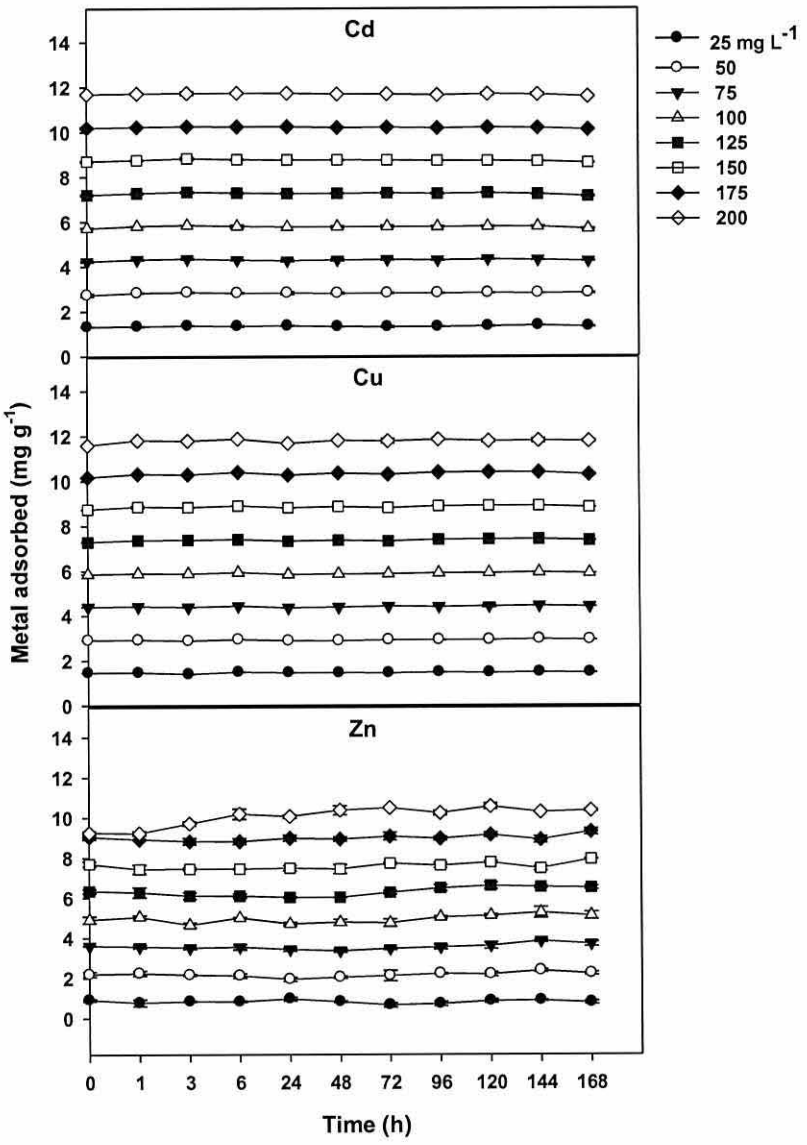

Figure 6. 11. The adsorption of Cd, Cu, and Zn by Eutric Cambisol soil treated with 25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1} of Cds, CuO, and ZnO NPs for 168 hours. The values are given as a mean ± SE of triplicate samples. The values of adsorption were equilibrated for 6 hours for all NPs.

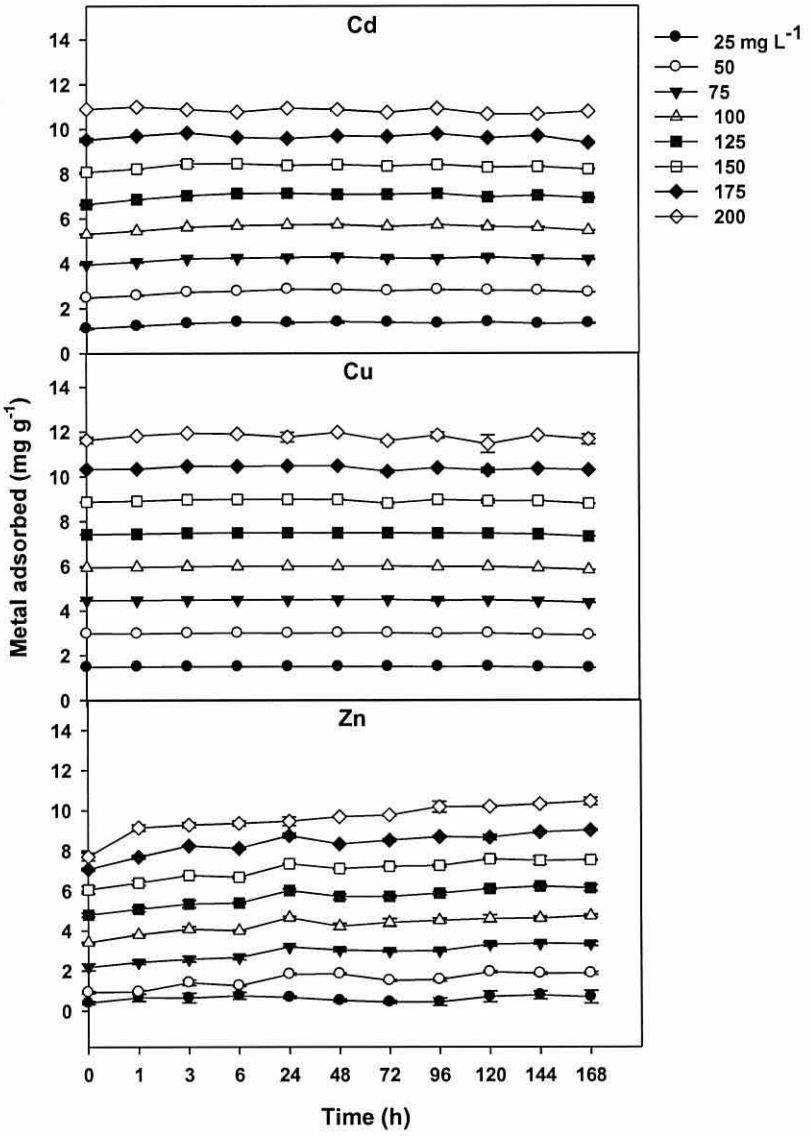

Figure 6. 12. The adsorption of Cd, Cu, and Zn by Sandy soil treated with 25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1} of Cds, CuO, and ZnO NPs for 168 hours. The values are given as a mean ± SE of triplicate samples. The values of adsorption were equilibrated for 6 hours for all NPs.

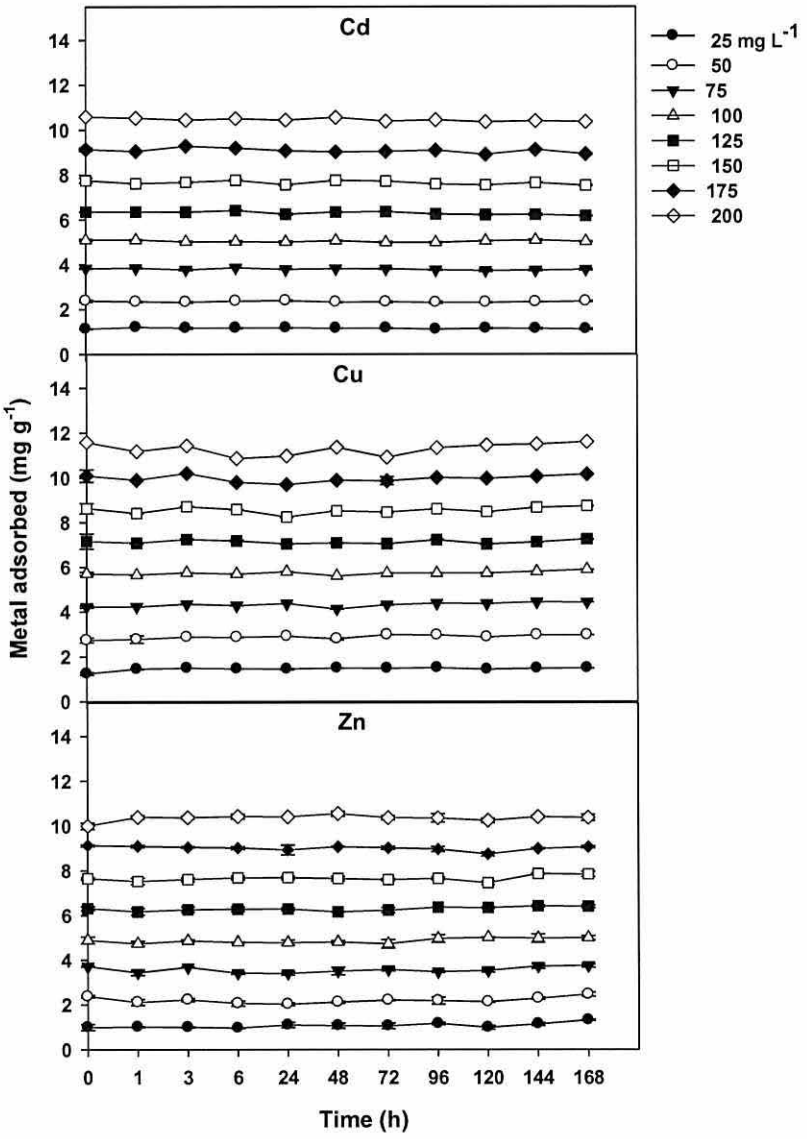

Figure 6. 13. The adsorption of Cd, Cu, and Zn by Haplic podzol soil treated with 25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1} of Cds, CuO, and ZnO NPs for 168 hours. The values are given as a mean ± SE of triplicate samples. The values of adsorption were equilibrated for 6 hours for all NPs.

The results of NP adsorption reactions suggest that the pseudo–second–order kinetic model fits linearly fit with Cd, Cu, and Zn adsorption for all soil types and amounts of adsorbed metals. Further, the calculated adsorption q (cal) for the three NPs using the second order equation were similar in value for the adsorbed amount in the batch experiment q (exp); this corroborates results of adsorption isotherms of the present study (see Figures 6.3–6.5): the adsorption rate in

Eutric Cambisol soil took longer time to reach equilibrium than did the other three soils; this can be related to the high organic matter content in this soil.

The adsorption kinetics of the three NPs for all soil types showed two phases for second order reaction with respect to their equilibrium times: a rapid metal adsorption stage and a slow one before the equilibrium was established. These results corroborate the finding that the adsorption data was well represented by the pseudo–second–order kinetic model. This suggests that the adsorption rate is proportional to the concentration of NPs (see Figures 6.3–6.5). At the beginning of the experiment, the concentration of Cd, Cu, and Zn ions was high. The adsorption rate of Cd in Libyan sandy soil was fast for the first 14.21 minutes; then it slowed until it reached 1.62 minutes. The equilibrium time for Cu and Zn was 10.89 and 10.57 minutes respectively. The adsorption rate of the soils, therefore, varied widely according to type of NP; this is consistent with results of adsorption isotherms of the present study. The equilibrium time for Zn in the Eutric Cambisol, a Haplic podzol, a Sandy soils was shorter in reaching equilibrium than were the times of the other three metals, Adsorption of the three NPs was linear, with a further increase in contact time after equilibrium had been reached; this took 6 hours for the majority of applied concentrations.

6.6. Leachability of sorbed metals

The leachability of NPs from different soils depends upon the properties of the extracting solution. The leachability percent is calculated with reference to the amount of the metal sorbed. The desorption percentage % (P_{des}) for the three NPs was calculated using the adsorption and the desorption data shown in Figures 6.14–6.16. See Equation 6.7.

$$P_{des} = \frac{M_d}{M_a} \times 100\% \qquad \text{Equation 6.7}$$

Where Md (mg g^{-1}) is the amount of NPs desorbed by different pH solutions and Ma (mg/g^{-1}) is the amount of NPs adsorbed by the soils.[15]

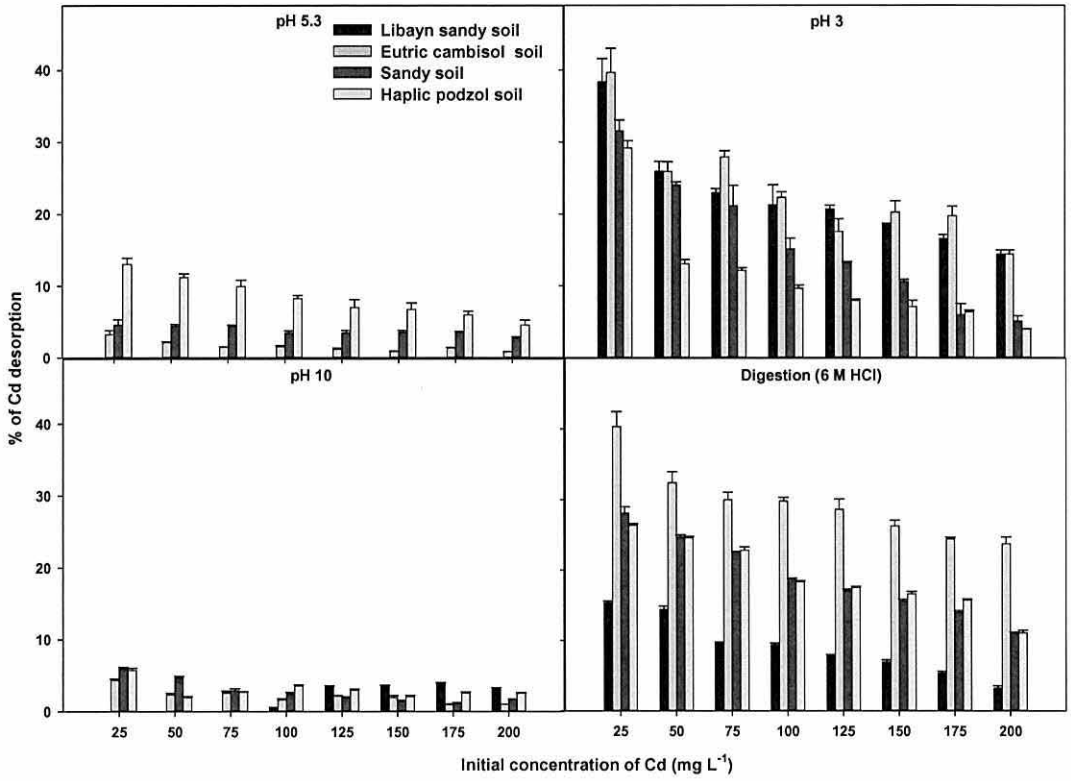

Figure 6. 14. The effect of pH on the desorption of Cd in Libyan sandy, Eutric Cambisol, Sandy, and Haplic podzol soils. Soils were treated with 25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1} CdS NPs. A leachability experiment (desorbed metals) was performed for 24 hours using different pH solutions. The values are given as a mean ±SEM of triplicate samples. Some of Cd concentrations were below the detection limit when in solutions at pH 5.3 and pH 10.

The results of statistical analysis observed a significant effect of concentration on desorption % of CdS NPs at pH 5.3 ($p < 0.001$). The ANOVA also revealed a significant difference of soil type on desorption % of CdS NPs at pH 5.3 ($p < 0.001$). *Post hoc* tests indicated differences between all soils compared to each other (each $p < 0.001$). The mean overall desorption % of CdS NPs in the Haplic podzol soil was 8.35 % (SE: ± 0.72); and that in Sandy soil was 3.77 % (SE: ± 0.18); that in Eutric Cambisol soil was 1.60 % (SE: ± 0.18); that in the Libyan sandy soil was 0 %. Desorption % of CdS NPs appeared highest in the Haplic podzol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of CdS NPs ($p < 0.001$) at pH 3. Statistical analysis showed a significant difference of soil type on desorption % of CdS NPs at pH 3 ($p < 0.001$). *Post hoc* tests showed differences were between Libyan sandy soil compared to Sandy and Haplic podzol soil (each $p < 0.001$), Eutric

Cambisol soil compared to Sandy and Haplic podzol soil (each $p < 0.001$), Sandy soil compared to all soils (each $p < 0.001$) and Haplic podzol soil compared to all soils (each $p < 0.001$). The mean overall desorption % of CdS NPs in the Eutric Cambisol soil was 23.47 % (SE: ± 1.81); that in Libyan sandy soil was 22.33 % (SE: ± 1.66); that in Sandy soil was 15.83 % (SE: ± 2.00); that in Haplic podzol soil was 11.18 % (SE: ± 1.75). Desorption % of CdS NPs appeared highest in the Eutric Cambisol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of CdS NPs ($p < 0.001$) at pH 10. There was no significant difference of soil type for desorption % of CdS NPs at pH 10. *Post hoc* tests observed differences between Libyan sandy soil compared to Sandy and Haplic podzol soil (each $p < 0.001$) and Eutric Cambisol soil compared to Sandy and Haplic podzol soil (each $p < 0.001$). Sandy soil compared to Libyan sandy and Eutric Cambisol soil (each $p < 0.001$) and Haplic podzol soil compared to Libyan sandy and Eutric Cambisol soil (each $p < 0.001$). The mean overall desorption % of CdS NPs in the Haplic podzol soil was 3.03 % (SE: ± 0.28); that in Sandy soil was 2.80 % (SE: ± 0.37); that in Eutric Cambisol soil was 2.14 % (SE: ± 0.24); and that in Libyan sandy soil was 1.82 % (SE: ± 0.39). Desorption % of CdS NPs appeared highest in the Haplic podzol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of CdS NPs ($p < 0.001$) at 6 M HCl. The ANOVA also revealed a significant difference of soil type on desorption % of CdS NPs at 6 M HCl ($p < 0.001$). *Post hoc* tests indicated differences between Libyan sandy soil and all soils (each $p < 0.001$) and Eutric Cambisol soil and all soils (each $p < 0.001$). Sandy soil compared to Libyan sandy and Eutric Cambisol soil (each $p < 0.001$) and Haplic podzol soil compared to Libyan sandy and Eutric Cambisol soil (each $p < 0.001$).

The mean overall desorption % of CdS NPs in the Eutric Cambisol soil was 29.44 % (SE: ± 1.19); that in Haplic podzol soil was 19.11 % (SE: ± 1.09); that in Sandy soil was 18.98 % (SE: ± 1.23); that in Libyan sandy soil was 8.98 % (SE: ± 0.89). Desorption % of CdS NPs appeared highest in the Eutric Cambisol soil.

