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Fate, accumulation and impact of metallic nanomaterials in the terrestrial environment

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Chapter 1

General Introduction

1.1 State of science of nanomaterials

The concept of nanotechnology was initiated by Richard P. Feynman in the lecture entitled “There’s Plenty of Room at the Bottom” in 1959^{1,2}, whereas the term “nanotechnology” was firstly created by Norio Taniguchi in 1974^{3,4}. Over the years, nanotechnology has emerged as a cutting-edge field of science with the potential to revolutionize the technology of the 21st century as it enables design, characterization and production of devices or materials at a nanometer scale. Nanotechnology is generally defined as “a science, engineering, and technology conducted at the nanoscale (1 to 100 nm in at least one dimension)⁵. In the last decades, a large number of research projects in the field of nanoscience and nanotechnology were funded by both industry and government over the world⁶, which makes nanotechnology become one of the most rapidly growing markets worldwide. Already, nanotechnology is changing the world in many ways and is applied in almost every field of science and household products⁷. The latest data reported that there are more than 5,169 nano-related products in the worldwide market⁸ and the estimated global market of nanotechnology will exceed US\$ 125 billion by 2024⁹.

The rapid development of nanotechnology extends the production and application of manufactured nanomaterials. The definition of nanomaterial according to European Commission Regulation (2011/696/EU) is “A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm”. With the advancement of nanotechnology, nanomaterials/nanoproducts have experienced several generations. The first generation of nanomaterials (2000-2005) usually refers to “passive nanostructures” that comprise relatively simple carbon-based and inorganic materials^{10,11,12}. These particles were used in products such as automobile tires (carbon and silica), cement (calcium silicate hydrate nanoplatelets), sunscreen (titanium dioxide) and antibacterial textiles (silver)^{11,12}. From 2005 to 2010, the second generation of active nanomaterials appeared on the market. These materials were characterized by nanoscale elements that serve as the functional ingredient in electronics, sensors and medicines et al^{10,11,12}. Examples of use are gold-silica

nanoparticles for cancer treatment, silver nanowires for the transparent conductive film of displays, surface-functionalized superparamagnetic iron oxide nanoparticles for the treatment of iron deficiency anemia in adult chronic kidney disease patients and brain tumors, and as contrast agents in magnetic resonance imaging¹². The third generation of nanomaterials (2010-2020) are constructed multifunctional nanosystems, in which the integration between organic and inorganic nanomaterials was increased. These combined nanomaterials/nanodevices were integrated into other systems^{10,11,12}. They can assemble or disassemble themselves, to some extent, to react to the environment¹¹. For example, the third generation of nanomaterials has been developed to deliver drugs to a targeted pharmacological site, thus reducing the amounts of the drug and potential adverse side effects¹¹. From 2020 onwards, the fourth generation of nanomaterials intends to build up molecular nanosystems that allow each nanomolecule in the system to have a specific structure and function^{10,11,12}.

The nanoscale size can transfer the classical physics to quantum effects in nanoparticles (NPs)⁶, and as a result NPs exhibit novel and unique properties, as compared to the individual molecules or their bulk counterpart. These include a high surface area to volume ratio, high chemical and catalytic properties, extraordinary electrical, optical, thermal and magnetic properties, easy functionalization et al.^{2,13,14}. These properties make that NPs are applied in a large number of fields, in particular cosmetics, textiles, electronics, energy, environment, agriculture, biology, pharmacy, healthcare and medicine^{7,13,15} (**Figure 1.1**). Among numerous NPs which have been produced, metals (e.g. silver, copper) and metal oxides (e.g. titanium dioxide, zinc oxide) are produced most commonly for diverse applications. These NPs have been categorized as metal-based NPs. With the increase in the manufacture and use of metal-based NPs, there is the likelihood of increased emissions of metal-based NPs to the environment^{14,16,17}. As a result, the metal-based NPs have been detected in a range of environments, including air, surface water, sediments, sludge and soil. Therefore there is a great necessity to improve the understanding of the impacts and potential risks of metallic NPs to the environment. In this thesis, I focus on the potential environmental risk of the most widely utilized metallic nanoparticles (NPs): silver nanoparticles (AgNPs) and titanium dioxide nanoparticles (TiO₂NPs)^{18,19}. The particles are of the 1st generation NPs and have an estimated global annual

production of 135-420 and 60,000-150,000 tons, respectively²⁰.

1.2 The application of nanomaterials in agriculture

Recent research has claimed that nanotechnology can potentially revolutionize the agriculture sector by producing a variety of nano-agrochemicals and nanosensors to enhance crop productivity and quality²¹. To date, metallic NPs such as CaNPs, MgNPs, ZnONPs, ZnNPs, CuNPs, Magnetite (Fe_2O_3)NPs and manganese zinc ferrite NPs have been applied as nanofertilizers²¹. These nanofertilizers can improve the accessibility of nutrients to the plant parts, prevent the loss of nutrients, and enhance the fertility of soil. Additionally, the application of metallic NPs with antimicrobial and insecticidal properties such as ZnONPs, AgNPs, CuNPs, TiO_2 NPs and Al_2O_3 NPs as nanopesticides against pests and plant pathogens has been documented^{15,21}. These nanoparticles can work as the active ingredients of pesticides or as a carrier for targeted pesticides delivery. Compared to traditional agrochemicals, nano-agrochemicals can significantly increase their efficiency and reduce the application dose, which is good for sustainable agriculture^{15,21}. However, the ecological and environmental risks associated with these new nano-agrochemicals must be carefully addressed before their wide applications.

1.3 Current understanding of nanoecotoxicology and risk assessment of nanomaterials

1.3.1 The development of nanotoxicology

The expected increase in emissions of nanomaterials into the environment will enhance the interactions between NPs and organisms, and this will possibly lead to unknown biological effects. This encouraged global scientific and governmental attention on assessing the safety of manufactured nanomaterials, resulting in the rapid development of the discipline of nanoecotoxicology. Nanoecotoxicology, a sub-discipline of ecotoxicology, is emerged to address the toxic effects of nano-scaled substances on organisms and the constituents of ecosystems²². Nanoecotoxicology aims to identify and predict the behavior, fate and adverse effects of NPs in/on the ecosystems, and hence to provide data and knowledge about making regulations on nanosafety. Three basic strategies for evaluating the toxicity of NPs have been

proposed by Oberdörster et al.: (1) physicochemical characterization (size, surface area, shape, solubility, aggregation), (2) elucidation of the biological effects from *in vitro* studies, (3) confirmation of the effects through *in vivo* studies²³. Based on these strategies, nanotoxicity tests have been performed using a range of aquatic and terrestrial organisms^{14,24}. Generally, negative impacts of NPs on bacteria have been reported. These impacts include cell membrane damage, inhibition of bacterial growth and biomass, alterations of bacterial community composition, structure and functions^{25,26}. The impacts of NPs on the survival, growth and reproduction of animals have been widely reported^{27,28}. Adverse responses on plants induced by NPs include the inhibition of seed germination and plant growth, reduction of biomass, and alterations in the photosynthesis of plants^{14,29}. Nowadays, investigations of the impacts of NPs on organisms are extenuatingly moving to the scale from the single laboratory-reared species to the natural communities. For example, microcosm studies of AgNPs and TiO₂NPs have been widely assessed to impact on soil microbial communities³⁰; Geitner et al.³¹ investigated the impact of the size of cerium oxide (CeO₂) NPs on their uptake, transport and distribution in a wetland mesocosm. The adverse effects of NPs are determined by a variety of parameters such as the physicochemical properties of NPs, the dynamic behaviour of NPs in the exposure medium, and the exposure route and dosage. Any modification of these parameters might change the biological fate of NPs, resulting in the risks of NPs being assessable only on a case-by-case basis³². Therefore, the full-scale understanding of the ecotoxicity of NPs is still a challenging task for environmental and ecotoxicological scientists. Evaluation of the characteristics and the fate of NPs in complex matrixes such as soil and organisms and the combined effects of different NPs in aquatic and terrestrial (eco)systems, are the major subject of ongoing research in the field of nanoecotoxicology³³. Moreover, research into the bioavailability, fate and mechanisms of action of NPs in organisms are largely lagging behind the knowledge about their observed adverse effects on organisms. Further, the trophic transfer of NPs within some simple aquatic and microbial food chains has been reported, but there is little known about the potential bioaccumulation and biomagnification of NPs in complex terrestrial food chains³⁴. These gaps call for more efforts on the ecotoxicity studies of NPs.