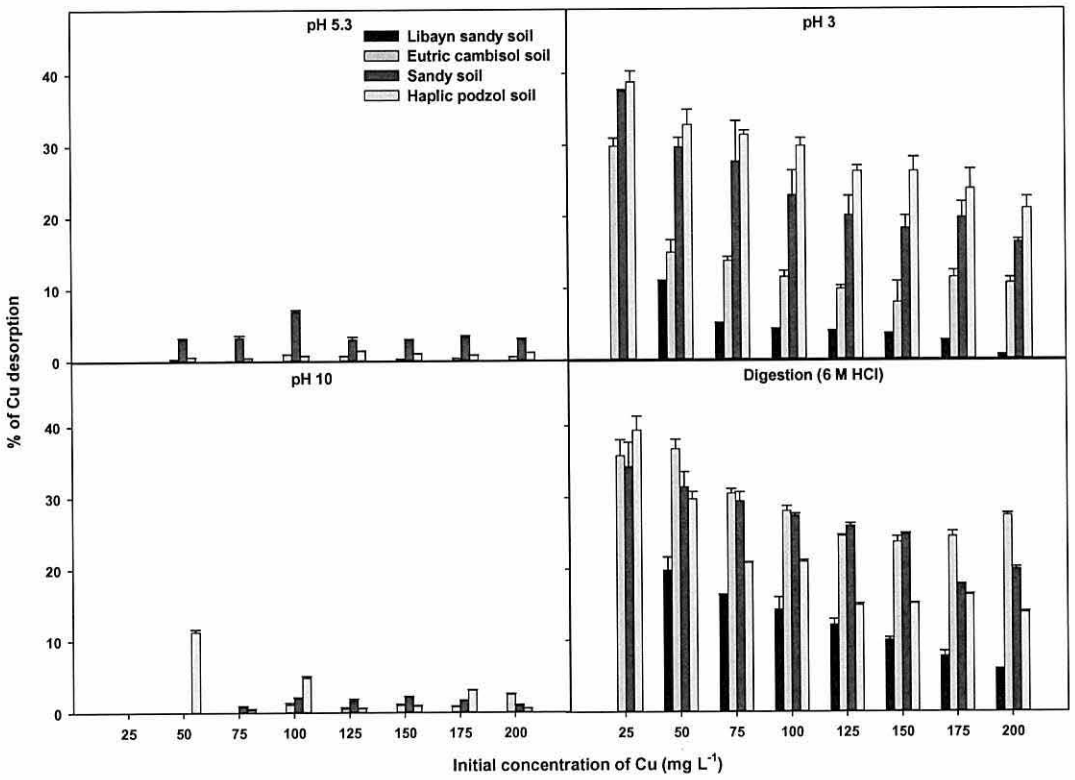

Figure 6. 15. The effect of pH on the desorption of Cu in Libyan sandy, Eutric Cambisol, Sandy and Haplic podzol soils. Soils were treated with 25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1} CuO NPs. A leachability experiment (desorbed metals) was performed for 24 hours using different pH solutions. The values are given as a mean ±SEM of triplicate samples. Some of Cu concentrations were below the detection limit when in solution at pH 5.3 and pH 10.

The results of statistical analysis observed a significant effect of concentration on desorption % of CuO NPs at pH 5.3 ($p < 0.001$). The ANOVA also revealed a significant difference of soil type on desorption % of CuO NPs at pH 5.3 ($p < 0.001$). *Post hoc* tests indicated differences between all soils compared to each other (each $p < 0.001$). The mean overall desorption % of CuO NPs in the Sandy soil was 3.19 % (SE: ± 0.40); that in Haplic podzol soil was 0.74 % (SE: ± 0.1); that in Eutric Cambisol soil was 0.36 % (SE: ± 0.07); that in Libyan sandy soil was 0 %. Desorption % of CuO NPs appeared highest in the Sandy soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of CuO NPs ($p < 0.001$) at pH 3. The ANOVA also revealed a significant difference of soil type on desorption % of CuO NPs at pH 3 ($p < 0.001$). *Post hoc* tests indicated differences were between all soils compared to each other (each $p < 0.001$). The mean overall desorption % of

CuO NPs in the Haplic podzol soil was 28.77 % (SE: ± 1.31); that in Sandy soil was 24.05 % (SE: ± 1.75); that in Eutric Cambisol soil was 13.76 % (SE: ± 1.52); that in Libyan sandy soil was 3.84 % (SE: ± 0.72). Desorption % of CuO NPs appeared highest in the Haplic podzol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of CuO NPs ($p < 0.001$) at pH 10. There was no significant difference of soil type for desorption % of CuO NPs at pH 10. *Post hoc* tests observed differences were between all soils compared to each other (each $p < 0.001$). The mean overall desorption % of CuO NPs in the Haplic podzol soil was 2.29 % (SE: ± 0.82); that in Sandy soil was 1.16 % (SE: ± 0.19); that in Eutric Cambisol soil was 0.74 % (SE: ± 0.19); that in Libyan sandy soil was 0 %. Desorption % of CuO NPs appeared highest in the Haplic podzol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of CuO NPs ($p < 0.001$) at 6 M HCl. There was a significant difference of soil type on desorption % of CuO NPs at 6 M HCl. *Post hoc* tests indicated differences were between all soils compared to each other (each $p < 0.001$). The mean overall desorption % of CuO NPs in the Eutric Cambisol soil was 28.91 % (SE: ± 1.13); that in Sandy soil was 26.35 % (SE: ± 1.28); that in Haplic podzol soil was 21.36 % (SE: ± 1.92); that in Libyan sandy soil was 10.70 % (SE: ± 1.37). Desorption % of CuO NPs appeared highest in the Eutric Cambisol soil.

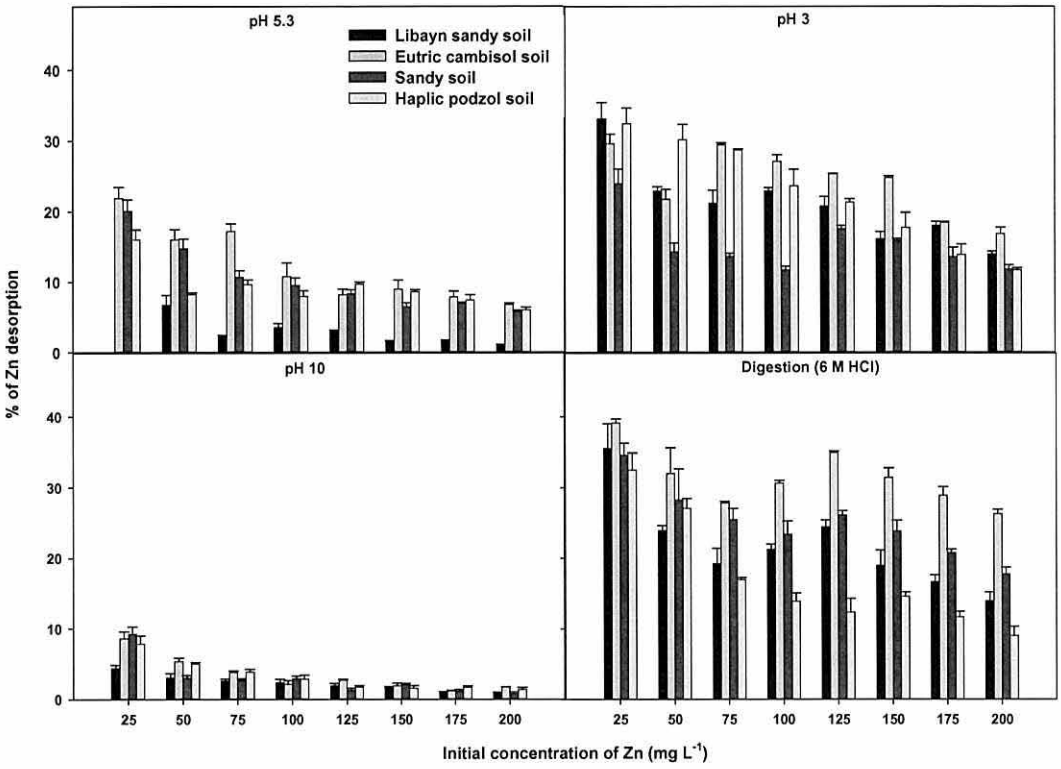

Figure 6. 16. The effect of pH on the desorption of Zn in Libyan sandy, Eutric Cambisol, Sandy, and Haplic podzol soils. Soils were treated with 25, 50, 75, 100, 125, 150, 175, and 200 mg L^{-1} ZnO NPs. A leachability experiment (desorbed metals) was performed for 24 hours using different pH solutions. The values are given as a mean ±SEM of triplicate samples. Some of Zn concentrations were below the detection limit when in solution at pH 5.3 and pH 10.

The results of statistical analysis observed a significant effect of concentration on desorption % of ZnO NPs at pH 5.3 ($p < 0.001$). There was a significant difference of soil type on desorption % of ZnO NPs at pH 5.3 ($p < 0.001$). *Post hoc* tests indicated differences were between Libyan sandy soil and all soils ($p < 0.001$) and Eutric Cambisol soil compared to Libyan sandy and Haplic podzol soil (each $p < 0.001$). Sandy soil and Libyan sandy soil ($p < 0.001$) and Haplic podzol soil compared to Libyan sandy and Eutric Cambisol soil (each $p < 0.001$). The mean overall desorption % of ZnO NPs in the Eutric Cambisol soil was 12.22 % (SE: ± 1.33); that in Sandy soil was 10.33 % (SE: ± 1.152); that in Haplic podzol soil was 9.20(SE: ± 0.68); that in Libyan sandy soil was 2.58 % (SE: ± 0.62). Desorption of NPs appeared highest in the Eutric Cambisol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of ZnO NPs ($p < 0.001$) at pH 3. Statistical analysis also showed a significant difference of soil type on desorption % of ZnO NPs at pH 3 ($p < 0.001$). *Post hoc* tests indicated differences were between Libyan sandy soil compared to Eutric Cambisol and Sandy soil (each $p < 0.001$) and Eutric Cambisol soil compared to Libyan sandy and Sandy soil (each $p < 0.001$). Sandy soil compared to all soils ($p < 0.001$) and Haplic podzol soil compared to Sandy soil ($p < 0.001$). The mean overall desorption % of ZnO NPs in the Eutric Cambisol soil was 24.15 % (SE: ± 1.05); that in Haplic podzol soil was 22.42 % (SE: ± 1.75); that in Libyan sandy soil was 21.10 % (SE: ± 1.32); that in Sandy soil was 15.29 % (SE: ± 0.92). Desorption % of ZnO NPs appeared highest in the Eutric Cambisol soil.

The results of statistical analysis revealed a significant effect of concentration on desorption % of ZnO NPs ($p < 0.001$) at pH 10. There was no significant difference of soil type for desorption % of ZnO NPs at pH 10. *Post hoc* tests observed no significant differences between soils. The mean overall desorption % of ZnO NPs in the Eutric Cambisol soil was 3.44 % (SE: ± 0.56); that in Haplic podzol soil was 3.25 % (SE: ± 0.67); that in Sandy soil was 2.90 % (SE: ± 0.63); that in Libyan sandy soil was 2.23 % (SE: ± 0.29). Desorption % of ZnO NPs appeared highest in the Eutric Cambisol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of ZnO NPs ($p < 0.001$) at 6 M HCl. The ANOVA also revealed a significant difference of soil type on desorption % of ZnO NPs at 6 M HCl. *Post hoc* tests indicated differences between all soils compared to each other ($p < 0.001$). The mean overall desorption % of ZnO NPs in the Eutric Cambisol soil was 31.39 % (SE: ± 1.0); that in Sandy soil was 25.00 % (SE: ± 1.25); that in Libyan sandy soil was 21.73 % (SE: ± 1.51); that in Haplic podzol soil was 17.24 % (SE: ± 1.80). Desorption % of ZnO NPs appeared highest in the Eutric Cambisol soil.

Results for Cu^{2+}, Cd^{2+}, and Zn^{2+} ions suggest that all desorption percentages were less than 100%; this agrees with results obtained by Nriagu.[40] This suggests that NP desorption was incomplete and not fully reversible. In this, NP desorption appears similar to that of bulk heavy metals in soils.

The desorption % of NPs varied among soils according to the pH of the extracting solutions, but most commonly desorption % of Cd NPs appeared highest in Haplic podzol soil using pH 5.3

and pH 10. Results of desorption % revealed that the desorption % of Cd NPs appeared highest in Eutric Cambisol soil using pH 3 and 6 M HCl. The desorption % of Cu NPs was highest in Sandy soil using pH 5.3 and that of Eutric Cambisol soil using 6 M HCl. Results of desorption % revealed that the desorption % of Cu NPs was highest in Haplic podzol soil using pH 3 and pH 10. The desorption % of Zn NPs was highest in Eutric Cambisol soil using all extracting solutions.

6.6.1. Effect of nanoparticles on desorption percentage

The results of statistical analysis observed a significant effect of concentration on desorption % of NPs at pH 5.3 ($p < 0.001$). Statistical analysis also showed a significant difference of NP types on desorption % at pH 5.3 ($p < 0.001$). *Post hoc* tests indicated differences between all NP types compared to each other (each $p < 0.001$). The mean overall desorption % of ZnO NPs was 8.58 % (SE: ± 0.58); that of CdS NPs was 3.43 % (SE: ± 0.37); that of CuO NPs was 1.07 % (SE: ± 0.16). Desorption % of ZnO NPs appeared higher than that of the other types of NP.

The results of statistical analysis observed a significant effect of concentration on desorption % of NPs ($p < 0.001$) at pH 3. There was a significant difference of NP types on desorption % at pH 3 ($p < 0.05$). *Post hoc* tests indicated the only differences were between CuO NPs and ZnO NPs ($p < 0.05$) and between ZnO and CuO NPs ($p < 0.05$). The mean overall desorption % of ZnO NPs was 20.74 % (SE: ± 0.68); that of CdS NPs in was 18.20 % (SE: ± 0.96); that of CuO NPs was 17.60 % (SE: ± 1.17). Desorption % of ZnO NPs appeared higher than that of the other types of NP.

The results of statistical analysis observed a significant effect of concentration on desorption % of NPs ($p < 0.001$) at pH 10. There was a significant difference of NP types on desorption % at pH 10 ($p < 0.05$). *Post hoc* tests indicated differences were between CdS NPs and CuO NPs ($p < 0.001$) and between CuO NPs and all NP types ($p < 0.001$) and between ZnO and CuO NPs ($p < 0.001$). The mean overall desorption % of ZnO NPs was 2.95 % (SE: ± 0.26); that of CdS NPs in was 2.45 % (SE: ± 0.16); that of CuO NPs was 1.15 % (SE: ± 0.22). Desorption % of ZnO NPs appeared higher than that of the other types of NP.

The results of statistical analysis observed a significant effect of concentration on desorption % of NPs ($p < 0.001$) at 6 M HCl. There was a significant difference of NP types on desorption % at 6 M HCl ($p < 0.05$). *Post hoc* tests indicated differences were between CdS NPs and CuO NPs

($p < 0.05$) and between CdS NPs and ZnO NPs ($p < 0.001$) and between CuO NPs and CdS NPs ($p < 0.05$) and between ZnO and CdS NPs ($p < 0.001$). The mean overall desorption % of ZnO NPs was 23.84 % (SE: ± 0.83); that of CuO NPs was 21.83 % (SE: ± 0.97); that of CdS NPs in was 19.13 % (SE: ± 0.90). Desorption % of ZnO NPs appeared higher than that of the other types of NP. The desorption % of ZnO was the highest, followed by that of CdS; the desorption % of CuO was the lowest using all the extracting solutions. The majority of leachability rates were slowest for the Cu^{2+} ions, followed by Cd^{2+}, and Zn^{2+} was the highest in the four test soils.

6.6.2. Effect of soil types on desorption percentage

The results of statistical analysis observed a significant effect of concentration on desorption % of NPs at pH 5.3 ($p < 0.001$). There was a significant difference of soil type on desorption % of NPs at pH 5.3 ($p < 0.001$). *Post hoc* tests indicated differences were between Libyan sandy soil compared to all soils (each $p < 0.001$), Eutric Cambisol soil and Libyan sandy soil ($p < 0.001$), Sandy and Libyan sandy soil ($p < 0.001$) and Haplic podzol soil and Libyan sandy soil ($p < 0.001$). The mean overall desorption % of Haplic podzol soil was 6.09 % (SE: ± 0.54); that in that in Sandy soil was 5.76 % (SE: ± 0.54); that in Eutric Cambisol soil was 4.73 % (SE: ± 0.75); that in Libyan sandy soil was 0.86 % (SE: ± 0.24). Desorption % appeared highest in the Haplic podzol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of NPs ($p < 0.001$) at pH 3. There was a significant difference of soil type on desorption % of NPs at pH 3 ($p < 0.001$). *Post hoc* tests indicated differences were between Libyan sandy soil compared to Eutric Cambisol and Haplic podzol soil (each $p < 0.001$), Eutric Cambisol soil and Libyan sandy soil ($p < 0.001$) and Haplic podzol soil and Libyan sandy soil ($p < 0.001$). The mean overall desorption % of Haplic podzol soil was 20.79 % (SE: ± 1.2); that in Eutric Cambisol soil was 20.46 % (SE: ± 0.96); that in Sandy soil was 18.39 % (SE: ± 0.97); that in Libyan sandy soil was 15.76 % (SE: ± 1.21). Desorption % appeared highest in the Haplic podzol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of NPs ($p < 0.001$) at pH 10. There was a significant difference of soil type on desorption % of NPs at pH 10 ($p < 0.001$). *Post hoc* tests indicated differences were between Libyan sandy soil and Sandy soil ($p < 0.05$), Libyan sandy soil and Haplic podzol soil ($p < 0.001$), Eutric Cambisol

soil and Haplic podzol soil ($p < 0.05$), Sandy soil and Libyan sandy soil ($p < 0.05$), Haplic podzol soil and Libyan sandy soil ($p < 0.001$) and Haplic podzol soil and Eutric Cambisol soil ($p < 0.05$). The mean overall desorption % of Haplic podzol soil was 2.99 % (SE: ± 0.33); that in Sandy soil was 2.29 % (SE: ± 0.25); that in Eutric Cambisol soil was 2.10 % (SE: ± 0.23); that in Libyan sandy soil was 1.35 % (SE: ± 0.19). Desorption % appeared highest in the Haplic podzol soil.

The results of statistical analysis observed a significant effect of concentration on desorption % of NPs ($p < 0.001$) at 6 M HCl. *Post hoc* tests indicated differences were between all soils compared to each other (each $p < 0.001$). The mean overall desorption % of Eutric Cambisol soil was 29.90 % (SE: ± 0.60); that in Sandy soil was 23.44 % (SE: ± 0.76); that in Haplic podzol soil was 19.24 % (SE: ± 0.88); that in Libyan sandy soil was 13.81 % (SE: ± 0.95). Desorption % appeared highest in the Eutric Cambisol soil.

The desorption % of Haplic podzol soil was higher than that of three soils using all the extracting solutions. The desorption % of Haplic podzol soil was highest, followed by decreasing levels of Sandy soil, Eutric Cambisol soil and Libyan sandy soil when using pH 5.3 and pH 10. The results of desorption % also showed Haplic podzol soil was highest, followed by decreasing levels of Eutric Cambisol soil, Sandy soil and Libyan sandy soil when using pH 3 and the desorption of Eutric Cambisol soil was highest, followed by Sandy soil, Haplic podzol and Libyan sandy soil, respectively using 6 M HCl. Desorption % appeared highest in Eutric Cambisol soil.

The decrease in the desorption % of NPs in Sandy texured and Eutric Cambisol soils using alkaline solutions, plausibly arises from the higher soil organic matter content in these soils. Cu^{2+} ions can form stable complexes with soil organic matter. This fraction of the adsorbed Cu cannot be desorbed completely by solutions.[41]

The amount of Cu^{2+} ions that form complexes with organic matter increases as a function of the soil's organic matter–the higher the soil organic matter, the higher the number of complexes. This plausibly accounts for the decline in the desorption percentage of previously adsorbed Cu^{2+} ions. Chen *et al.*[42] observed similar leachability behaviour for metals sorbed on to hydroxyapatite. In the present study, under alkaline extraction conditions, sorbed NPs were more

stable than they were in acidic conditions. Further, only a few NP desorbed values in alkaline conditions (pH 10) were under the detection limit of atomic absorption (AAS).

6.3. Conclusion

The adsorption of CdS, CuO and ZnO NPs increased with increasing levels of added NP concentrations in soil solutions, with higher levels of test NP concentration leading to progressively higher levels of adsorption. Study of nano–metal adsorption revealed that adsorption of Cu appeared highest, followed by that of Cd; the adsorption of Zn NPs was the lowest across all soil types and NP concentrations. Results also indicated adsorption in Libyan sandy, Eutric Cambisol, Sandy and Haplic podzol soil increased with increasing NP concentrations across all NP types, The highest adsorption was of Libyan sandy soil, followed by decreasing levels of Eutric Cambisol soil, Sandy texured soil and Haplic podzol soil respectively.