1.3.2 The toxicity mechanisms of metallic nanoparticles

Oxidative damage is the most frequently suggested pathway for many adverse effects induced by NPs. The physical interaction between living organisms and NPs can induce the generation of excess reactive oxygen species inside organisms, which can cause lipid peroxidation, cell membrane damage, cell structure alteration, protein and DNA damage, and inhibit the growth of organisms^{35,33}. Moreover, the formation of biologically active NPs–protein corona complexes is also considered as a possible mechanism for the toxicity of NPs³⁶. Once NPs are in contact with protein-containing biological fluids like plasma, the NPs–protein corona complexes will be formed on the surface of NPs³⁶. This can govern the fate and functions of NPs in an organism as the NPs–protein corona complexes rather than the “bare” NPs are ‘seen’ as the effective unit by cells in the cell–nanoparticle interactions processes^{36,37}. Additionally, the accumulation and internalization of NPs in organisms has been suggested as the major determinant driving their toxicological effects³⁸. The cellular uptake of NPs can occur by means of attachment of the NPs to cell membranes or clog the pores and barriers of cells wall/membranes, thereby causing mechanical damage and inhibiting nutrient uptake and transport^{35,38}.

1.3.3 Regulations and policies of nanosafety

Undoubtedly, the environmental risk assessment of NPs is a worldwide issue, and therefore global efforts have been taken in assessing nanosafety. For example, the EU incorporated the risk assessment of NPs into the generic EU–policies such as the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and the CLP (Classification, Labelling and Packaging) regulations¹¹. Further, the European Commission is funding numerous research projects about the potential impacts of NPs and NPs-containing products on environmental health¹¹. Similar, in the United States, the Environmental Protection Agency (EPA) administered a special regulation for NPs in the Toxic Substances Control Act (TSCA) and created the US National Nanotechnology Initiative (NNI)³⁹. As the biggest developing country, China also takes many actions in nanosafety assessment. For instance, some regulations related to nanosafety have been created by China, such as Occupational risk management applied to engineered nanomaterials (GB/T 38091.2-2019),

Nanomaterial risk evaluation (GB/T 37129-2018), Guidance on toxicological screening methods for nanomaterials (GB/Z 39262-2020). Also, the National Natural Science Foundation of China (NSFC) has launched numerous research projects with aims ranging from the methodology of NPs characterization, assessment of the hazards of NPs to the environment and to human beings, to recommendations regarding the regulation of nanosafety to the Chinese government. China has founded many institutions that are dedicated to fundamental and applied researches in the field of nanoscience and technology, such as “The National Center for Nanoscience and Technology” and the “Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Chinese Academy of Sciences (CAS)”. Global financial support in nanosafety speeds up the development of the risk assessment of NPs. Generally, the current existing frameworks and tools for risk assessment of NPs are modified from the risk assessment paradigm for chemicals (**Figure 1.1**)⁴⁰. Basically, the risk assessment of chemicals is composed of a four-step workflow including problem formulation, exposure assessment, hazard assessment and identification, and risk characterization⁴⁰. The MARINA risk assessment framework is for instance suggested as a flexible approach for efficient information collection and risk assessment of NPs⁴¹. This framework is composed of two phases: phase 1 is the problem framing with the specific goal to identify relevant exposure scenarios throughout the life cycle of NPs; phase 2 is the iterative risk assessment, consisting of four steps^{11,41}. The four steps of phase 2 are risk characterization, defining data needs, data gathering, and data evaluation^{11,41}. Risk assessment of NPs can help policymakers and managers to make regulations about managing the safe production and use of NPs in the industry by integrating risk considerations with scientific and socioeconomic information.

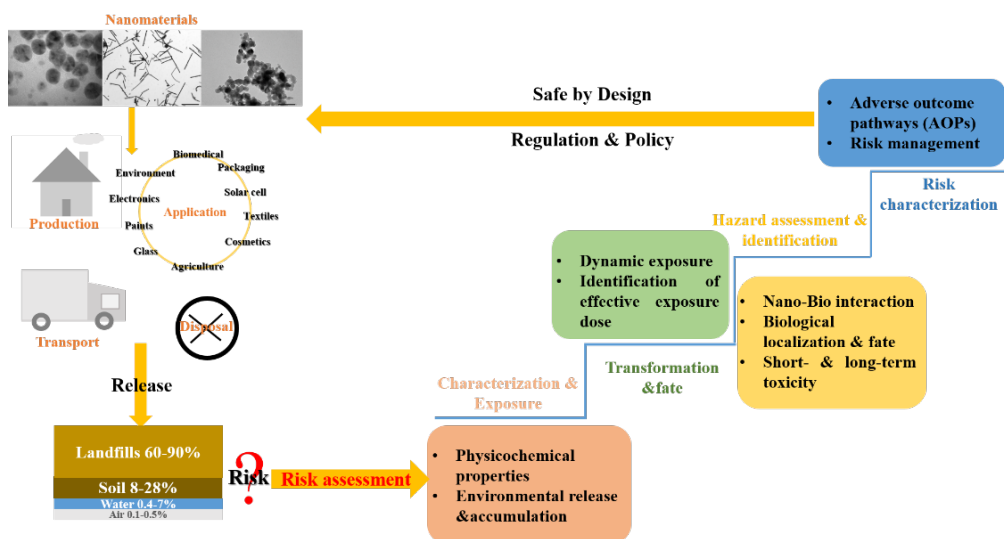


Figure 1.1 Schematic diagram showing the applications and emissions of NPs in environment and risk assessment paradigm for NPs.

1.4 The fate of metallic nanoparticles in the terrestrial environment

The release of metallic nanoparticles into the environment can occur intentionally or unintentionally throughout the lifespan of NPs-containing products, during synthesis, production, transport, usage and disposal^{19,42,43,44} (**Figure 1.1**). A large part of the released NPs is expected to end up and accumulate in soil⁴⁵. NPs can be introduced into soil directly by the application of NPs for soil remediation and the application of nano-agrochemicals such as nano-pesticides, nano-fertilizers and nano-amendments to agricultural fields^{46,47,48,30}. In addition, the discharge of sewage or biosolids from wastewater treatments to soil and the runoff from nano-agrochemicals are important indirect major pathways for NPs to enter soil^{42,30,49,50}. It is still a challenging task to quantify the concentrations of metallic NPs in soil due to analytical limitations. Several studies have predicted the concentrations of NPs in the environmental compartments using mathematical modeling. The concentration of TiO₂NPs and AgNPs in soil has been estimated to be in the range of 1-10 µg/kg and 0.01-0.1µg/kg⁵¹. Sun et al.⁵² predicted that the concentration of TiO₂NPs and AgNPs increases in the range of 0.09-0.24 µg/kg/y and 0.9-1.8 ng/kg/y for natural and urban

soil, and 940-3600 $\mu\text{g/kg/y}$ and 0.09-0.65 $\mu\text{g/kg/y}$ for sludge treated soil. Given the growing commercial applications of these nanoparticles and hence increasing emission of NPs into the soil, there is an emergent need to evaluate their fate, ecotoxicity and environmental risks in soil.