The results obtained from the Freundlich equation well represented the adsorption data compared with that of Langmuir equation. The kinetic behaviour of Cd, Cu and Zn NPs towards Libyan sandy, Eutric Cambisol, Sandy and Haplic podzol soils revealed that pseudo–second order provided better correlations rather than that of pseudo–first order kinetics.

In contrast to CuO, the desorption % of Zn was the highest, followed by that of Cd; the desorption % of Cu was the lowest using all the extracting solutions across all soil types. This high leaching of Cd and Zn suggests may possibly have adsorbed on the available surface sites of hydroxyapatite with little diffusion into apatite structure. The desorption % of Haplic podzol soil was higher than that of three soils using all the extracting solutions across all NP types. The desorption % of Haplic podzol soil was highest, followed by decreasing levels of Sandy soil, Eutric Cambisol soil and Libyan sandy soil when using pH 5.3 and pH 10. The results of desorption % also indicated Haplic podzol soil was highest, followed by decreasing levels of Eutric Cambisol soil, Sandy soil and Libyan sandy soil when using pH 3 and the desorption of Eutric Cambisol soil was highest, followed by Sandy soil, Haplic podzol and Libyan sandy soil, respectively using 6 M HCl. Desorption % appeared highest in Eutric Cambisol soil.

The results of this study indicated that the four soils had a different adsorption capacity for nanoparticles. Thus, the availability of NPs in soil may increase to toxic levels for soil biota. However, little information is available regarding the behaviour and environmental risk of MNPs to terrestrial ecosystems. The fate, environmental effects, and transformation of NPs in soils most likely depends on the physicochemical characteristics of these nanometals in the soil as indicated in section 2.6. Soils also contain materials such as organic matter, clays at nano and

micro scales, iron oxides, and diverse kinds of minerals; these resources play important roles in the biogeochemical interactions of soil systems. Therefore, there is a large range of issues to be addressed. These issues strongly overlap with the fate and behaviour of natural and manufactured nanoparticles in soil.

6.4. References

1. Maynard, A. D., Aitken, R. J., Butz, T., Colvin, V., Donaldson, K., Oberdorster, G., Philbert M. A., Ryan, J ., Seaton, A ., Stone, V ., Tinkle, S.S ., Tran, L ., Walker, N.J., and Warheit, D. B. (2006). Safe handling of nanotechnology. *Nature.* 444 (7117): 267–269.

2. Roco, M. (2005). The emergence and policy implications of converging new technologies integrated from the nanoscale. *Journal of Nanoparticle Research.* 7 (2–3): 2–3.

3. Zhu, J., Li, D., Chen, H., Yang, X., Lu, L., and Wang, X. Highly dispersed CuO nanoparticles prepared by a novel quick–precipitation method. (2004). *Materials Letters.* 58 (26): 3324.

4. Marino, E., Huijser, T., Creyghton, Y., and van der Heijden, A. (2007). Synthesis and coating of copper oxide nanoparticles using atmospheric pressure plasmas. *Surface and Coatings Technology.* 201 (22–23): 9205–9208.

5. Brayner, R., Dahoumane, S. A., Yepremian, C., Djediat, C., Meyer, M., Coute, A., and Fievet, F. (2010). ZnO nanoparticles: Synthesis, characterization, and ecotoxicological studies. *Langmuir.* 26 (9): 6522–6528.

6. Lin D., and Xing, B. (2008). Root uptake and phytotoxicity of ZnO nanoparticles. *Environmental Science and Technology.* 42 (15): 5580–5585.

7. Arab, K.C. (2003). Nanomaterials set for explosive growth. (http://news.thomasnet.com/IMT/archives/2003/09/nanomaterials_s.html).

8. Singh, V., and Chauhan, P. (2009). Synthesis and structural properties of wurtzite type CdS nanoparticles. *University of Allahabad, India.* 6 (8): 421–426.

9. Yoffe, A. D. (1993). Low–dimensional systems: quantum size effects and electronic properties of semiconductor microcrystallites (zero–dimensional system) and some quasi–two–dimensional system. *Adavnced Physics.* 42: 173.

10. Mubarak, A., D., Gopinath, V., Rameshbabu, N., and Thajuddin, N. (2012). Synthesis and characterization of CdS nanoparticles using C–phycoerythrin from the marine cyanobacteria. *Materials Letters.* 74: 8–11.

11. El–Tantawy, F. (2001). Influence of solvent transport on physico–chemical properties of crosslinked butyl rubber filled with TiC ceramic. *Polymer Degradation and Stability.* 73 (2): 289–299

12. Faez, R., Martin, I. M., De Paoli, M. A., and Rezende, M. C. (2002). Influence of processing time and composition in the microwave absorption of EPDM/PAni blends. *Journal of Applied Polymer Science.* 83 (7): 1568–1575.

13. Arias, M., Perez–Novo, C., Lopez, E., and Soto, B. (2006). Competitive adsorption and desorption of copper and zinc in acid soils. *Geoderma.* 133 (3–4): 151–159.

14. Abat, M., McLaughlin, M.J., Kirby, J.K., and Stacey, S.P. (2012). Adsorption and desorption of copper and zinc in tropical peat soils of Sarawak, Malaysia. *Geoderma.* 175–176: 58–63.

15. Ma, L., Xu, R., Jiang, J. (2010). Adsorption and desorption of Cu(II) and Pb(II) in paddy soils cultivated for various years in the subtropical China. *Journal of Environmental Sciences.* 22 (5) 689–695.

16. McBride, M. B. (1994). Environmental Chemistry in Soils. New York: *Oxford University Press.*

17. Pardo, M. T. (2000). Adsorption of lead, copper, zinc, and cadmium by Soils: Effect of nitriloacetic acid on metal retention. *Communications in Soil Science and Plant Analysis.* 31 (1): 31–40.

18. Sparks, D. L. (2003). Environmental soil chemistry (2nd ed.). *Academic Press*, New York.

19. Spark, K. M., Wells, J. D., and Johnson, B. B. (1995). Characterizing trace metal adsorption on kaolinite.*European Journal of Soil Science.* 46 (4): 633–640.

20. Gerritse, R. G., and van Driel, W. (1984). The Relationship between adsorption of trace Metals, organic matter, and pH in temperate soils1. *Journal of Environment Quality.* 13 (2): 197–204.

21. Kabata–Pendias, A. (1993). Behavioural properties of trace metals in soils. *Applied Geochemistry.* 8: 3–9.

22. Batley, G. E., and McLaughlin, M. J. (2010). Fate of manufactured nanomaterials in the Australian environment. *CSIRO Niche Manufacturing Flagship Report.* the Department of the Environment, Water, Heritage and the Arts.pp:1–75.

23. Chaturvedi, P.K., Seth, C.S., and Misra, V. (2006). Adsorption kinetics and leachability of heavy metal from the contaminated soil amended with immobilizing agent (humus soil and hydroxyapatite). *Chemosphere.* 64 (7): 1109–1114.

24. Nightingale, E.R.(1995). (1959). Phenomenological theory of ion solvation. Effective radii of hydrated ions. *Journal of Physical Chemistry.* 63: 1381–1387.

25. Wang, Q., Huang ,X.F., Li, C.X., Cao, Z.X., Sun, G., Lu, K.Q., Pan, L.Q., Wu, Z.H., Hu, T.D., Jiang, Z., and Huang Y.Y. (2012). Coordination variation of hydrated Cu^{2+}/Br^{1-} ions traversing the interfacial water in mesopores. *American Institute of Physics, AIP Advances.* 2 (2): 022107.

26. Pearson. R. G. (1963). Hard and Soft Acids and Bases.*Journal of The American Chemical Society.* 1(85): 3533– 3539.

27. Aylward, G. H., and Findlay, T. J. V. (2008). SI chemical data. 6 th edition. *Milton, Qld: Wiley,* Australia.

28. Liu, C.C., Wang, M.K., Chiou, C.S., Li, Y.S., Yang, C.Y., and Lin, Y.A. (2009). Biosorption of chromium, copper and zinc by wine–processing waste sludge: Single and multi–component system study. *Journal of Hazardous Materials.* 171 (1–3): 386–392.

29. Covelo, E.F., Andrade, M.L., and Vega, F.A. (2004). Heavy metal adsorption by humic umbrisols: selectivity sequences and competitive adsorption kinetics. *Journal of Colloid and Interface Science.* 280 (1): 1–8.

30. Gomes, P. C., Fontes, M. P. F., Da Silva, A. G.., De SMendonca, E., and Netto, A. R. (2001). Selectivity sequence and competitive adsorption of heavy metals by Brazilian soils. *Soil Science society of American Journal.* 65: 1115–1121.

31. Fontes, M.P.F., and Gomes, P.C. (2003). Simultaneous competitive adsorption of heavymetals by the mineral matrix of tropical soils. Appl. *Geochem.* 18: 795–804.

32. Jalali, M., and Moharrami, S. (2007). Competitive adsorption of trace elements in calcareous soils of western Iran. *Geoderma.* 140 (1–2): 156–163.

33. McBride, M. (1989). Reactions controlling heavy metal solubility in soils, Advanced Soil Science. 10: 1–56.

34. Echeverria, J.C., Morera, M.T., Mazkiaran, C., and Garrido, J.J. (1998). Competitive adsorption of heavy metal by soils. Isotherms and fractional factorial experiments. *Environmental Pollution.* 101 (2): 275–284.

35. Morera, M.T., Echeverria, J. C., Mazkiaran, C., and Garrido, J.J. (2001). Isotherms and sequential extraction procedures for evaluating adsorption and distribution of heavy metals in soils. *Environmental Pollution.* 113 (2): 135–144.

36. Legeroes, R.Z., Legeroes, J.P. (1984). Phosphate minerals in human tissues. In: Nariagu, J.O. (Ed.), Phosphate Minerals. Springer–Verlag, Berlin, pp. 351–385.

37. Zhou, X., Xue, X., and Jiang, W. (2011). Study on adsorption of heavy metal ion in metallurgical wastewater by sepiolite. *2nd International Conference on Environmental Science and Development.* 4:100–103.

38. Chen, J.P., and Wang, L. (2004). Characterization of metal adsorption kinetic properties in batch and fixed bed reactors. *Chemosphere.* 54: 397–404.

39. Ho, Y., and Chiang, C. (2001). Adsorption studies of acid dye by mixed sorbents. *Adsorption.* 7: 139–147.

40. Nriagu, J. O. (1979). Zinc in the environment. *Wiley–Interscience*, New York.

41. Guo, X., Zhang, S., Shan, X.Q., Luo, L.E., Pei, Z., Zhu, Y.G., Liu, T., Xie, Y.N., and Gault, A. (2006). Characterization of Pb, Cu, and Cd adsorption on particulate organic matter in soil. *Environmental Toxicology and Chemistry.* 25 (9): 2366–73.

42. Chen, X., Wright, J. V., Conca, J. L., and Peurrung, L. M. (1997). Effects of pH on heavy metal adsorption on mineral apatite. *Environmental Science and Technology.* 31 (3): 624.

Chapter 7. Do manfacured nanaoparticles impact soil nitrogen cycling

7.1. Introduction

There is little knowledge concerning the influence of MNPs discharge on ecosystems.[1,2] Specially, the impact of these materials on soil nitrogen cycling. Soil microorganisms perform an essential role in geological, hydrological, and ecological cycles; thus any change brought about by the release of MNPs to the microbial diversity and function could potentially influence the quantity of plant available nitrogen in the soil matrix.[3,4] The fate of soil nitrogen is difficult to determine as regards the behaviour of ecosystems and their responses to natural and anthropogenic sources of pollution.[5] Thus it is important to know whether the annual production of tons of NPs affects the terrestrial nitrogen cycle; nitrogen is among the most important nutrients for plants and the microbial community.[1,6]

The effect of manufactured metal NPs on terrestrial microbial communities has been reported under laboratory conditions.[7–9] Under pot field conditions, the effects of ZnO and Cu NPs on microbial communities have been investigated in pot soils.[1] The fate and production of inorganic nitrogen in the form of nitrate (NO_3^-) and ammonium (NH_4^+) ions have been well reported in different soils, but without assessing the ecological effect of NPs.[5,10,11] The breakdown of organic nitrogen to dissolved organic nitrogen (DON) and low molecular weight (LMW) followed by conversion to ammonium ions and finally to nitrate ions has been determined for contrasting grassland soils.[5,12,13]

Jones *et al.*[5] have indicated that the processes of inorganic nitrogen (NH_4^+ and NO_3^-) are well recognized; however, the stages prior to the production of ammonium ions through the nitrogen cycle remain poorly understood. The production of inorganic nitrogen in soil (without additive NPs) is controlled by several factors, including the rate of plants residues decomposition above and below soil surface; however, the most important factors that appear to control inorganic nitrogen in soil systems are the interaction of inorganic nitrogen with decomposer communities, the availability of nitrogen in different soluble forms, and environmental conditions.[14] The release of nitrogen in different forms from organic residues to the soil and decomposition process is determined by chemical factors; these include the primary nitrogen ratio of carbon to nitrogen, amino acids, soluble carbohydrates, active polyphenols, and lignin.[15]

7.2. Objectives

The aims of this research is to study the effect of CdS, CuO, and ZnO NPs on the rate of nitrogen mineralization over 28 days and to determine whether the breakdown of soil organic nitrogen to dissolve organic nitrogen, then to ammonium ions, and finally to nitrate ions; the ions represent the most significant pool of nitrogen to agricultural grassland soils impacted by different concentrations of MNPs. A further aim was to illustrate the influence of different soil types on the degree of NP toxicity.

7.3. Materials and methods

7.3.1. Site description of the soils

Three contrasting agricultural soils – Eutric Cambisol, Haplic podzol, and Sandy soil were selected from the Henfaes Research Centre, North Wales. The characteristics of these soils were as described in Chapter 2, Section 2.16.

7.3.2. Preparation of soil samples

The preparation of the soil samples prior to experimentation was as described in Chapter 3, Section 3.2.2. The soils were stored in plastic bags at 4°C within 24 hours of their collection to prevent any microbial activity that might affect mineralization. The total carbon and nitrogen content of the soils was determined using a CHN–2000 analyzer (Leco Corporation, St Joseph, MI) as described in Chapter 3, Section 3.2.10. Nitrate and ammonium ion content were also analyzed as described in Chapter 3, Section 3.2.8. The soluble N was extracted by the centrifugal drainage technique for soil solution. The pH and electrical conductivity of soils were measured as described in Chapter 3, Section 3.2.5. The soils' Cation Exchange Capacities (CEC) and particle size distributions were tested as described in Chapter 3, Sections 3.2.6 and 3.2.7 respectively. The soils' moisture content and organic matter content (OM) (%) were determined as described in Chapter 3, Section, 3.2.4; their water holding capacities (WHC) were also evaluated as described in Chapter 3, Section, 3.2.3. Table 7.1 shows the properties of the soils.

Table 7. 1. Selected properties of the three grassland soils in the mineralization study

Soil properties	Haplic podzol soil	Eutric Cambisol soil	Sandy soil
texture	loamy sand	Clay loam	Sand
Moisture content (%)	7.33±0.03	28.92±0.03	7.52±0.05
pH	4.7±0.05	5.5±0.03	5.7±0.01
EC (ms cm^{-1})	0.11±0.001	0.64±0.004	0.28±0.001
Total C (g kg^{-1})	16±2.9	49.0±6.1	38±2.3
Total N (g kg^{-1})	1.6±0.3	7.7±0.1	3.9±0.3
C–to–N ratio	10	6.3	16.5
Soil respiration (μmol CO_2 kg^{-1} h^{-1})	127±5.0	243±5.0	240±5.0

Values represent the means of three replicates ($n = 3$).

7.3.3. Chemical treatments

CdS, ZnO, and CuO NPs were chosen to study their effect on soil processes. Table 7.2 shows the properties of these NPs. Three concentrations of each metal NP (0.01, 0.10, and 1.0 g kg^{-1}) were selected to identify the threshold at which the tested NPs influence the rate of nitrogen mineralisation and soil respiration. Three replicates for each concentration were used to help ensure experimental accuracy. Table 7.3 lists the relevant calculations of the metal NPs that were used in the present study.

Table 7. 2. Characteristic of NPs used for the phytotoxity experiments

Compound	Size (nm)	Purity (%)	Surface area (m^2/g)	Particular morphology	Molecular weight (g/mol^{-1})
ZnO	90–210	99.9	5–7	irregular	81.39
CuO	40–80	99.9	–	–	79.55
CdS	~ 7.6– 17.7	–	–	–	144.48

The parameters of ZnO and CuO were measured by the IoLitec Nanomaterials Company. The size of CdS was calculated using XRD and SEM.

Table 7. 3. The concentration calculations used to calculate the quantity of metal NPs applied to the sand and the quantity of metal NP/sand mixture applied to the soil.

0.01 g metal/kg of soil	0.1g/kg of soil	1 g/kg of soil
0.01 g MNP to 99.99 g sand	0.1 g MNP to 99.9 sand	1 g MNP to 9.0 g sand
1.00 g MNP/sand to 10 g soil	0.10 g MNP/sand to 10 g soil	0.10 g MNP/sand to 10 g soil

MNP = Manufactured NPs

Pure sand was used to minimise the aggregation of the tested NPs. Sand was used because, at least for the purposes of the present study, it was considered chemically inert. The sand and NP mixtures were shaken at 250 rpm for 30 minutes using a SW2 Shaker Table (Edmund Buhler Swip), and subsequently sonicated for 15 minutes to attempt an even dispersal throughout the application medium.[16] Table 7.3 shows the measures that were used to calculate the compositions of the sand and NPs mixtures and application rates for each soil. In the case of the control samples, the blank soils contained the inert sand without the NPs. The same amount of sand was used with the metal NPs when applied to the test soils.

7.3.4. Influence of nanoparticles on nitrogen mineralization

Each soil type (10 g) was placed in 50 mL centrifuge tubes in the dark chamber at 10°C; each tube held a single soil type. Each soil was treated with the different concentrations of CdS, CuO, and ZnO NPs (0, 0.01, 0.1 and 1 g metal kg^{-1} soil). Each concentration was repeated three times. The soil samples were stored in a dark chamber at 10°C. After incubating for 0, 2, 7, 14, and 28 days, the tubes were removed; the soluble nitrogen was extracted by adding 25 ml of distilled water to 10 g of each soil.[5] The soil solution was shaken for 30 minutes using a Gallenkamp shaker incubator (Orbital incubator shaker. 2010, England; 250 rev min^{-1}). The mixed solutions were then centrifuged for 15 minutes using a Hettich–Zentrifugen centrifuge (Rotanta, 460 R.) at 9050 rcf. The supernatant solutions were recovered and frozen at –20°C prior to nitrogen analysis.

7.3.5. Influence of NPs on the respiration rates of soil

10 g of each soil type were placed in 50 mL centrifuge tubes; each tube held a single soil type. The same concentrations (0, 0.01, 0.1 and 1 g metal kg^{-1} soil) of CdS, ZnO, and CuO NPs were applied to each soil. The preparation of NPs concentrations was described in Table 7.3. Each concentration was repeated three times. Rates of CO_2 efflux from each soil were measured with a PP system SRI soil respirometer (PP–system, Hitchin, UK) at ± 20° C within 48 hours. The measurement of CO_2 was determined immediately in the soils after the NPs exposure.