After being released into soil, NPs will undergo a series of transformation processes including physical, chemical and biological transformations^{49,53,24,54} (**Figure 1.2**). Physical transformations of NPs usually refer to their aggregation, agglomeration, sedimentation in soil, and ad/de-sorption to/from the soil matrix¹¹. Dissolution, oxidation, sulfidation, chlorination, precipitation and changes of surface properties are the main chemical transformations of NPs in soil¹¹. Biological transformations are the processes mediated by the interaction with organisms, such as biological oxidation, complexation with the exudates/excretes by organisms to form a new surface coating¹¹. These transformation processes of NPs in soils can be affected by both environmental conditions such as soil pH, the content of soil organic matter, and the intrinsic physicochemical properties of NPs including size, shape, surface charge and coating^{17,24,55}. The dynamic transformations of NPs in soil may alter their behavior, fate, bioavailability, and hence their cellular uptake and toxicity on organisms^{17,24}. For instance, the dissolution of the soluble metallic NPs (e.g. AgNPs, CuNPs, CuONPs and ZnONPs) plays a vital role in governing the toxicological effects. When exposed to soluble NPs, both the particulate form and the metal ions released from the NPs may contribute to the overall toxicity as the tested organisms always interact with both of them. But whether the nanoparticles themselves or the released metal ions dominate the observed toxicity of soluble metallic NPs is still under investigation and can differ between species and type of NPs. For example, the dominant role or sole role of dissolved ions to the toxicity have been reported for AgNPs in maize (*Zea mays*)⁵⁶, Onion (*Allium cepa*), alga *Chlamydomonas reinhardtii*²⁹ and *Euglena gracilis*^{57,58}, and bacteria *Escherichia coli*⁵⁹, CuONPs in wheat (*Triticum aestivum* L.)⁶⁰ and ZnONPs in maize (*Zea mays* L.) and cabbage (*Brassica oleracea* var. *capitata* L.)⁵⁶. In contrast, particle-dominant toxicity has also been reported for AgNPs in *Lolium multiflorum*⁶¹, ZnONPs in *Scenedesmus obliquus*²⁸ and CeO₂NPs in *Arabidopsis thaliana*⁶². The inconsistent results require more effort in exploring the relative contribution of particulate and ionic forms to the overall

toxicity of metallic NPs. The relative contribution of particulate and ionic forms of NPs suspensions to the overall toxicity of the suspension of the NPs, is most likely attributable to the fate of the particles in the exposure medium and the processes occurring at the exposure – bio interface. It hence is crucial to determine both fate as well as responses of NPs in organisms when performing exposure experiments.

Similarly, the processes of aggregation/agglomeration and sedimentation also play the main role in affecting the state of NPs in the dispersion medium and hence impact the bioavailability and toxicity of NPs to biota⁶³. Based on the classic Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, the aggregation of NPs occurs once the electrostatic repulsive forces were lower than the van der Waals attractive forces between particles⁶⁴. Aggregation of NPs in exposure medium results in larger size of NPs and affects the sedimentation rate of NPs. Both of the aggregation and sedimentation processes may ultimately lead to the changes of the effective exposure dose and uptake rates of NPs, and hence affect the biological impacts of NPs⁶⁵. Several studies have reported that the size of NPs agglomerates instead of the primary size of NPs plays the predominant role in the cytotoxic of NPs^{66,67}. Therefore, it is critical to consider the dynamic transformations of NPs into their environmental risk assessment, which enables to more accurately capture the actual effective exposure of organisms to NPs and offer a better and more comprehensive assessment of their impacts on the ecosystem and environment.

1.5 Consideration of factors affecting the impacts of nanoparticles on biota

In addition to the impacts of the dynamic transformations (dissolution aggregation and sedimentation) of NPs on their toxicity, the physicochemical characteristics of the NPs and the exposure duration may influence the responses of organisms to NP . Further, it should be pointed out that the NPs co-exist with numerous pollutants including other nanoparticles once they entered the environment. This may result in modulation of the effects of individual NPs. The observed toxicity of NPs is the outcome of the combined effects exerted by the characteristics of NPs, the exposure route, the dynamic behavior of NPs in the exposure medium, and the intrinsic toxicity of the NPs (**Figure 1.2**). In this section, how these factors can affect the

impacts of NPs on biota will be documented.

1.5.1 Physicochemical characteristics of nanoparticles

The physicochemical properties of NPs including shape, size and coating are crucial factors influencing their impacts on organisms. The size of NPs is one of the determinant factors affecting the uptake, translocation and phytotoxicity in organisms as the barriers that inhibited the entrance of NPs into organisms have a certain size-exclusion limit^{68,55,69}. This size-exclusion limit results in the plants being inclined to take up NPs with smaller sizes^{55,69} and hence induce higher phytotoxicity. For example, AuNPs with a size of 3.5 nm can be taken up by tobacco, whereas AuNPs with an average diameter of 18 nm remained on the outer surfaces of the root of tobacco plants⁷⁰. Smaller sizes NPs were found to be more phytotoxic as exemplified for AgNPs in *Allium Cepa*⁷¹, *Spirodela Polyrliza*⁷² and *Arabidopsis*⁷³, ZnONPs in broad bean (*Vicia faba*)⁷⁴, maize (*Zea mays*)⁷⁵, Chinese cabbage (*Brassica rapa var. pekinensis*)⁷⁶ and CuONPs in soybean (*Glycine max cv. Kowsar*)⁷⁷. However, contradictory results about the impacts of size on plants have been reported, such as size-independent toxicity⁷⁸ or larger size inducing more intense adverse effects^{79,80}. The inconsistent results suggest that the impacts of NPs on organisms are not only related to their size.

Next to the size, the surface modification of NPs, for example by using citrate, tannic acid, or polyvinyl alcohol (PVP) as coating, can affect their impacts on organisms^{55,69}. These coatings can change the toxicity of NPs by affecting their dispersion, stability and agglomeration of NPs in the exposure medium^{55,69}. For example, Navarro et al.²⁹ comprehensively assessed the effects of nine different coated AgNPs on the photosynthesis of the algae *Chlamydomonas reinhardtii* and demonstrated that the role of different coatings in affecting the toxicity of AgNPs to algae was related to their complexation with the released Ag ions. Similarly, the stability, uptake, translocation and biological responses of soybean to cadmium sulfide quantum dots were also found to be dependent on their surface coatings⁸¹. Nevertheless, no generic coating-dependent impact of NPs can be concluded. Reports of coated NPs inducing comparable, lower or higher toxicity than uncoated NPs have been published^{82,81,83}. How the coatings change the interactions between NPs and biota and the associated

(eco)toxicological effects depends also on the other physicochemical properties of the NPs and on the testing conditions. Therefore, investigating the joint effects of coating and other characteristics of NPs is important in order to comprehensive understand their bioavailability and toxicity on biota.

In addition to the size- and coating-dependent toxicity of NPs, the shape-dependent impacts of NPs on organisms have also been investigated. For example, shape-dependent transformation and translocation of CeO₂NPs in Cucumber plants have been demonstrated⁸⁴. To date, Ag nanospheres have been reported to induce higher toxicity in Arabidopsis plants⁷³, ryegrass *Lolium multiflorum*^{85,86}, alga *Raphidocelis subcapitata*^{87,88} as compared to other shapes, including triangular, decahedral, nanocubes, and nanowires composed of Ag. However, some contradictory results about the shape-dependent toxicity have been obtained as well. For instance, Ag nanoplates and Ag nanowires were found to be more toxic to the algae *Chlorococcum infusionum* as compared to Ag nanospheres⁸⁹, and no obvious shape-related impacts of AgNPs were observed on bacteria (*Escherichia coli*⁵⁹, *Bacillus cereus*, and *Pseudomonas aeruginosa*) after exposure to Ag nanospheres, Ag nanowires and Ag nanocubes⁸⁶. These results indicate that the test species plays an important role in affecting the shape-dependent impacts of NPs. Even though the results regarding the effects of physicochemical characteristics of NPs on their bioavailability and toxicity are growing, the picture of the ecotoxicity of NPs is still far from clear. This thesis compared the uptake, translocation and phytotoxicity of Ag nanospheres and Ag nanowires in lettuce, and investigated how the joint effects of size, length and coating of Ag nanowires influence the uptake and phytotoxicity of nanowires in plants.

1.5.2 Long-term exposure

The impacts of NPs on organisms are highly related to the variability of exposure time. For example, Ag₂SNPs had no adverse effects on cowpea (*Vigna unguiculata* L. Walp.) and wheat (*Triticum aestivum* L.) during 24h exposure, but reduced the root biomass of these plants by more than 30 % after 2 weeks of exposure⁹⁰. Zhai et al.⁹¹ also observed that the soil bacterial composition was significantly altered by TiO₂NPs after exposure of 1 and 60 days, but no obvious shifts were observed after 15 days of exposure. Similarly, the impacts of NPs on plants and soil microbes vary over time

have been reported for CuNPs⁹², CuONPs^{93,94}, ZnONPs^{93,95,96,97}, AgNPs^{92,98}, and Fe₂O₃⁹⁹. These results highlight that assessing the toxicity of NPs using a single timepoint should be done with caution and short-term assessment may not fully reveal the potential toxicity of NPs. Ecotoxicological study of NPs over a longer exposure period is needed. Additionally, the dynamics of both exposure and biological responses should be considered for a better understanding of the interactions between NPs and biota. Therefore, this thesis investigated the long-term impacts of AgNPs on plants and the soil bacterial community with a series of different exposure times.