7.3.6. Chemical analysis

The total dissolved nitrogen (TDN) in the extracted solution was measured with a Shimadzu TOCV–TNM1 analyzer (Shimadzu Corp., Kyoto, Japan). A series of standard solutions of

carbon and nitrogen were prepared to obtain a calibration curve as described in Chapter 3, Section (3.2.10). Some of the extracted solutions were diluted 10–fold with de–ionized water prior to analysis to prevent the presence of high ion concentrations in the soils.

The concentrations of the ammonium ions (NH_4^+) in the soil solutions were determined by colorimetric analysis using the salicylate–nitroprusside method as described in Chapter 3, Section 3.2.8. Measurements were taken using a microplate spectrophotometer (Biotek PowerWave XS). The concentration of the nitrate ions (NO_3^-) in the soil solutions were also determined by colorimetric analysis using the same spectrophotometer. The procedure used for nitrate ion determination was as described in Chapter 3 Section, 3.2.8. Soil nitrogen was extracted in soil solution by the centrifugal drainage technique using deionized water. The extraction of soil nitrogen using KCl method was not used because this would have caused a build up of excess salt in the catalyst column and artificially increased the anion content of soil samples.

The amount of dissolved organic nitrogen was calculated as the difference between the total dissolved nitrogen and the combined ammonium and nitrite /nitrate ion concentrations (Dissolved inorganic nitrogen–DIN). The method used to determine the total amino acids followed procedure of Jones *et al.*[17] as described in Chapter 3, Section, 3.2.9. Measurements were taken using a fluorescence spectroscopy (Perkin Elmer Corp., Boston, MA).

7.4. Statistical analysis

The experiments were performed with three replicates of each concentration in the laboratory. Experimental means and standard errors were calculated using Microsoft Excel. The concentrations of NO_3^-, NH_4^+, AA and DON in each soil and NP concentrations, moreover the soil respiration of each soil and NP concentrations were subjected to an one way analysis of variance (ANOVA) and differences identified with a Tukey test using the software package SPSS 20.0 for Windows (SPSS Inc., Chicago, IL). Data normality was tested using Shapiro–Wilk test.

Two–way ANOVA was used to test for significant differences between NP type (CdS, CuO, and ZnO) and concentrations (0, 0.01 and 1.0 g kg^{-1}) for all nitrogen concentrations; significant differences between soil type (Eutric compisol, Sandy, and Haplic podzol) and concentrations for all nitrogen concentrations; and significant differences between incubation time (0, 2, 7, 14

and 28 days) and concentrations for all nitrogen concentrations. *Post hoc* tests for between measures were performed using Tukey's HSD. All statistical analyses were conducted using SPSS for Windows, Version 20 (Chicago, IL). Significant differences were accepted at the ($p < 0.05$) level. Graphs were constructed using Sigma Plot 12.3 for Windows using means and standard.

7.5. Results and discussion

7.5.1. Nitrogen of soil solution

Figures 7.1–7.9 summarise concentrations of nitrate (NO_3^-) and ammonium (NH_4^+) ions, amino acids (AA) and dissolved organic nitrogen (DON) in soil solutions of Haplic podzol, Eutric Cambisol and Sandy soils incubated with different concentrations of CdS, CuO and ZnO NPs (0, 0.01, 0.1 and 1 g kg^{-1}) without leaching for 28 days.

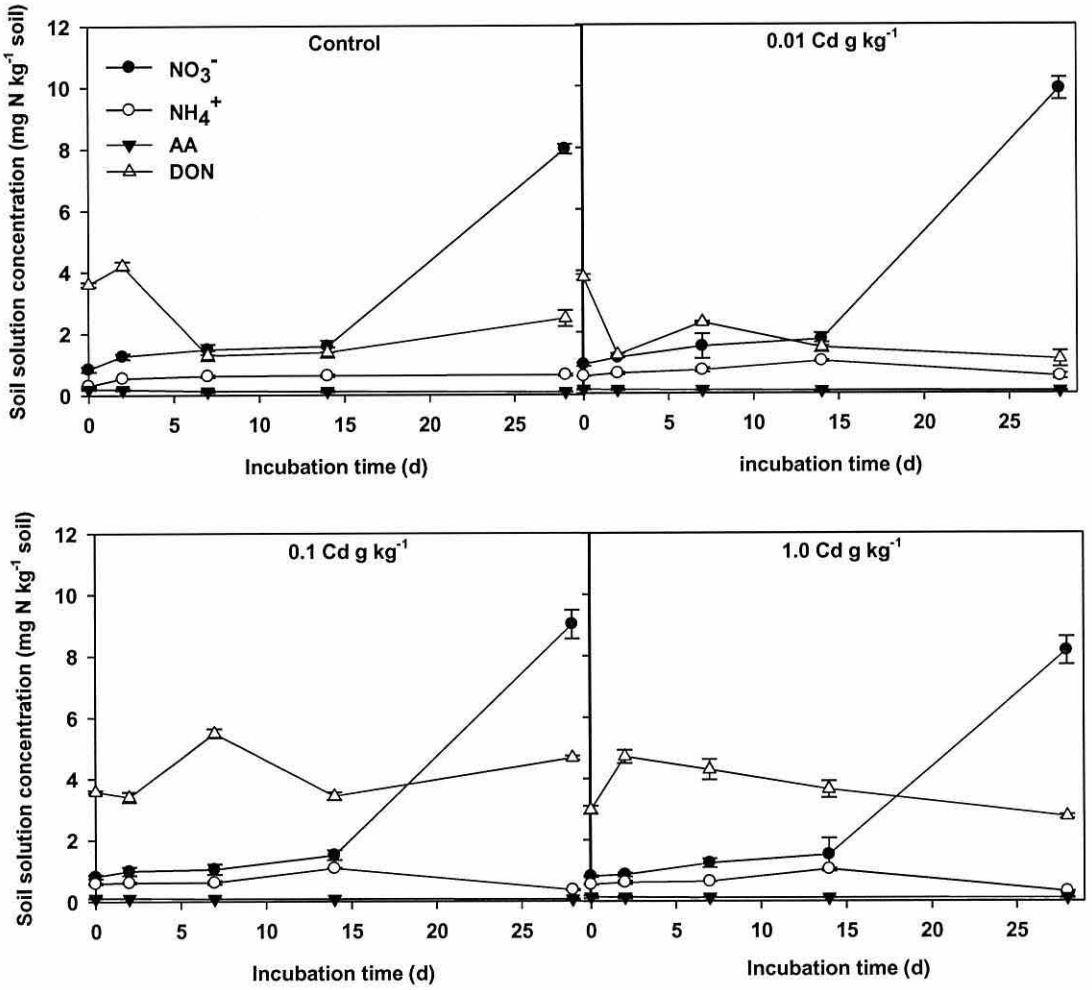

Figure 7. 1. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) ions, amino acids (AA), and dissolved organic nitrogen (DON) in soil solutions of Haplic podzol soil. Soil was incubated with different concentrations of CdS NPs (0, 0.01, 0.1, and 1.0 g kg^{-1}) without leaching for 28 days at 10°C. All values represent means ± SEM (n = 3). The concentrations of NH_4^+ and AA were small in all samples (< 1.0 mg N kg^{-1} soil). Error bars represent one standard error.

The concentrations of NO_3^-, NH_4^+, DON and AA in the tested soils were subjected to the analysis of variance (one way ANOVA). Results of statistical analysis observed no significant effects for all treatments of CdS NPs (0–1.0 g kg^{-1}) on the total concentration of NO_3^- and NH_4^+ in the Haplic podzol soil. However, statistical analysis showed a significant difference ($p < 0.001$) for CdS NPs on the total concentration of DON at a level of 0.1 g kg^{-1} when compared with that of control sample (Figure 7.1). Statistical analysis showed significant difference ($p < 0.05$) for CdS NPs on the total concentration of AA at the treatment of 0.01 g kg^{-1} and significant difference ($p < 0.001$) at a level of 0.1 g kg^{-1} compared with that of control groups.

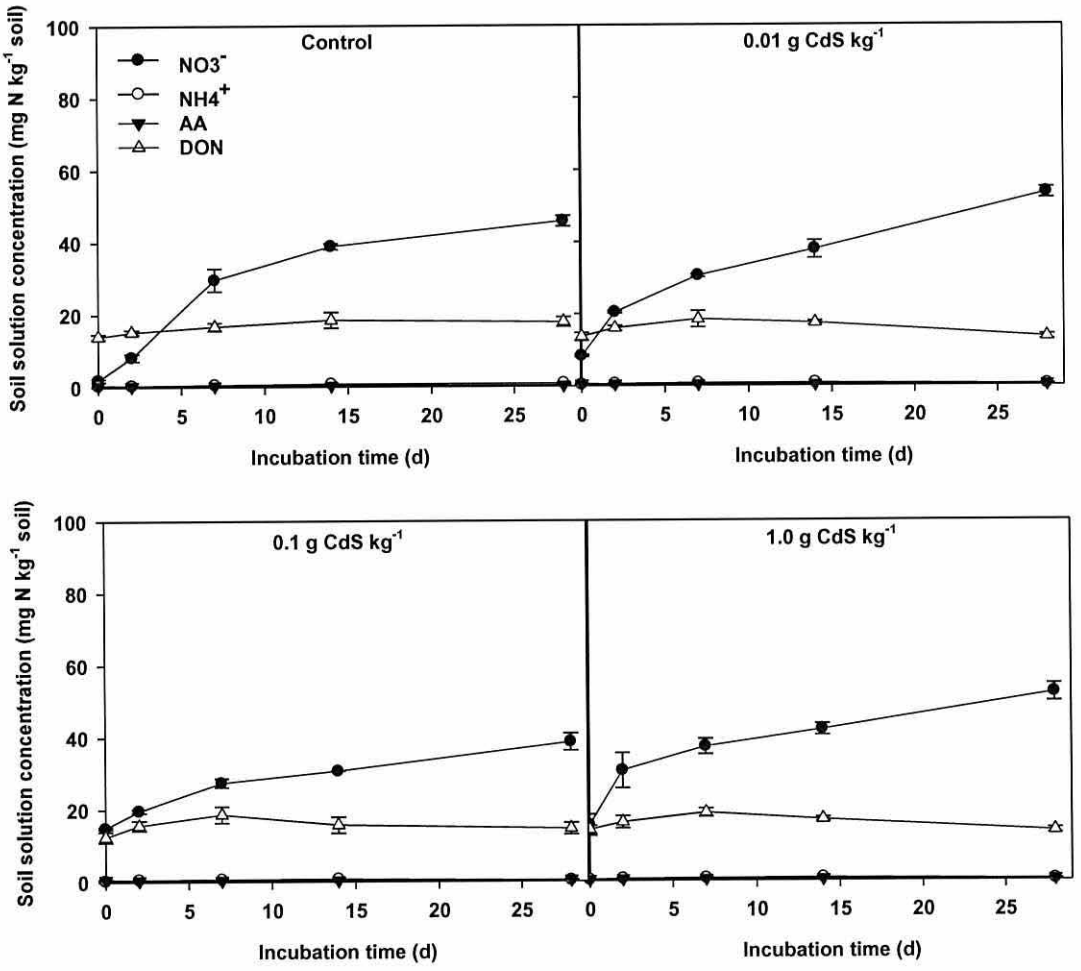

Figure 7. 2. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) ions, amino acids (AA), and dissolved organic nitrogen (DON) in soil solutions of Eutric Cambisol soil. Soil was incubated with different concentrations of CdS NPs (0, 0.01, 0.1, and 1.0 g kg^{-1}) without leaching for 28 days at 10°C. All values represent means ± SEM ($n = 3$). The concentrations of NH_4^+ and AA were small in all samples (< 1.0 mg N kg^{-1} soil). Error bars represent one standard error.

Results of statistical analysis observed no significant effects for all treatments of CdS NPs on the total concentration of NO_3^-, NH_4^+ and DON in the Eutric Cambisol soil. However, statistical analysis showed a significant difference ($p < 0.05$) for CdS NPs on the total concentration of AA at a treatment of 0.1 g kg^{-1} when compared with control sample (Figure 7.2).

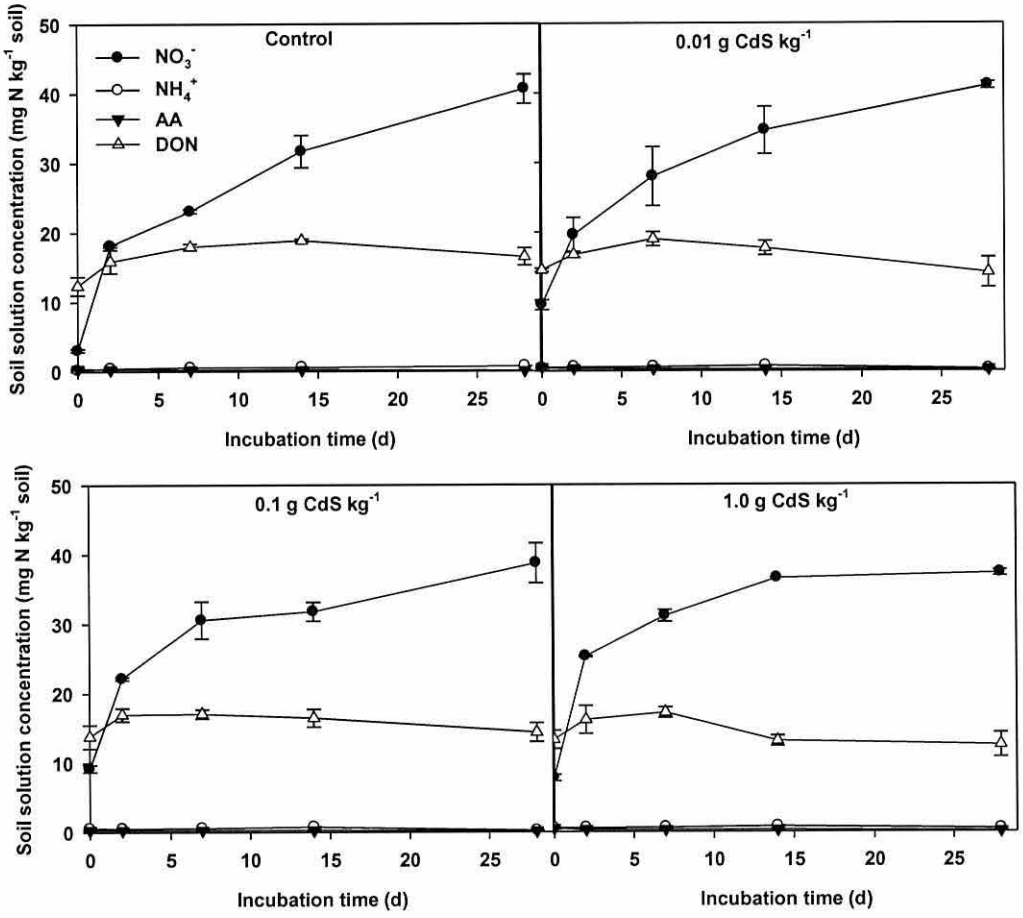

Figure 7. 3. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) ions, amino acids (AA), and dissolved organic nitrogen (DON) in soil solutions of Sandy soil. Soil was incubated with different concentrations of CdS NPs (0, 0.01, 0.1, and 1.0 g kg^{-1}) without leaching for 28 days at 10°C. All values represent means ± SEM (n = 3). The concentrations of NH_4^+ and AA were small in all samples (< 1.0 mg N kg^{-1} soil). Error bars represent one standard error.

Results of statistical analysis showed no significant effects for all treatments of CdS NPs on the total concentration of NO_3^-, NH_4^+, DON and AA in the Sandy soil (Figure 7.3).

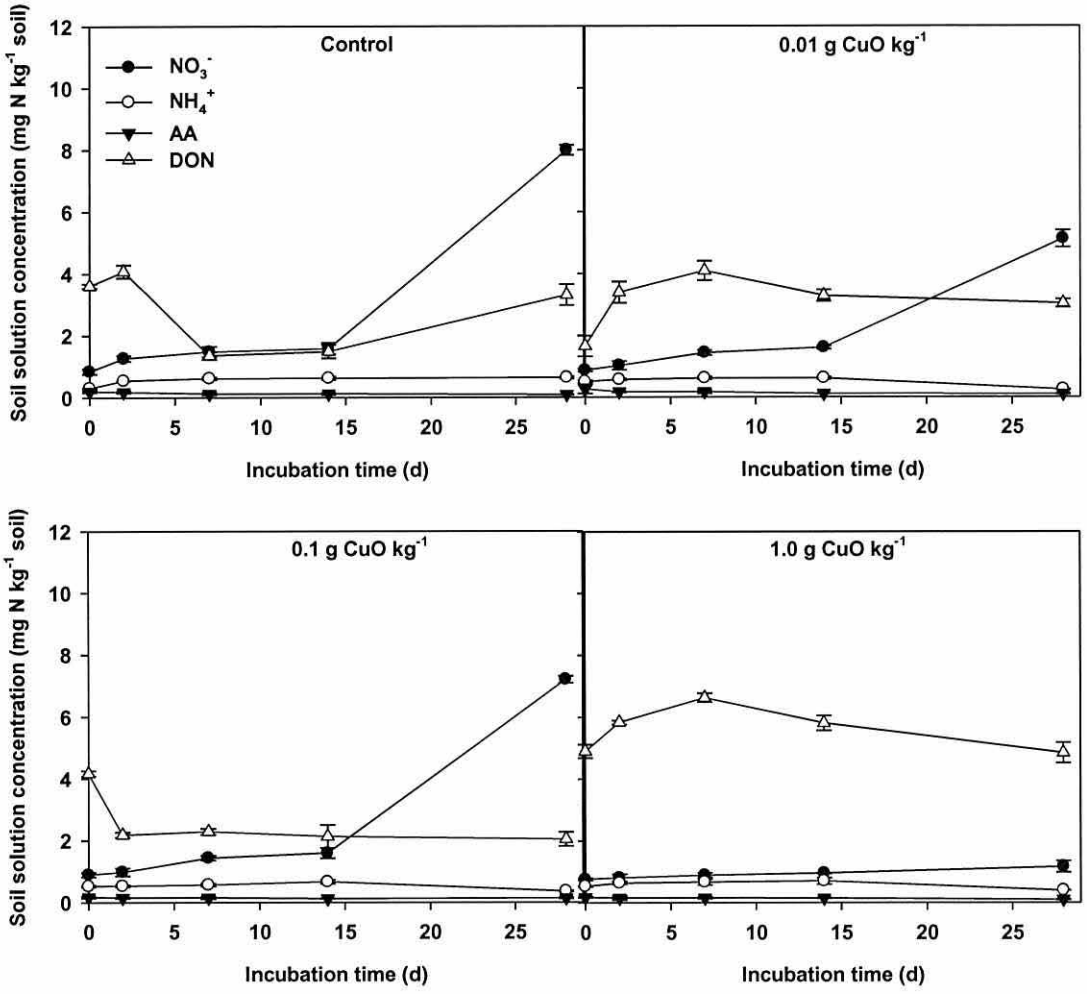

Figure 7. 4. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) ions, amino acids (AA), and dissolved organic nitrogen (DON) in soil solutions of Haplic podzol soil. Soil was incubated with different concentrations of CuO NPs (0, 0.01, 0.1, and 1.0 g kg^{-1}) without leaching for 28 days at 10°C. All values represent means ± SEM (n = 3). The concentrations of NH_4^+ and AA were small in all samples (< 1.0 mg N kg^{-1} soil). Error bars represent one standard error.

Results of statistical analysis observed no significant effects for all treatments of CuO NPs on the total concentration of NO_3^-, NH_4^+ and AA in the Haplic podzol soil. However, statistical analysis showed a significant difference ($p < 0.001$) for CuO NPs on the total concentration of DON at a level of 1.0 g kg^{-1} when compared with control sample (Figure 7.4).

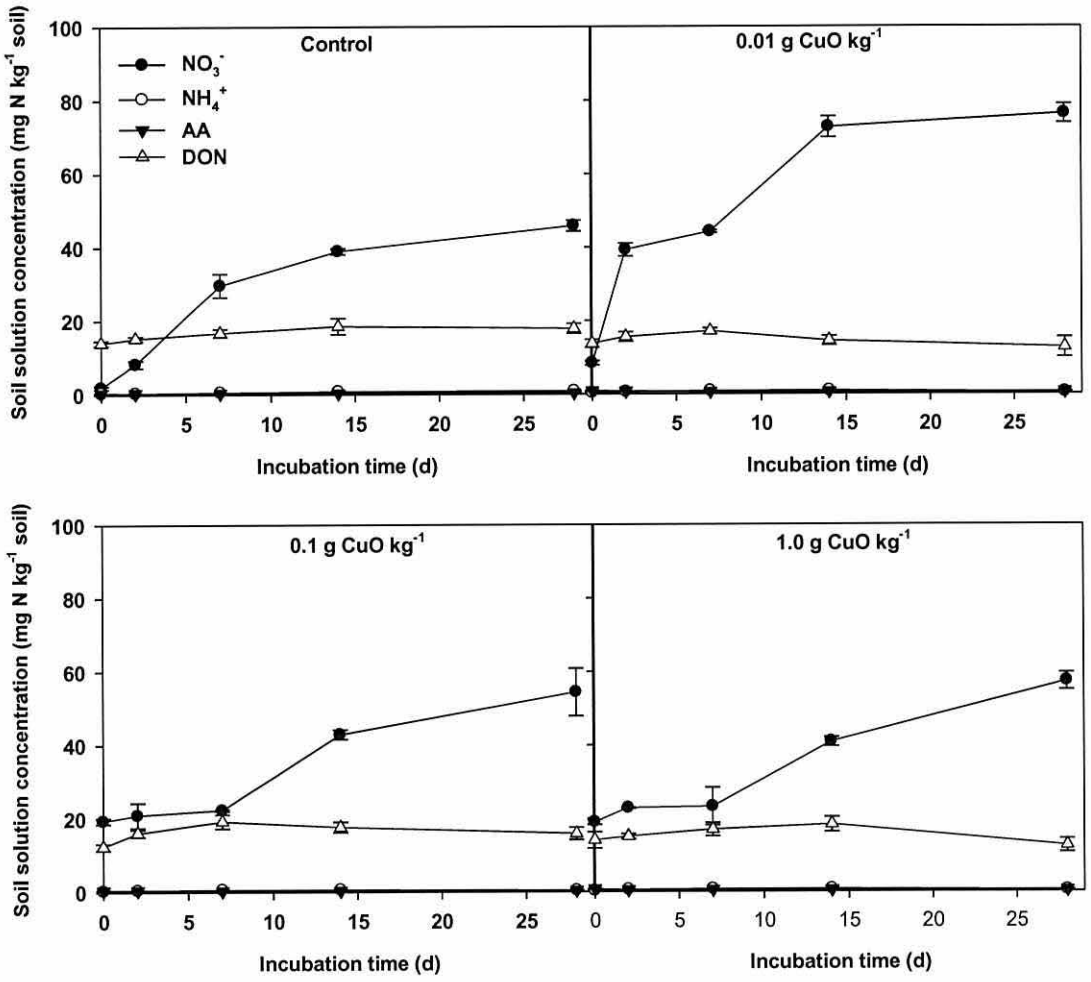

Figure 7. 5. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) ions, amino acids (AA) and dissolved organic nitrogen (DON) in soil solutions of Eutric Cambisol soil. Soil was incubated with different concentrations of CuO NPs (0, 0.01, 0.1, and 1.0 g kg^{-1}) without leaching for 28 days at 10°C. All values represent means ± SEM (n = 3). The concentrations of NH_4^+ and AA were small in all samples (< 1.0 mg N kg^{-1} soil). Error bars represent one standard error.

Results of statistical analysis showed no significant effects for all treatments of CuO NPs on the concentration of NH_4^+ and DON in the Eutric Cambisol soil. However, *Post hoc* tests showed a significant difference ($p < 0.001$) for CuO NPs on the total concentration of NO_3^- at a treatment of 0.01 g kg^{-1} when compared with that of control sample. Results of statistical analysis observed a significant difference ($p < 0.001$) for CuO NPs on the total concentration of AA at a treatment of 0.01 and 1.0 g kg^{-1} compared control group (Figure 7.5).

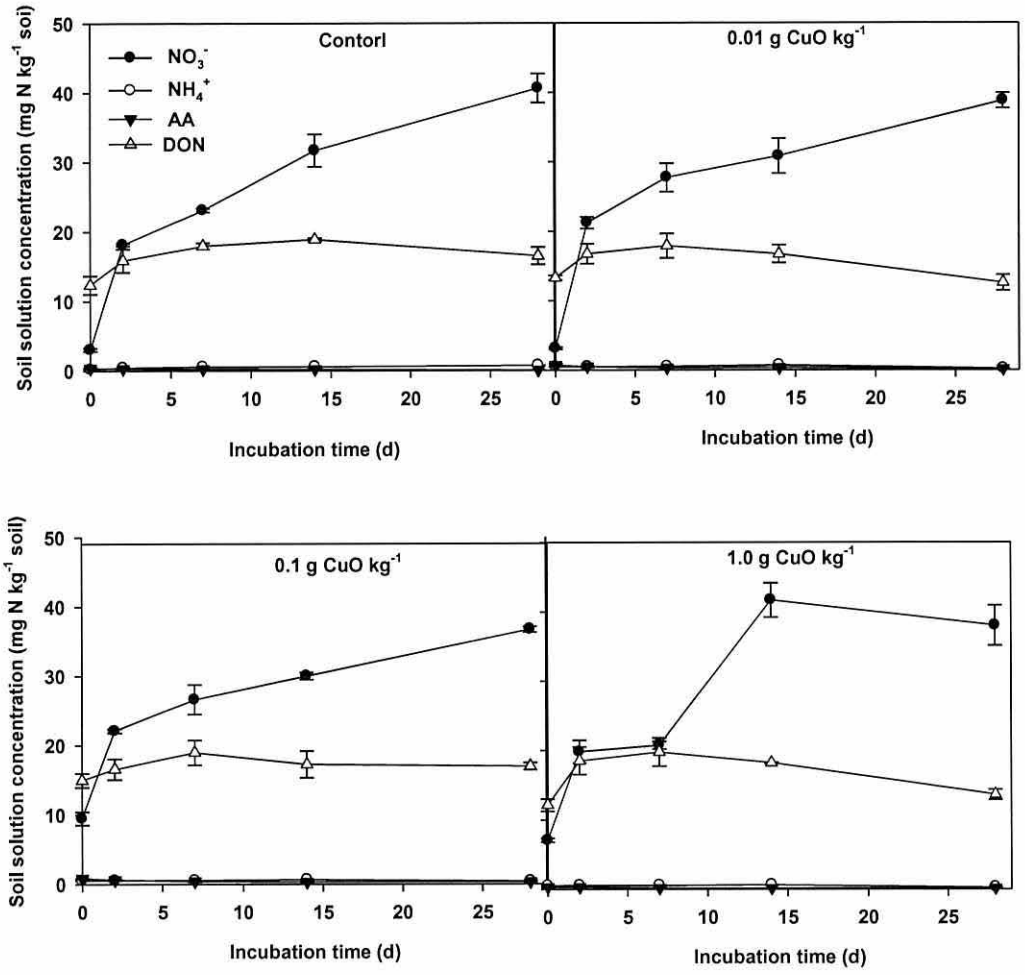

Figure 7. 6. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) ions, amino acids (AA), and dissolved organic nitrogen (DON) in soil solutions of Sandy soil. Soil was incubated with different concentrations of CuO NPs (0, 0.01, 0.1, and 1.0 g kg^{-1}) without leaching for 28 days at 10°C. All values represent means ±SEM (n = 3). The concentrations of NH_4^+ and AA were small in all samples (< 1.0 mg N kg^{-1} soil). Error bars represent one standard error.

Statistical analysis observed no significant effects for all treatments of CuO NPs on the total concentration of NO_3^-, NH_4^+ and DON in the Sandy soil. However, statistical analysis showed a significant difference ($p < 0.001$) for CuO NPs on the total concentration of AA at a treatment of 0.1 g kg^{-1} when compared with that of control sample (Figure 7.6).

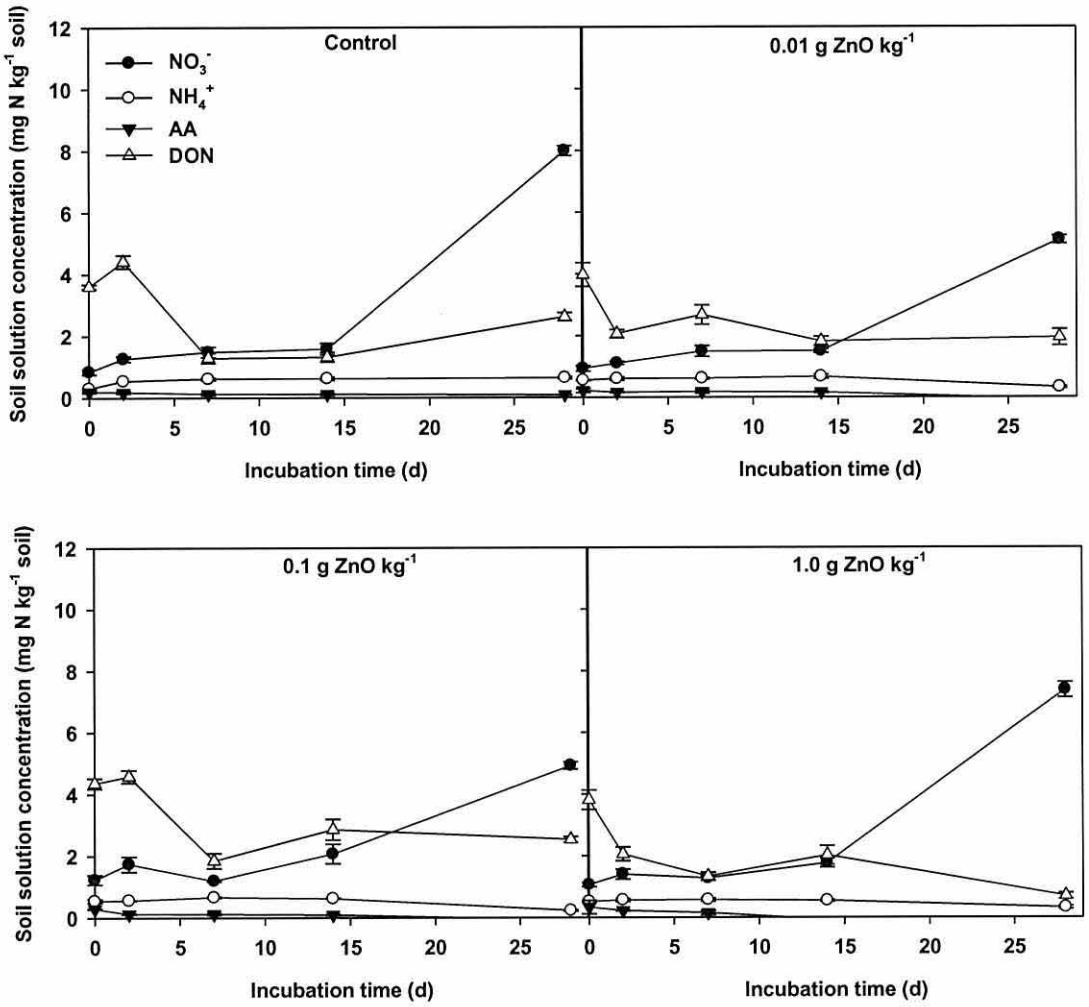

Figure 7. 7. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) ions, amino acids (AA), and dissolved organic nitrogen (DON) in soil solutions of Haplic podzol soil. Soil was incubated with different concentrations of ZnO NPs (0, 0.01, 0.1, and 1.0 g kg^{-1}) without leaching for 28 days at 10°C. All values represent means ±SEM (n = 3). The concentrations of NH_4^+ and AAs were small in all samples (< 1.0 mg N kg^{-1} soil). Error bars represent one standard error.

Results of statistical analysis showed no significant effects for all treatments of ZnO NPs on the total concentration of NO_3^-, NH_4^+, DON and AA in the Haplic podzol soil (Figure 7.7).

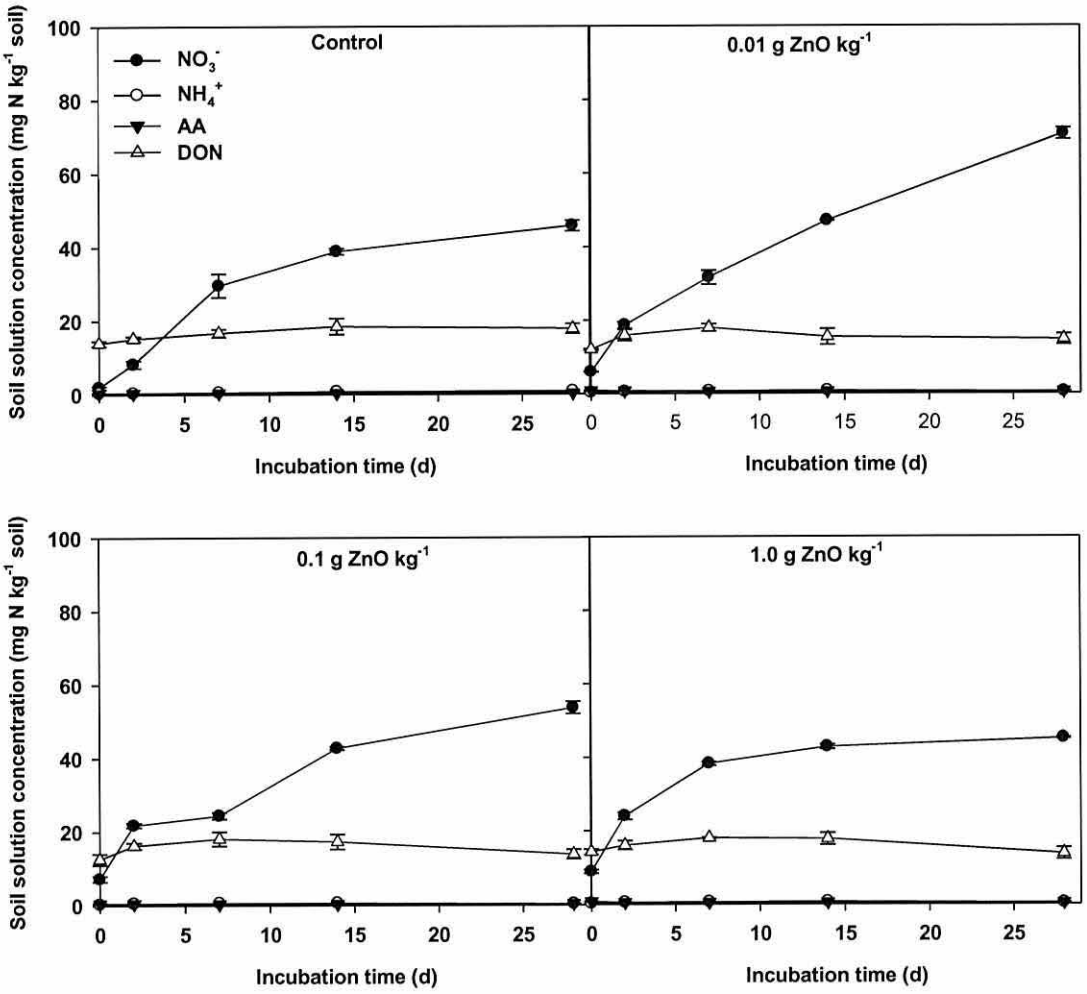

Figure 7. 8. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) ions, amino acids (AA), and dissolved organic nitrogen (DON) in soil solutions of Eutric Cambisol soil. Soil was incubated with different concentrations of ZnO NPs (0, 0.01, 0.1, and 1.0 g kg^{-1}) without leaching for 28 days at 10°C. All values represent means ±SEM (n = 3). The concentrations of NH_4^+ and AAs were small in all samples (< 1.0 mg N kg^{-1} soil). Error bars represent one standard error.

Results of statistical analysis observed no significant effects for all treatments of ZnO NPs on the total concentration of NO_3^-, NH_4^+ and DON in the Eutric Cambisol soil. However, Statistical analysis showed a significant difference (p < 0.001) for ZnO NPs on the total of AA concentration at a treatment of 0.01 g kg^{-1} when compared with that of control sample (Figure 7.8).

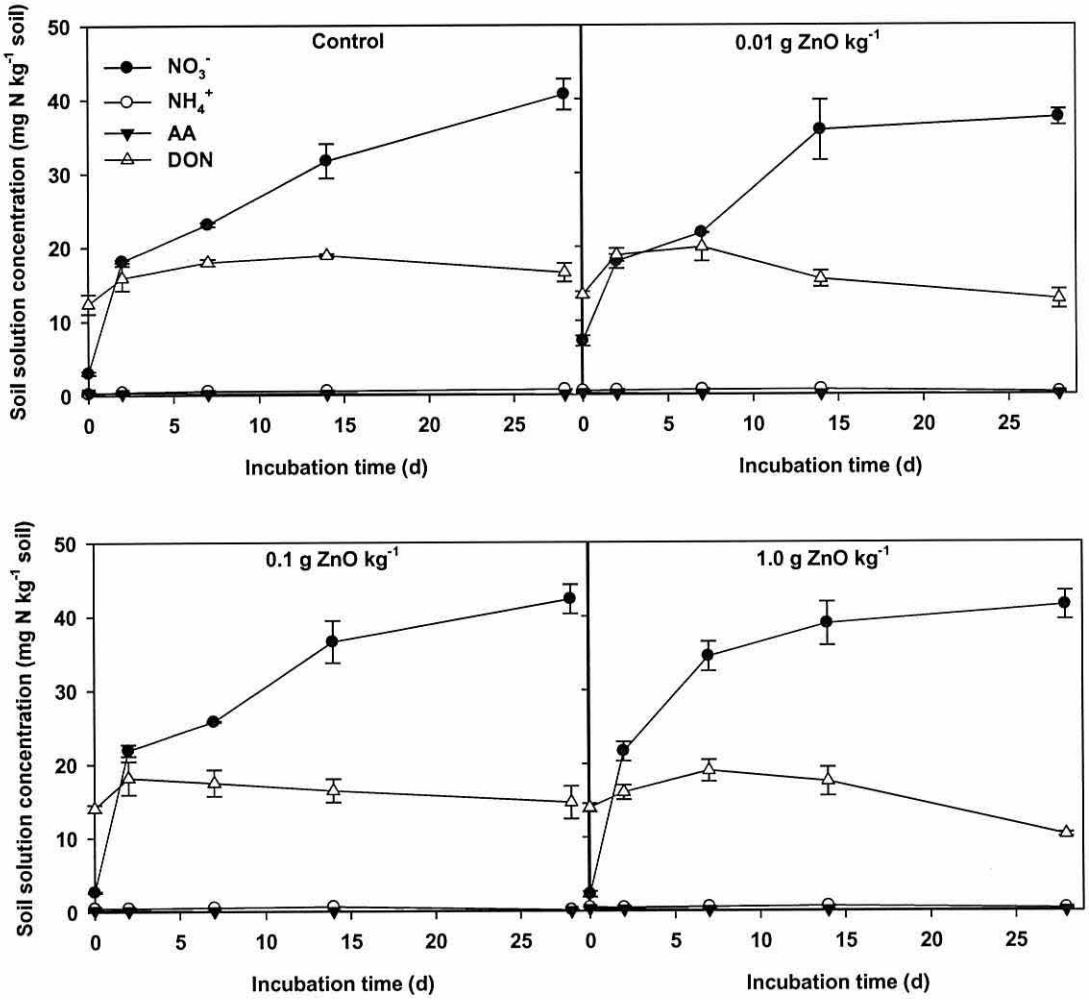

Figure 7. 9. Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) ions, amino acids (AA), and dissolved organic nitrogen (DON) in soil solutions of Sandy soil. Soil was incubated with different concentrations of ZnO NPs (0, 0.01, 0.1, and 1.0 g kg^{-1}) without leaching for 28 days at 10°C. All values represent means ±SEM (n = 3). The concentrations of NH_4^+ and AAs were small in all samples (< 1.0 mg N kg^{-1} soil). Error bars represent one standard error.