1.5.3 Co-existing contaminants

Importantly, after entering the environment, NPs inevitably come to contact with other contaminants, including NPs of different composition and/or of different morphology that are also present in the environment. Therefore, in the natural environment, biota are rarely exposed to single NPs but rather to a mixture of NPs. The interactions between different NPs can influence their bioavailability and toxicity to biota by forming NPs-NPs complexes through electrostatic interaction and/or surface complexation/adsorption^{100,101,102}, and/or by affecting their respective transformations such as dissolution, aggregation/agglomeration and sedimentation^{101,103,104}. This may result in the impacts of mixtures of NPs on biota being completely different from the summed impacts of the single NPs. For example, antagonistic effects have been found for TiO₂NPs and CeO₂NPs in *Nitrosomonas europaea*¹⁰¹, AgNPs and hematite nanoparticles in *E. coli*¹⁰⁴, TiO₂NPs and ZnONPs in *E. coli* and *A. hydrophila*¹⁰². Joško et al. compared the toxicity of each combination of binary mixtures of NPs including ZnONPs, CuONPs, TiO₂NPs, Cr₂O₃NPs and Fe₂O₃NPs to the toxicity of the individual NPs using the cress *Lepidium sativum*, flax *Linum usitatissimum*, wheat *Triticum aestivum* and cucumber *Cucumis sativus* as test species¹⁰⁵. These authors also found that all of the binary mixtures induced significantly lower toxicity to the plants in comparison to the summed toxicity of the individual NPs¹⁰⁵. However, some research indicated synergistic effects, which is contradictory to the above results. For instance, the co-exposure of CuONPs and ZnONPs caused severe inhibition of the growth and photosynthesis of spinach

Spinach oleracea as compared to the exposure of single NPs¹⁰⁶. The mixture of ZnONPs and CeO₂NPs had synergistic effects in *Nitrosomonas europaea*¹⁰¹. Understanding the interactions between different NPs and how the interactions affect their mixture toxicity can provide a more complete picture for the risk assessment of NPs in the natural environment. However, current understanding of the mixture toxicity of multiple NPs is largely lacking. Even less is understood regarding the impacts on plants and the trophic transfer. It is therefore demonstrated in this thesis how a mixture of NPs will affect the transfer of NPs along a terrestrial food chain and the associated impacts on consumers. This advances the current understanding of the fate of NPs and their possible risks in/to terrestrial ecosystems.

1.6 Interactions of metallic nanoparticles with terrestrial biota

Soil organisms including plants and soil microbes are exposed to NPs, producing the associated nano-bio interface that plays a dominant role in determining the adverse effects of NPs. The interactions occurring at the nano-bio interfaces are greatly influenced by the physicochemical properties of NPs, the exposure conditions, and the tested species¹⁰⁷ (**Figure 1.2**). For example, the dynamic nano-bio interactions result in the formation of protein coronas, particles wrapping, cellular uptake and internalization of NPs. These phenomena will affect the biological fate of NPs and hence potentially influence whether or not adverse biological effects of NPs are observed^{108,107}. Therefore, a good understanding of the interactions between NPs and terrestrial organisms is paramount for their environmental risk assessment.

1.6.1 Uptake, translocation and impacts of nanoparticles in plants

Plants, being primary producers, form the foundation of the terrestrial ecosystem. They play a vital role in regulating nutrient cycling, maintaining the functioning and stability of ecosystems, and supplying food within food webs. Given the potential adverse effects of NPs and the importance of plants, it is therefore necessary to comprehensively understand the uptake, translocation and phytotoxicity of NPs in plants.