Results of statistical analysis showed no significant effects for all treatments of ZnO NPs on the total concentration of NO_3^-, NH_4^+, DON and AA in the Sandy soil (Figure 7.9).

In general, results of statistical analysis (one way ANOVAs) showed that soil type has an influence on the nitrogen content of soils but that NP types and their concentrations has little effect. This is not to say that NPs have effects on soil nitrogen content; it is to assume, however, such effects, at least as regards the NPs used in the present study, any such effects appear broadly the same regardless of NP type. The results of this study agree with the findings of

Jones *et al.*[5] who found the total concentrations of NO_3^-, NH_4^+, DON and AA in Dystric gleysol, Eutric Cambisol, and Haplic podzol soils was similar. However, their findings cover an incubation period of over 56 days without addition NPs to the soils. The present study showed that the total concentration of DON did not increase significantly during the incubation period compared to the total concentration of NO_3^- across all NP or soil types; all nitrogen concentrations stabilized after an initial increase on Day 0. It appears that the tested NPs do not affect the rate of nitrogen mineralization in the tested soils.

7.5.2. Influence of soil types on nitrogen concentration

The results of statistical analysis observed a significant difference of NPs treatments on NO_3^- concentration ($p < 0.05$) and a significant difference between soil types and concentrations ($p < 0.05$). There was a significant effect ($p < 0.001$) on soils type regards NO_3^- concentration. *Post hoc* tests revealed significant differences ($p < 0.001$) for all three soils ($p < 0.001$ in each case) compared to each other. Nitrate concentration was highest in Eutric Cambisol soil (mean = 31.31 mg kg^{-1}; SE = ± 1.34); next highest in Sandy soil (mean = 25.25 mg kg^{-1}; SE = ± 0.94); and lowest in Haplic podzol (mean = 2.26 mg kg^{-1}; SE = ± 0.18).

The results of statistical analysis showed no significant difference of NPs treatments on NH_4^+ concentration and no significant difference between soil types and concentrations. Statistical analysis on soils NH_4^+ concentration observed a significant effect ($p < 0.001$). *Post hoc* tests revealed significant differences for all comparison soils save that between Euric camiposl and Sandy soils. The comparison between Haplic podzol and Eurtic Cambisol soils was significant ($p < 0.001$). That between Haplic podsol and Sandy was significant ($p < 0.001$). NH_4^+ concentration was highest in Haplic podzol soil (mean = 0.57 mg kg^{-1}; SE = ± 0.01); next highest in Sandy soil (mean = 0.50 mg kg^{-1}; SE = ± 0.01); and lowest in Eutric Cambisol soil (mean = 0.50 mg kg^{-1}; SE = ± 0.01).

The results of statistical analysis observed no significant difference of NPs treatments on DON concentration and no significant difference between soil types and concentrations. Statistical analysis on soils DON concentration revealed a significant effect ($p < 0.001$). *Post hoc* tests revealed significant differences for all comparison soils save that between Euric camiposl and Sandy soils. The comparisons between Haplic podzol and Eurtic Cambisol soils and between Haplic podsol and Sandy soil were each significant ($p < 0.001$). Dissolved organic nitrogen

concentration was highest in Sandy soil (mean = 16.0 mg kg^{-1}; SE = ± 0.22); next highest in Eutric Cambisol soil (mean = 15.80 mg kg^{-1}; SE = ± 0.17); and lowest in Haplic podzol soil (mean = 3.03 mg kg^{-1}; SE = ± 0.11).

The results of statistical analysis observed a significant difference of NPs treatments on AA concentration ($p < 0.001$) and a significant difference ($p < 0.001$) between soil types and concentrations. Statistical analysis on soils AA concentration revealed a significant effect ($p < 0.001$). *Post hoc* tests revealed significant differences for all three comparison soils ($p < 0.001$ in each case). Amino acid concentration was highest in Eutric Cambisol soil (mean = 0.43 mg kg^{-1}; SE = ± 0.01); next highest in Sandy soil (mean = 0.26 mg kg^{-1}; SE = ± 0.01); and lowest in Haplic podzol (mean = 0.14 mg kg^{-1}; SE = ± 0.01).

7.5.3. Influence of nanoparticle types on nitrogen concentration

All statistical analysis failed to reveal any significant differences for all NP types on soils N concentrations, save that for AA concentration ($p < 0.01$). The results of analysis observed a significant difference of NPs treatments on AA concentration ($p < 0.001$) and no significant difference between NP types and concentrations. *Post hoc* tests ondicated significant differences between CdS and CuO ($p < 0.01$) and between CuO and ZnO ($p < 0.05$). Soil amino acid concentration was highest in soils inoculated with CuO (mean = 0.31 mg kg^{-1}; SE = ± 0.02); next highest soil inoculated with ZnO (mean = 0.26 mg kg^{-1}; SE = ± 0.02); and lowest in soil inoculated with CdS (mean = 0.25 mg kg^{-1}; SE = ± 0.01).

7.5.4. Influence of incubation times on nitrogen concentration

The results of statistical analysis observed no significant difference of NPs treatments on NO_3^- concentration and no significant difference between incubation times and concentrations. The ANOVA on NO_3^- concentration revealed a significant effect of time after inoculation ($p < 0.001$). *Post hoc* tests indicated significant differences for all comparison times involving Day 0 ($p < 0.001$ in each case); significant differences for all comparisons involving Day 2 ($p < 0.001$ in each case), save that between Day 2 and Day 7 (insignificant); all comparisons involving Day 7 ($p < 0.001$ in each case), save that involving Day 2 (obviously) and all comparisons involving Day 14 ($p < 0.001$ in each case)–that between Day 14 and Day 28 was significant difference ($p < 0.05$); all comparisons involving Day 28 ($p < 0.001$ in each case) – that between Day 28 and Day 14 ($p < 0.05$). Nitrate concentration was highest at Day 28 (mean = 33.02 mg kg^{-1}; SE = ± 1.56),

next highest at Day 14 (mean = 26.30 mg kg^{-1}; SE = ± 1.42); next highest for Day 7 (mean = 19.44 mg kg^{-1}; SE = ± 1.05); next highest for Day 2 (mean = 13.96 mg kg^{-1}; SE = ± 0.80); and least for Day 0 (mean = 5.32 mg kg^{-1}; SE = ± 0.40). These results suggest a broadly linear trend in NO_3^- concentration, with NO_3^- concentration steadily decreasing as a function of time after inoculation.

The results of statistical analysis observed no significant difference of NPs treatments on NH_4^+ concentration and a significant difference ($p < 0.001$) between incubation times and concentrations. There was a significant effect of time after inoculation ($p < 0.001$). *Post hoc* tests showed significant differences for all comparisons save that between Day 0 and Day 28 (insignificant). NH_4^+ concentration was highest at Day 14 (mean = 0.69 mg kg^{-1}; SE = ± 0.01), next highest at Day 7 (mean = 0.59 mg kg^{-1}; SE = ± 0.01); next highest for Day 2 (mean = 0.51 mg kg^{-1}; SE = ± 0.01); next highest for Day 28 (mean = 0.41 mg kg^{-1}; SE = ± 0.02); and least for Day 0 (mean = 0.40 mg kg^{-1}; SE = ± 0.02). These results suggest a broadly quadratic trend in NH_4^+ concentration over time, with NH_4^+ concentration increasing for the first 14 days after inoculation, but thereafter decreasing.

The results of statistical analysis observed no significant difference of NPs treatments on DON concentration and no significant difference between incubation times and concentrations. Statistical analysis on soils DON revealed a significant effect of time after NPs inoculation ($p < 0.001$). *Post hoc* tests revealed significant differences only for the comparison between Day 0 and Day 7 ($p < 0.001$). Dissolved organic nitrogen concentration was highest at Day 7 (mean = 13.0 mg kg^{-1}; SE = ± 0.55), next highest at Day 14 (mean = 12.29 mg kg^{-1}; SE = ± 0.54); next highest for Day 2 (mean = 12.01 mg kg^{-1}; SE = ± 0.47); next highest for Day 28 (mean = 10.61 mg kg^{-1}; SE = ± 0.45); and least for Day 0 (mean = 10.09 mg kg^{-1}; SE = ± 0.36). These results suggest a broadly quadratic trend in dissolved organic concentration over time, with dissolved organic nitrogen concentration increasing for the first 14 days after inoculation, but thereafter decreasing.

The results of statistical analysis observed a significant difference of NPs treatments on AA concentration ($p < 0.001$) and no significant difference between incubation times and concentrations. Statistical analysis on soils AA concentration revealed a significant effect of time after inoculation ($p < 0.001$). *Post hoc* tests revealed significant differences for all comparisons

involving Day 0 ($p < 0.001$ in each case), save that between Day 0 and Day 2 (insignificant); significant differences between Day 2 and Day 14 ($p < 0.05$) and between Day 2 and Day 28 ($p < 0.001$); a significant difference between Day 7 and Day 0 ($p < 0.001$) and between Day 7 and Day 28 ($p < 0.05$); significant differences between Day 14 and Day 0 ($p < 0.001$) and between Day 14 and Day 2 ($p < 0.05$); and significant differences comparisons involving Day 28 ($p < 0.001$), save that between Day 28 and Day 14 (insignificant). Amino acid concentration was highest at Day 0 (mean = 0.37 mg kg^{-1}; SE = ± 0.02), next highest at Day 2 (mean = 0.32 mg kg^{-1}; SE = ± 0.01); next highest for Day 7 (mean = 0.27 mg kg^{-1}; SE = ± 0.02); next highest for Day 14 (mean = 0.24 mg kg^{-1}; SE = ± 0.01); and least for Day 28 (mean = 0.19 mg kg^{-1}; SE = ± 0.01). These results suggest a broadly linear trend between amino acid concentration and time after inoculation, with amino acid concentration decreasing as a function of time after inoculation.

7.5.5. Influence of different NP concentrations on nitrogen concentration

The only statistical analysis to reveal a significant effect of concentration of inoculation was that involving amino acids concentration ($p < 0.001$). *Post hoc* tests revealed significant diferences only between 0 and 0.01 g kg^{-1} ($p < 0.001$) and between 0.01and 1.0 mg kg^{-1} ($p < 0.05$). Amino acid concentration was highest for the 0.01 g kg^{-1} level of inoculation (mean = 0.33 mg kg^{-1}; SE = ± 0.01), next highest for 0.1 g kg^{-1} (mean = 0.28 mg kg^{-1}; SE = ± 0.001); next highest for 1.0 g kg^{-1} (mean = 0.27 mg kg^{-1}; SE = ± 0.01); and least for 0 g kg^{-1} (mean = 0.23 mg kg^{-1}; SE = ± 0. 0.01). These results suggest a broadly quadratic trend in amino acid concentration as a function of concentration of inoculation, with amino acid concentration increasing with a slight increase in concentration of inoculation, but progressively decreasing with larger concentrations of inoculation.

Figures 7.1–7.9 show that, at the start of time collocation (Day 0), the majority of dissolved organic nitrogen (DON) was the main component of the soil's nitrogen pool for all NP concentrations and soil types. This agrees with results by Jones *et al.*[5] who found that DON to be the major component of the nitrogen pool in Dystric gleysol, Eutric Cambisol, and Haplic podzol soils–this over an incubation period of 56 days without additive NPs.

Results of the present study, however, suggest a quadratic trend for DON over time, soils DON concentration first increasing but thereafter decreasing, and this appeared differently for Eutric

Cambisol soil treated with CdS and CuO NPs at concentrations of 0.1 and 1.0 g kg^{-1}, in this case the concentration of DON was not the main component of nitrogen pool (see Figures 7.2 and 7. 5). Soil pH could be relevant. Low soil pH increases the availability of Cd^{2+} and Cu^{2+} ions and decreases microbial activity.

Collins *et al.*[1] have argued that Cu NPs and ZnO NPs changes the soil microbial community's structure; the authors used two cultures (dependent and independent) over 162 days. Their results suggetsted *Flavobacteriales* spp and *Sphingomonadales* spp in rhizosphere are sensitive to Cu and ZnO NPs. Ellis has reported that iron oxide and dysprosium oxide NPs have significant negative effects on mineralisation rates in Eutric Cambisol soil at 0.1 g kg^{-1}.[16] Results of the present study suggest that the concentration of DON is approximately the same for all three soils–concentration ranged from 1.3 to 12 mg of N kg^{-1} (Figures 7.1–7.9). This result agrees with that obtained by Jones *et al.*[5] These researchers, however, used untreated Eutric Cambisol and Haplic podzol soils.

Results of DON analysis in Eutric Cambisol and Sandy soils indicated that CdS, CuO, and ZnO have little to no adverse effect (the results were all insignificant) on DON concentrations for any of the applied concentrations. Results as they stand results agree with those found by Ellis.[16]

By contrast, in the present study, analysis of Haplic podzol soil suggests that CdS NPs has a significant effect ($p < 0.001$) on DON concentration at a level of 0.1 g kg^{-1} (Figure 7.1). CuO NPs had a significant effect ($p < 0.001$) on the concentration of DON at 1.0 g kg^{-1} in Haplic podzol soil (Figure 7.4). The effect of CdS and CuO on soil DON was thus clear in Haplic podzol soil. This could be related to the low content of organic matter; this decreased the binding sites for these NPs and plausibly resulted in increased toxicity to soil microbes. In this regard, previous research suggests that fulvic and humic acids bind to MNPs.[18,19] In addition, French *et al.*[20] and Franklin *et al.*[21] have suggested that a moderately acid pH, ionic strenght, and the solubility of TiO_2 and ZnO NPs may play significant roles in their aggregation and availability and in aqueous suspensions.

Results of the present study suggest significant differences ($p < 0.001$) for the majority of incubation times on DON concentration across all NPs treatments and soil types. Incubation time, however, appeared to show no significant difference to the concentration of DON in Haplic podzol soil when the soil was treated with CdS and CuO NPs.

A comparison (two–way ANOVA) of the total concentration of DON in the three test soils shows significant differences ($p < 0.001$) between Haplic podzol and the Eutric Cambisol soils and there was a significant difference ($p < 0.001$) between Haplic podzol and Sandy soils, but no significant difference between Eutric Cambisol and Sandy soils. By contrast, the total concentration of nitrate ions appeared dependent on incubation time and soil type: the NO_3^- concentration varied from 0.8 to 76.2 mg kg^{-1} (Figures 7.1–7.9). The majority of ammonium ions and free amino acid concentrations, however, were, less than 1 mg of N kg^{-1} for any incubation time, added NP and soil type. These results broadly agree with those of Jones *et al.*[5]

7.5.6. Dynamics of inorganic nitrogen mineralization

The majority of nitrate ion concentrations in the three tested soils gradually increased over the 28 days of incubation; this was across all NP concentrations (Figures 7.1–7.9). The concentration of NO_3^- reduced in Haplic podzol soil when treated with CuO NPs at 1.0 g kg^{-1}; this was in contrast to NO_3^- concentration in Eutric Cambisol and Sandy soil (Figure 7.4). This is plausibly because the physiochemical properties of Haplic podzol soil play an important role on metal behaviour, especially that of CuO in Haplic podzol soil.[18,21] In addition, the smaller size of CuO NPs (40–80 nm) compared with that of the other two nanomaterials increases their surface reactivity with the soil, with subsequent increase of toxicity to the performance of the microbial biomass.[22] Choi *et al.*[23] have indicated that soil bacteria are affected by silver NPs. These NPs are toxic to soil bacteria; they also affect nitrification. Results of the present study suggest the trend in nitrate ion concentration was approximately linear (increasing over time and the NP concentrations); this was in Eutric Cambisol and Sandy soils as shown in Figures 7.2–7.9. This agrees with results obtained by Jones *et al.*[5] In the present study, however, nitrate ion concentrations dramatically increased by incubation day 14 in Haplic podzol soil for all NP concentrations. This increase in nitrate (NO_3^-) concentration could be related to the distinct properties of soil and vegetation in Haplic podzol soil when compared with those of the other two soils.[24,25]

Some research suggests that the production of inorganic nitrogen in any soil is regulated by several factors–thus the rate of plant residue decomposition, interaction between inorganic nitrogen and decomposer communities, the availability of soluble forms of nitrogen, and environmental conditions all play important roles in controlling of the nitrogen cycle.[14] Results of the present study suggest that, in general, the rate of increase of inorganic nitrogen

mineralisation in Eutric Cambisol soil was similar to that of Sandy soil; however, the rate of conversion of nitrate in Haplic podzol soil appeared slower. This result plausibly relates to the properties of Haplic podzol soil, as discussed above. This result agrees with that of Jones *et al.*[5] who found similar behaviour in Haplic podzol soil in relative to day zero incubation time for the control samples (i.e. soil without NP inoculation). In the present study, the concentration of nitrate ions increased 25–fold in Eutric Cambisol soil, 13–fold in Sandy soil, and 10–fold in Haplic podzol soil. Jones *et al.*[5] have suggested that a threshold for organic nitrogen is established within the first two weeks in Haplic podzol soil, and therefore results in a low nitrate accumulation because of net immobilization – after 14 days, the nitrogen limitation may be slowly removed to allow nitrate concentration to increase in the soil's solution.

The present study, however, found that the concentration of ammonium ions and free amino acids remained at a low level (< 1.0 mg kg^{-1}) when compared to DON and nitrate concentrations in soil solution throughout the 28 days of incubation; this was for all NP concentrations and soil types (Figures 7.1–7.9). In contrast, nitrate ions regularly concentrated in the tested soil, which agrees with results of Jones *et al.*[5] who suggested that the level of nitrate increase was due to lack of microbial demand and available carbon; there was absence of roots in their experiments.

The majority of NH_4^+ concentrations in three soils increased regularly during the first 14 days of incubation approximately 2–fold when compared to concentrations at Day 0. By contrast, ammonium ion concentrations gradually decreased after the first 14 days of incubation–this by about 1.5–fold for all metal NP concentrations and soil types; however, this concentration could not be sustained over 28 days of incubation. The most ammonium ion concentrations reduced at high concentrations of NP treatments across the different soil types. Thus the trend appeared quadratic.

7.6. Impact of NPs on soil respiration for in the short term

Results of statistical analysis observed no significant effects on soil respiration for the majority of tested NPs across all the applied concentrations and soil types. However, there is a significant positive effect ($p < 0.05$) of CdS NPs on respiration of Haplic podzol soil at a concentration of 0.01 g kg^{-1} (see Figure 7.10). However, the respiration rate of Haplic podzol soil was deacreased at the higest concentrations of CdS NPs (0.1 and 1.0 g kg^{-1}). As indicated, this soil's properties appear to play an important role in increasing NPs toxicity to the soil microbial community. It is

therefore possible that Cd is a toxic metal with unknown biological function.[26] By contrast, Cu and ZnO NPs appeared to have no significant effects on respiration rate in Haplic podzol soil. By contrast, there appeared no significant effects on Sandy and Eutric Cambisol soil respiration as regards any of the three NP types (Figure 7.10). This agrees with results of Ellis.[16]

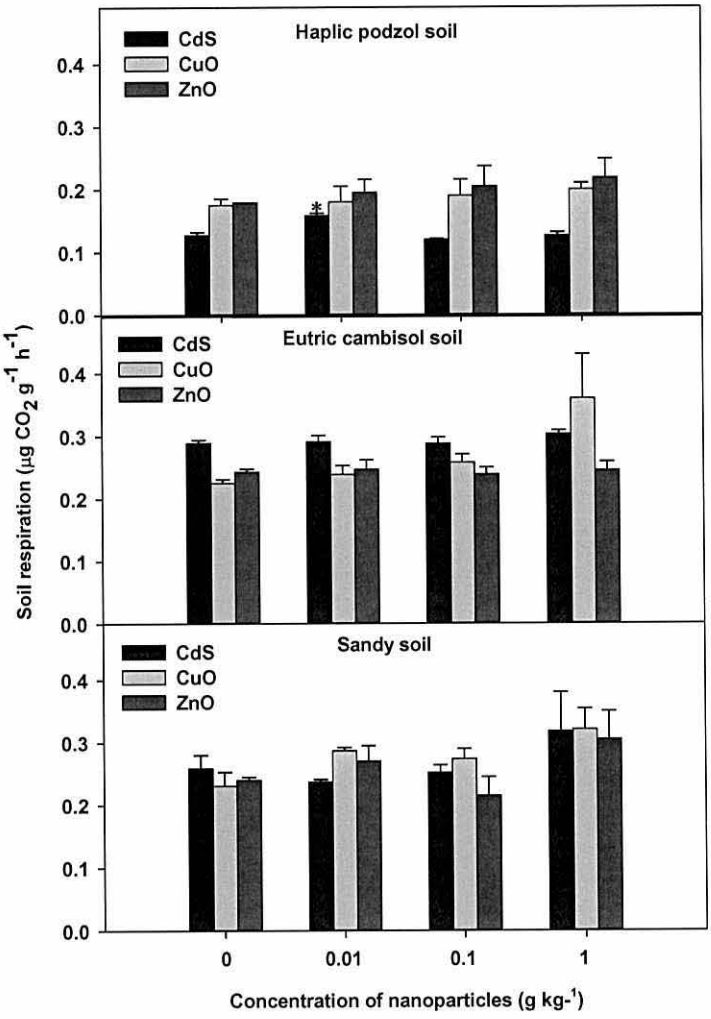

Figure 7. 10. The short term effects on soil respiration in Haplic podzol, Eutric Cambisol, and Sandy soils. Effects were measured after the addition of three concentrations of metal NP (CdS, CuO, and ZnO). (Mean ±SEM, n =3).

7.6.1. The effect of soil types on respiration rates

The results of statistical analysis observed no significant difference of NP concentrations on soil respiration ($p < 0.001$) and no significant difference between soil types and concentrations ($p < 0.05$). There was a significant effect ($p < 0.001$) on soils type regards soil respiration. *Post hoc*

tests revealed significant differences ($p < 0.001$) for all three soils ($p < 0.001$ in each case) compared to each other, save that between Euric Camiposl and Sandy soils (insignificant). Soil respiration was highest in Eutric Cambisol soil (mean = 0.27 μg CO_2 g^{-1} h^{-1}; SE = ± 0.01); next highest in Sandy soil (mean = 0.27 μg CO_2 g^{-1} h^{-1}; SE = ± 0.01); and lowest in Haplic podzol (mean = 0.17 μg CO_2 g^{-1} h^{-1}; SE = ± 0.01).

7.6.2. The effect of NP types on respiration rates

The results of two–ANOVA revealed no significant difference of concentration on soil respiration and no significant difference between NP types and concentrations. The results of statistical analysis showed no significant effect of NPs on soil respiration. Results of the present study suggest that the majority of soil respiration rates increased slightly with increasing concentrations of nanopraticles. This agrees with results of Hansch and Emmerling,[9] who observed similar results for sliver NPs in Sandy loam soil; their results suggested that soil respiration increases with increased silver concentration in the soil. A study by Fliesbach *et al.*[27] obtained similar results for soil that has been treated with sewage sludge containing different concentrations of Cu, Zn, Ni, and Cd as bulk materials.

Results of the present study, however, suggest that CdS decreases the respiration rate in Haplic podzol soil at concentrations of 0.1 and 1.0 g kg^{-1} compared with that of control samples. The negative effects of CdS can be related to the properties of this soil (see Section 7.6.1). The bioavailability of Cd depends on such factors as soil type, pH, and redox conditions. These factors are thought to play significant roles in increasing the toxicity of heavy metals to soil microbial communities.[26] Results of the present study suggest that respiration in Haplic podzol soil was lower than in the other two soils. This could be due to the relatively low content of organic material in Haplic podzol soil (see Table 7.1).

Results of the present study suggest no significant effects on soil respiration as regards the majority of tested NPs across all concentrations and soil types. This agrees with results of Ellis.[16] who reported that the majority of twelve metal oxide MNPs, including ZnO and CuO, do not appear to significantly affect respiration rates in Eutric Cambisol, Haplic podzol, and Sandy soils for concentrations of 0.01, 0.1 and 1.0 g kg^{-1}. Ellis used two doses of ^{14} C–U– glucose to increase soil respiration and thereby to clarify soil respiration processes. The soil samples were incubated for 56 days at 21°C. Similar results were obtained by Tong *et al.*[28] who evaluated the

toxicity of n–C_{60} in aqueous suspension in granular shape on soil microorganisms by analyzing soil respiration. These researchers suggested that the findings could be related to the strong binding of carbon NPs to organic matter within the soil matrix.

7.7. Conclusion

The results of present study indicated that, the concentration of NO_3^- accumulated readily in three soils. However, NH_4^+, DON and free amino acids concentrations show low levels of accumulation across all of soil and NP types. This revealed that they do not limit the rate of nitrogen mineralization. The large majority of DON was the main component of nitrogen pool in the treated soils at day 0. DON concentration did not increase significantly over the 28 days across all NP or soil types; all concentrations appeared steady when compared with those at Day 0.

The results of one way ANOVAs and *Post hoc* tests showed that no significant differences exist for the majority of NPs on NO_3^- and NH_4^+ concentrations across all soil types, CuO, however, appeared to have a negative effect on NO_3^- concentration at a treatment of 0.01 g kg^{-1} in the Eutric Cambisol soil when compared with the control groups. The results from Haplic podzol soil suggest that CdS NPs has a significant effect on DON concentration at a treatment of 0.1 g kg^{-1}. CuO NPs had a significant effect on the concentration of DON at 1.0 g kg^{-1} in Haplic podzol soil.

CdS also appeared to have had a negative effect on the concentration of free amino acids (AAs) at a treatment of 0.01 and 0.1 g kg^{-1} in Haplic podzol soil compared with control samples. CdS NPs appeared to have a significant effect on AAs concentration at a CdS level of 0.1g kg^{-1} in Eutric Cambisol soil compared with controls. CuO appeared to have analogous significant effects on the concentration of AA at concentrations of 0.01 and 1.0 g kg^{-1} in the Eutric Cambisol soil compared with that of control samples. ZnO revealed a significant effect on AAs concentration at a treatment of 0.01 g kg^{-1} compared with controls. CuO appeared to significantly affect AA concentrations at 0.1 g kg^{-1} in Sandy soil compared with the control.

The rate of nitrogen mineralization varied widely across all soils under any of the NP levels. The results of soil comparisons (two way ANOVA) as regards the nitrogen mineralization suggest that the concentration of NO_3^- and AAs are highest in Eutric Cambisol soil followed by Sandy soil and Haplic podzol was the lowest. The concentration of NH_4^+ was highest in Haplic podzol soil, next highest in Sandy soil and lowest in Eutric Cambisol soil. Furthermore, the concentration of DON was highest in Sandy soil followed by Eutric Cambisol soil; Haplic podzol soil was the lowest.

The results of NPs comparison suggest that the large majority of NPs failed to reveal any significant effect upon nitrogen mineralization under any of the NP concentrations when using two way ANOV and *Post hoc* tests, save that for AAs concentration. Soil amino acids concentration was highest in soils treated with CuO followed by soil treated with ZnO; soil treated with CdS was the lowest. The results of time incubation comparison suggest that there is a significant effect of time on the majority of nitrogen mineralization across all soil and NP types.

The comparison results of NP concentrations suggest that the only a significant effect of NP concentrations is that involving the levels of amino acid, Amino acid concentration was highest for the 0.01 g kg^{-1} level of treatment; next highest for 0.1 g kg^{-1}; next highest for 1.0 g kg^{-1}; and least for 0 g kg^{-1}. The results of soil respiration indicated no significant effects of the majority of tested NPs on soil respiration across all NP concentrations and soil types when using a one way ANOVA and *Post hoc* test. However, CdS NPs shows a significant positive effect on respiration of Haplic podzol soil at a treatment of 0.01 g kg^{-1}. The results of soils comparison (two way ANOVs) as regards the respiration rate suggest that there are significant differences between all soils. Soil respiration was highest in Eutric Cambisol soil followed by Sandy soil and Haplic podzol soil is the lowest. The comparison of NP types show that no significant effect of NPs on soil respiration across any of soil types and concentration levels. The lack of soils response to the NPs concentrations for all three soil types indicates that during the short incubation time (48 hours) there was limited nitrogen and carbon cycling. Further work is needed to fully quantify the influence of these NPs on three tested soils for long periods.

7.8. References

1. Collins, D., Luxton, T., Kumar, N., Shah, S., Walker, V. K., and Shah, V. (2012). Assessing the impact of copper and ZnO nanoparticles on soil: a field study. *Plos One.* 7 (8).

2. Lead, J. R., and Smith, E. (2009). Environmental and human health impacts of nanotechnology. *Chichester*, U.K.: Wiley.

3. Hill, G., Mitkowski, N., Aldrich–Wolfe, L., Emele, L., Jurkonie, D., Ficke, A., Maldonado–Ramirez, S., Lynch, S.T., and Nelson, E.B. (2000). Methods for assessing the composition and diversity of soil microbial communities. *Applied Soil Ecology.* 15 (1): 25–36.

4. Colwell, R. R. (1997). Microbial diversity: the importance of exploration and conservation. *Journal of Industrial Microbiology and Biotechnology.* 18 (5): 302–307.

5. Jones, D. L., Shannon, D., Murphy, D. V., and Farrar, J. (2004). Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. *Soil Biology and Biochemistry.* 36 (5): 749–756.

6. Lambers, H., Chapin, S.F., and Pons, T., (1998). Plant physiological ecology. *Springer*, New York.

7. Kumar, N., Shah, V., and Walker, V. K. (2011). Perturbation of an arctic soil microbial community by metal nanoparticles. *Journal of Hazardous Materials.* 190 (1–3): 816–822.

8. Kumar, N., Shah, V., and Walker, V. K. (2012). Influence of a nanoparticle mixture on an arctic soil community. *Environmental Toxicology and Chemistry.* 31 (1): 131–5.

9. Hansch, M, and Emmerling, C. (2010). Effects of silver nanoparticles on the microbiota and enzyme activity in soil. *Journal of Plant Nutrition and Soil Science*.173 (4): 554–558.

10. Jarvis, S.C., Stockdale, E.A., Shepherd, M.A., and Powlson, D.S., (1996). Nitrogen mineralisation in temperate agricultural soils: processes and measurement. *Advances in Agronomy*. 57: 187–235.

11. Murphy, D.V., Recous, S., Stockdale, E.A., Fillery, I.R.P., Jensen, L.S., Hatch, D.J., and Goulding, K.W.T. (2003). Gross nitrogen fluxes in soil: theory, measurement and application of ^{15}N pool dilution techniques. Advances in Agronomy. 79: 69–118.

12. Jones, D.L., and Kielland, K. (2002). Soil amino acid turnover dominates the nitrogen flux in permafrost–dominated taiga forest soils. *Soil Biology and Biochemistry*. 34 (2): 209–219.

13. Jones, D.L., Willett, V.B., Stockdale, E. A., Macdonald, A.J., and Murphy D.V. (2012). Molecular weight of dissolved organic carbon, nitrogen, and phenolics in grassland soils. *Soil Science Society of America Journal.* 76 (1): 142–150.

14. Tate, R.L. (2000). Soil Microbiology. *Wiley*, New York.

15. Handayanto, E., Cadisch, G., and Giller, K.E. (1995). Manipulation of quality and mineralisation of tropical legume tree prunings by varying nitrogen supply. *Plant and Soil.* 176: 149–160.

16. Ellis.S.C. (2011). Ecotoxicology of metal oxide nanoparticles: Effects on soil process and plant growth. University of Wales, Bangor, *Master thesis*, pp: 24–40.

17. Jones, D.L., Owen, A.G., and Farrar, J.F. (2002). Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. *Soil Biology and Biochemistry.* 34 (12): 1893–1902.

18. Domingos, R.F., Tufenkil, N. and Wilkinson, K. (2008). Aggregation of Titanium Dioxide Nanoparticles: Role of a Fulvic Acid. *Environmental Science and Technology.* 43(5). 1282–1286.

19. Ghosh, .S.G., Mashayekhi, H., Pan, B., Bhowmik, P., and Xing, B. (2008). Colloidal Behaviour of Aluminium Oxide Nanoparticles as Affected by pH and Natural Organic Matter. *Langmiur.*24 (21): 12385–12391.

20. French, R.A., Jacobson, A.R., Kim, B., Isley, S.L., Penn, R.L. and Baveye, P.C. (2009). Influence of Ionic Strength, pH and Cation Valence on Aggregation Kinetics of Titanium Dioxide MPs. *Environmental Science and Technology.* 43 (5): 1354–1359.

21.Franklin, N.M., Rogers, N.J., Apte, S.C., Batley, G.E., Gadd, G.E., and Casey, P.S. (2007). Comparative toxicity of nanoparticulate ZnO, bulk ZnO and $ZnCl_2$ to a freshwater microalga (*Pseudokirchnerilla subcapitata*): the importance of particle solubility. *Environmental Science and Technology.* 41(24): 8484–8490.

22. Batley, G. E., and McLaughlin, M. J. (2010). Fate of Manufactured Nanomaterials in the Australian Environment. CSIRO niche Manufacturing flagship Report. The Department of the Environment, Water, Heritage and the Arts.pp:1–58.

23. Choi, O., Deng, K.K., Kim, N.J., Ross, L., Surampalli, R.Y., and Hu, Z. (2008). The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Research.* 42 (12): 3066–3074.

24. Pranagal, J., Podstawka–Chmielewska, E., and Slowinska–Jurkiewicz, A. (2007). Influence on selected physical properties of a Haplic podzol during a ten–year fallow period. *Polish journal of environmental studies.* 16: 875–880.

25. Uwah, E.I., Abah, J., Ndahi, N.P., and Ogugbuaja, V.O. (2009). Concentration levels of nitrate and nitrite in soils and some leafy vegetables obtained in Maiduguri, Nigeria. *Journal of Applied Sciences in Environmental Sanitation.* 4 (3): 233–244.

26. Lazzaro, A. (2008). Impact of zinc and cadmium on the microbial community in soils. ETH Zurich. *Science and Technology University* (Switzerland). pp: 1–21.
scee2012.ethz.ch/abstracts_new/SCEE12_Abstract_51_talk_Bandlow.pdf

27. Fliesbach, A., Martens, R., and Reber, H. (1994). Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. *Soil Biology and Biochemistry.* 26 (9): 1201–1205.

28. Tong, Z.H., Bischoff, M., Nies, L., Applegate, B., and Turco, R.F. (2007). Impact of fullerene (C–60) on a soil microbial community. *Environmental Science and Technology*. 41 (8): 2985–2991.

Chapter 8. General conclusions and future work

This study sought to provide some insight into the uptake and partitioning of different nanoparticles in the maize plant grown in Eutric Cambisol soil and in hydroponic culture, with particular focus on their effects on the maize growth parameters over defined period (21 days). It also sought to examine the adsorption and desorption of these materials on surface of Eutric Cambisol (clay loam), Haplic podzol (loamy sandy), Sandy and Libyan sandy soil. In addition, the effect of nanoparticles on the rate of nitrogen mineralization was tested in agricultural Eutric Cambisol, Haplic podzol and Sandy soil.

The samples of Eutric Cambisol, Haplic podzol and Sandy soils have been exposed to a coastal climate, North Wales. However, Libyan sandy soil is found in the climate range from arid to semi–arid. Contrasting soil types were selected because they have different physical and chemical properties which were used to test the behaviour and influence of CdS, ZnO and CuO NPs. Eutric Cambisol soil was also chosen to grow maize because the majority of NPs experiments were performed during the germination stage or in hydroponic culture. Maize was used because it is an important cereal crop and is adaptable over a range of agricultural climatic zones.

The pots used (84 pots) were classified into four main groups (21 pots for each main group) in first experiment (Chapter 4). In second experiment (Chapter 4), the Petri dishes (42 Petri dishes) were divided into two main groups (21 Petri dishes for each main group). Furthermore, the pots (72 pots) were divided into six main groups (12 pots for each main group) in the Chapter 5 experiment. For the hydroponic cultures, maize seedlings were planted in plastic pot (9.0 cm diameter, 14.0 cm depth, capacity 570 mL). These pots were painted by black spray to avoid the exposure of light to the maize roots. Completely block randomized design was used in the Chapter 4 and Chapter 5 experiments. All the statistical analyses of variances (one and two way ANOVA) were applicable to identify the significant differences between and within measurements compared to the control samples (reference). The investigation consisted of two main parts in Chapter 4. In the first part uptake of nanoparticles and their bulk materials was examined in maize plants grown in Eutric Cambisol soil. It has been possible to determine the concentration of all metals in plant parts (roots and shoot) and soil after 21 days. The results of Chapter 4 indicated that the concentration (mg kg^{-1}) of assimilated Cd and Zn increased in maize

roots and shoots following initial treatments with increasing concentrations of either NPs or bulk corresponding materials in the Eutric Cambisol soil. The absorption of Cd and Zn was accumulated in roots more than in shoots across all chemical compounds as shown in Figure 4.8 and Figure 4.12. The concentration of bulk Cd and Zn was higher than NP materials in the maize roots and shoots. The low concentration of Cd found in maize roots and shoots could be related to their low levels in the soil, and it is possible that aggregation and the presence of NOM strongly influenced their bioavailability within the Eutric Cambisol soil (Section 4.10). However, the concentrations of Zn in the maize roots and shoots following treatments of soil with either bulk or nanoparticulate Zn sources were higher compared with initial Zn treatments. This could be attributed to the residual effect of the fertilizers applied to the soil during sample collection; fertilizers might increase the Zn concentration in maize parts (roots and shoots). On the other hand, the results of Chapter 5 showed that the absorption of Cd, Cu and Zn NPs greatly increased in the maize roots and shoots for both soil and hydroponic culture (Figure 5.8 and Figure 5.12). The results indicated that the content of NPs in maize roots and shoots increased with increasing concentrations of the NPs in the growth mediums. The concentration (mg kg^{-1}) of CdS, CuO and ZnO NPs also showed a similar trend of accumulation behaviour in maize roots and shoots grown in Eutric Cambisol soil and nutrient solution. These results agreed with those of Chapter 4. Furthermore, the concentration of Zn NPs in roots and maize shoots was higher than Cu NPs and the concentration of Cd NPs was the lowest in the maize root and shoots.

The results of second part in Chapter 4 showed that, the concentrations of Cd and Zn NPs increased in maize seeds and roots with increased the levels of either CdS (0–100 mg L^{-1}) or ZnO NPs (0–1000 mg L^{-1}) in the suspension solution (Figure 4.17 and Figure 4.18). The accumulation of Cd and Zn NPs in maize roots was higher than that of seeds for all the applied concentrations. The concentration of Zn NPs in maize seeds and roots was higher than that of Cd NPs at any of the additional concentrations.

The effect of metals on the length and dry biomass of maize roots and shoots was reported across all growth mediums, metals types and additional concentrations. The majority of the nanoparticles and their bulk compounds investigated did not significantly impact upon maize growth parameters; however, both Cd compounds (CdS and $CdCl_2$) had negative effects on the length of maize roots and shoots at the highest metal soil ratios (Figure 4.9). Thus, confirming that toxicity of Cd on maize plants. CdS NPs had negative effects on the length of maize roots

more than those found at bulk Cd across the majority of Cd concentrations. This can be related to the small size (6–10 nm) of CdS NPs being more reactivity phases with maize roots than that of their bulk counterparts. In addition, ZnO NPs also had a negative impact on the length of maize shoots at the highest concentration (Figure 4.13). However, ZnO NPs had no negative effects on the length and dry biomass of maize roots and shoots grown in the high concentrations of ZnO NPs in soil and hydroponic culture (0–1.0 g kg^{-1}) as shown in Figures 5.9, 5.10, 5.13 and 4.14. However, ZnO NPs reduced the growth of maize shoots at the concentrations of 1.25 mg kg^{-1} (Chapter 4). This could be related to the low agglomeration of ZnO NPs at low levels (0–1.25 mg kg^{-1}), resulting in more NPs and metal ions available for plant uptake. An increasing Zn NPs availability affects the maize growth. As previously reported in the literature review that uptake of Zn can be driven by the agglomeration, concentration and solubility of the ZnO NPs in the media. ZnO NPs showed an agglomeration resulting in floc formation with increasing the concentration irrespective of particle size. Although Zn is an essential element for plant development and growth, however, above a certain concentration Zn becomes toxic, causing plants to decrease their biomass or to activate defence mechanisms (Section 411.1). These results contradicted those of Chapter 5, which show that the majority of CdS, CuO and ZnO concentrations had no negative effects on maize parameters in both soil and hydroponic culture; however, CuO and CdS NPs appeared to reduce the length of maize roots and shoots at the highest concentration of both cultures (Figure 5.9 and Figure 5.13). Moreover, the dry weight of maize shoots was significantly inhabited by CdS NPs at the highest concentration of hydroponic culture (Figure 5.14). Moreover, the results of second part (Chapter 4) showed that, CdS and ZnO NPs have negative effects on the length and dry weight of maize roots in the highest concentration (Figures 4.19–4.22). CdS NPs showed a significant inhibition on the elongation and dry weight of roots greater than ZnO NPs at any of the additional concentrations. Seed germination was inhibited by the suspension of ZnO NP more than that of CdS. It is difficult to clarify whether the phytotoxicity of these NPs caused by NPs compounds or from the dissolved ions in both maize mediums (soil and nutrient solution), which warrant further research.

The conclusions above, in addition to previous knowledge on NPs toxicity, suggests that the main factors influence toxicity in food crops appear to be as follows: (1) concentration of NPs; (2) particle size; (3) physicochemical properties of NPs; (4) plant species and plant age; (5) growth media (soil and hydroponic); and (6) stability of NPs and the dilution agent as described

in Section 2.15. In general, CdS, CuO and ZnO NPs had reduced the parameters of maize roots and shoots grown in hydroponic culture more than those of the soil culture. This is not surprising given that maize roots interact with NPs directly.

The total concentrations of all metals in the Eutric Cambisol soil increased with increases in the levels of all metals. The total concentration of Cd NPs and Zn NPs was higher than bulk Cd and Zn when the Eutric Cambisol soil treated with nanoparticles and bulk compounds (Figure 4.11, Figure 4.15 and Figure 5.11). The same results were found when the soil was treated with high concentrations of CdS, CuO and ZnO NPs. The total concentrations of Cd NPs in the soil increased more than those of Cu and Zn NPs. This can be attributed to the former's low solubility and precipitation, and the aggregation of NPs compared with that of bulk materials. Further testing of NPs availability in Eutric Cambisol soil is required before undertaking any maize experimentation. However, little is known about the fate of NPs in soil, NPs are small enough to travel through soil pores. They can be adsorbed to soil particles due to their high surface area and, therefore, become immobile. In reality, it is often stated that it is very difficult to determine or measure the NPs in environmental samples. Hence, there are many advanced analytical methods (e.g. dispersion, stability, soil simulation, sediment simulation, hydrolysis and bioaccumulation potential test) that are required but, as importantly, sampling, handling, storage protocols, extraction and digestion methods also needed to optimize and validate the results.

The majority of calculated BCR, uptake and uptake % increased in the roots and maize shoots with increased in the concentration rate for nanoparticles and bulk compounds when the Eutric Cambisol soil was treated with low concentrations (0–1.25 mg kg^{-1}) (Tables 4.3 and 4.7). In addition, the results of these parameters were higher in maize roots than those found in the shoots across all the applied concentrations and metal types. These calculated parameters of soluble bulk counterparts in roots and maize shoots were higher than that of nanoparticles. These results agreed with those found in soil and hydroponic culture (Tables 5.5 and 5.6). The BCR of ZnO was the highest, followed by that of CuO; the BCR of CdS was the lowest in both soil and hydroponic culture. The tolerance index (TI) of maize roots and shoots appeared to decrease with increasing concentrations of all metals for the majority of growth mediums and metal types (Tables 4.4, 4.8, 5.7, and 5.8). However, the TI of maize roots for Zn NPs slightly increased across all of the ZnO NP concentrations. The TI of maize shoots was higher than TI of roots in

the most concentrations across all compounds and growth media. The tolerance index of roots and maize shoots for bulk Cd was higher than that of Cd NPs. However, maize roots showed a high tolerance for Zn NPs in the most used concentrations compared to those for bulk Zn. On the other hand the results of soil and nutrient solution culture showed that the TIs of all NPs in maize roots were higher than that of maize shoots in most concentrations under study. The TIs of Zn NPs in root and maize shoots was higher than the TIs of Cu and Cd NPs respectively. The majority of RI (%) and AE indicators were decreased with increased levels of metal concentration. These calculated parameters showed negative values across all growth medias and metal types (Tables 4.4, 4.8, 5.7, and 5.8). This may have been because the dry biomass of treated plant parts (roots and shoots) was reduced by the different compounds of these metals compared to control samples.

Adsorption and desorption reactions on the surfaces of soils are factors that control the concentration of heavy metals and their availability in soil solutions for plants and soil microorganisms. Thus, the potential toxicity of heavy metals in a soil may mostly depend on the composition of the soil's solids, particularly the amounts and types of clay minerals and organic matter. The bio–availability and mobility of heavy metals in soils appear mainly to be a function of their physicochemical forms (Section 6.1).

The results of soil adsorption indicated that the adsorption of CdS, CuO and ZnO NPs increased with increasing NP concentrations across all soil types (Figures 6.3–6.5), the adsorption of Cu appeared highest, followed by that of Cd; the adsorption of Zn NPs was the lowest across all soil types and NP concentrations (Fgure 6.1). The highest adsorption was of Libyan sandy soil, followed by decreasing levels of Eutric Cambisol soil, Sandy texured soil and Haplic podzol soil respectively (Figure 6.2). The Freundlich equation well represented the adsorption data compared to Langmuir equation (Figure 6.9). The kinetic behaviour of all NPs towards soils showed that pseudo–second order provided better correlations rather than pseudo–first order kinetics (Tables 6.4–6.7). The desorption % of Zn was the highest, followed by that of Cd; the desorption % of CuO was the lowest using all the extracting solutions across all soil types. This high leaching of Cd and Zn suggested possible adsorption on the available surface sites of hydroxyapatite with little diffusion into apatite structure. The desorption % of Haplic podzol soil was higher than that of three soils using all the extracting solutions across all NP types (Figures 6.14–16).

The soil microorganisms perform an essential role in geological, hydrological, and ecological cycles; therefore any change caused to the microbial diversity and function by the release of MNPs could potentially influence the quantity of plant available nitrogen in the soil (Section 7.1). Thus, the effect of NPs on the breakdown of organic nitrogen to dissolved organic nitrogen (DON) and low molecular weight (LMW) followed by conversion to ammonium ions and finally to nitrate ions was determined for three grassland soils. The results of the nanoparticles' impact on nitrogen mineralisation indicated, that the concentration of NO_3^- accumulated readily in three soils; however, NH_4^+, DON and free amino acids concentrations show low levels of accumulation across all of soil and NP types (Figures 7.1–7.9). This revealed that they do not limit the rate of nitrogen mineralization. The great majority of DON was the main component of the nitrogen pool in the treated soils at day 0. DON concentration did not increase significantly over the 28 days across all NP or soil types; all concentrations appeared steady when compared with those at Day 0. The rate of nitrogen mineralization was varied widely across all soils under NP concentrations, the results of soils comparison as regards the nitrogen mineralization suggest that the concentration of NO_3^- and AAs are highest in Eutric Cambisol soil followed by Sandy soil; Haplic podzol was the lowest. The concentration of NH_4^+ was highest in Haplic podzol soil, the next highest in Sandy soil and the lowest in Eutric Cambisol soil. Furthermore, the concentration of DON was highest in Sandy soil followed by Eutric Cambisol soil; Haplic podzol soil was the lowest. The results of soil respiration indicated that no significant impacts for all NPs. However, CdS NPs increased the respiration rate in Haplic podzol soil at the first addition concentration. The lack of soils response to the concentrations of CdS, CuO, and ZnO NPs for all three soil types showed that during the short incubation time (48 hours) there was limited nitrogen and carbon cycling which reduce the activity of soil microbial. Further study is needed to fully quantify the influence of these NPs on three tested soils for long periods.

Most of the analytical techniques (e.g. AAS, ICP–OES, UV–Vis) used in this study were applicable to dispersed NPs. There are various chemical methods (e.g. digestions and separations) applicable for characterization and analysis of NPs in different media (plant and maize). These methods have potential, under the experimental condition used to test for the presence of NPs in environmental samples. Although, free NPs and suspended aggregated are the most important fraction in many applications, deposited or adhered NPs are also important in some matrices (e.g soil and sediments). For detection and analysis of NPs in the environment

there still remains a major challenge to develop suitable methods. Therefore, further studies are required to consider methods to extract or detach NPs from solid matrices, as well as methods to characterize the attached NPs. Such detachment methods include addition of dispersion agents (e.g. sodium pyrophosphate) and sonication prior to analysis. The same considerations also apply to the interaction of NPs with natural materials, however, natural NPs should be extracted and detached before analysis. The promising analytical technique of electron microscopy, (elemental analysis within the TEM), permits a quantitative analysis of the X–rays absorption within the samples which need to be pre-fractionated. Image analysis would need to be developed.

Future work

The results of this study pose several interesting questions that future work could seek to clarify. Study of the translocation, distribution and mechanism of NP materials in maize plant using transmission electron microscopy (TEM), synchrotron micro X–ray fluorescence (μ–XRF) and isotopic labelling, would allow the detection of the presence and distribution of nanomaterials in the tissues of maize roots and shoots. Further investigations may need to consider other types of NPs, soils and plants. This would allow for comparison with results obtained in this work. To fully clarify the adsorption of MNPs in the soil, adsorption columns utilizing media of similar composition could be used in conjunction with different concentrations, pH and flow rates. It would be interesting to investigate the adsorption of MNPs in other soil of and NPs types as this would assess the behaviour and fate of these materials in the environment. It may also be usefule to investigate how nanoparticle materials behave in their immediate environment–for instance treating maize plant or soil with NPs in field; this would elucidate the impact of NPs on maize growth parameters and could also show the effect of NPs upon localized communities, normal organic matter and nutrient turnover and their availability.

Appendix 1

Scanning electron microscopy (SEM) of nanoparticles with the observation of these nanoparticles on surface of maize roots

As part of the uptake of Cd, Cu and Zn NPs in maize roots and shoots grown in the hydroponic culture, plant samples treated with a high concentration (1.0 g L^{-1}) of CdS, CuO and ZnO NPs were selected. The SEM had performed with the tip of roots and shoots surface. Fresh maize roots and shoots were thoroughly washed with deionized water after seedlings growth immediately (21 days). The sections of root and shoot were taken manually from control and samples treated with NPs. The fresh maize roots and shoots samples were cut into small pieces with blunt knife, the pieces were freeze-dried overnight at –80°C. The plant samples were coated with platinum/palladium (4 nm thickness of platinum/palladium). The morphology of maize roots was examined by scanning electron microscope (HITACHI 4700 FE–SEM).

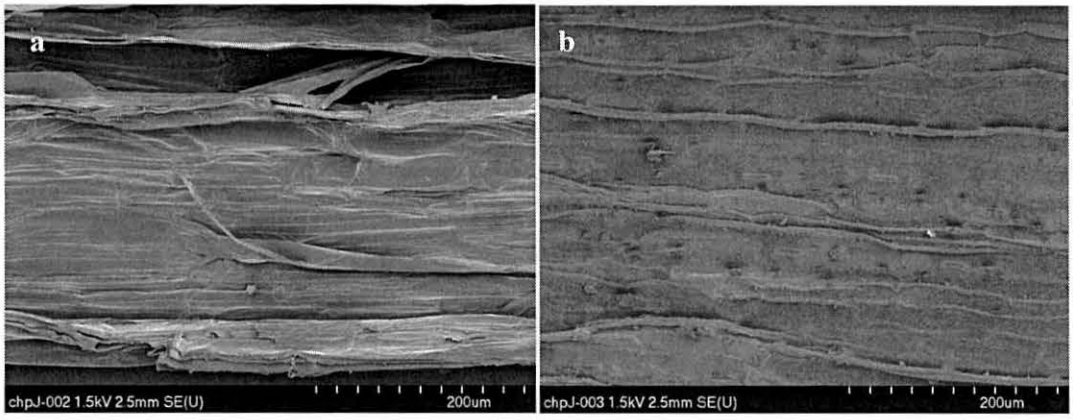

Figure A1. SEM images of maize root and shoot tissues grown in nutrient solution without nanoparticles treatments (control samples), (a) control roots and (b) control shoots, respectively. Bars =200 μm. The samples observed at 1.5 kV

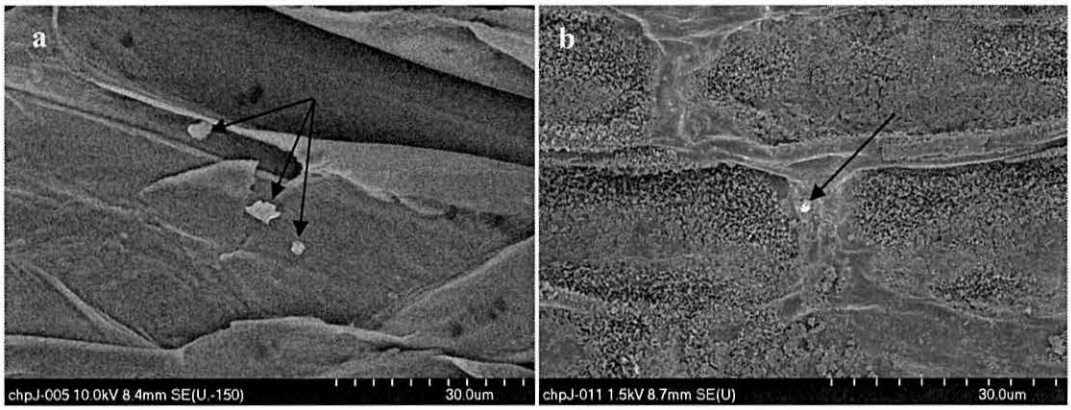

Figure A2. SEM images of maize root and shoot tissues grown in Cd solution treated with 1.0 g CdS L^{-1} nutrient solution, (a) maize roots observed at 10 kV and (b) maize shoots observed at 1.5 kV, respectively. Bars =200 μm.

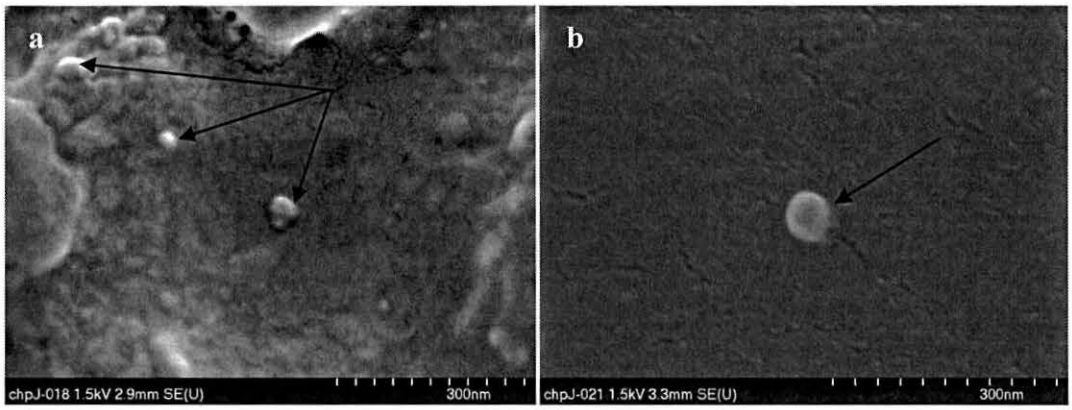

Figure A3. SEM images of maize root and shoot tissues grown in Cu solution treated with 1.0 g CuO L^{-1} nutrient solution, (a) maize roots and (b) maize shoots, respectively. Bars = 300 μm. The samples observed at 1.5 kV.

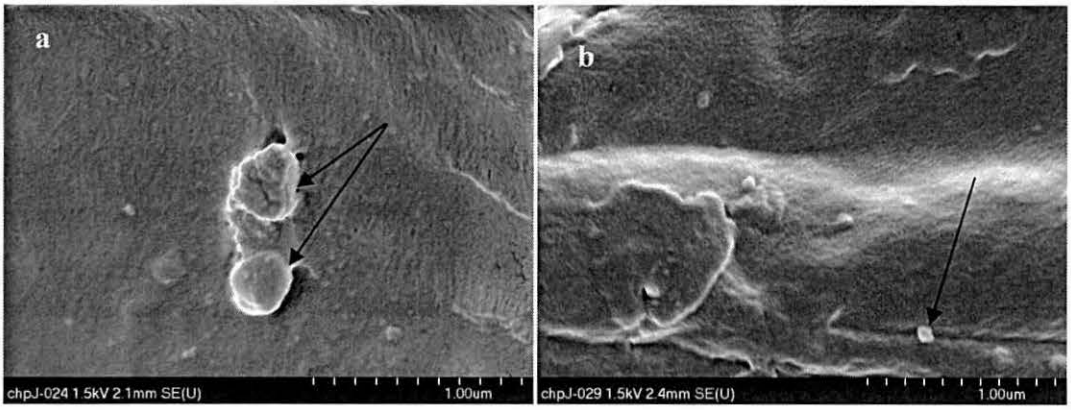

Figure A4. SEM images of maize root and shoot tissues grown in Zn solution with 1.0 g ZnO L^{-1} nutrient solution, (a) maize roots and (b) maize shoots, respectively. Bars =1μm. The samples observed at 1.5 kV.