After exposure, NPs are first adsorbed/absorbed onto the surface of the plant root. For the uptake of NPs by plants, the adsorbed/absorbed NPs need to penetrate a

series of physiological barriers from the root surface to vascular tissues (xylem). These barriers include the root surface cuticle, cell wall, epidermis, cortex, endodermis and Casparian strip. For example, Ma et al.¹⁰⁹ observed La_2O_3 NPs (22 nm) and their aggregates in the intercellular spaces, middle lamellas, cytoplasm and vacuoles of cucumber roots using TEM-EDS. Geisler-Lee et al.¹¹⁰ confirmed the presence of 20-40 nm AgNPs in the cell walls and plasmodesmata of *Arabidopsis thaliana* roots. Theoretically, only the NPs smaller than 20 nm can be taken up via the pores of the cell wall of plant roots as the estimated pore diameters are in between 5 and 20 nm¹¹¹. However, many studies have reported the presence of NPs larger than 20 nm inside the plant roots^{110,111}. The possible explanations for the uptake of NPs larger than 20 nm by plants are 1) the NPs might enlarge the pore size by inducing the destruction of the cell wall¹¹²; 2) the NPs might enter the intercellular spaces or even the xylem using a crack-entry pathway¹¹³. Also, NPs can be taken up by plant roots through endocytosis and intercellular plasmodesmata^{111,114,115}.

Once inside the plant roots, NPs can be translocated via the vascular system to the other organs like stems, leaves, and fruits^{55,69}. Even though a large number of studies have reported the uptake and translocation of metallic NPs in plants, the majority of them focused on the total metal concentrations in plant shoots^{116,117}. Knowledge of whether the NPs taken up by plant roots will translocate upwards in the form of particles or ions is thus still scarce. In fact, both particles and the released ions can be taken up by plant roots, have a different biodistribution, and will therefore end up in different parts of the plant and in different biochemical forms, and will subsequently contribute differently to the adverse effects induced by exposure to metallic NPs. It is therefore important to differentiate the relative contributions of the particulate form and dissolved ionic forms to the overall toxicity of suspensions of metallic NPs.

After being taken up in plants, metallic NPs can induce adverse impacts on plants at the morphological, physiological and biochemical levels. Morphological damages caused by metallic NPs were not only observed at the contact zone of the roots but also in other organs including stem and leaves. Morphological changes including the inhibition of the formation of the lateral root and root elongation, the reduction of biomass and leaf surface area and chlorosis were observed in a range of plants

exposed to metallic NPs^{35,118,60}. Metallic NPs can also affect the transpiration rate, the fluidity and permeability of the membrane, the production of chlorophyll and the photosynthetic activity in plants^{35,114,119,120}. Moreover, exposure to metallic NPs can induce excess production of reactive oxidative species including singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radicals inside plant cells^{119,120}. This can cause oxidative stress and hence activate the antioxidative defense mechanism of plants^{119,120}. Further, the phytotoxicity of metallic NPs at the genomic level including DNA damage, the increase of chromosome aberration and micronuclei was also observed for various NPs^{121,122}. In contrast to the negative impacts on plants, metallic NPs were also reported to have positive effects on plants growth, including the increase of biomass, protein content and the photosynthesis rate in plants¹²³. For example, Kaveh et al.¹²⁴ observed a significant increase in biomass of *Arabidopsis thaliana* after exposure to low concentrations of AgNPs (below 2.5 mg/L) for 10 days, whereas a significant decrease in plant biomass at a higher concentration of AgNPs (from 5-20mg/L). Similar positive impacts on the growth of plants were also observed for Cu, CuO, ZnO, TiO₂ NPs¹²⁵. These contradictory results indicate that assessment of the impacts of NPs on plants needs to consider the properties of NPs, the experimental methodology, and the plant species.

Aside from root exposure, plants also can be exposed to metallic NPs through their leaves. Nowadays, more and more metallic NPs are being applied in agriculture directly as fertilizers^{48,126}, pesticides⁴⁸, plant protectors¹²⁷ and growth regulators²¹ or as a carrier¹²⁸ to increase the efficiency of traditional agrochemicals. Foliar application of nano-enabled agrochemicals is considered to be a more promising tool than soil application¹²⁹. Foliar application of nano-agrochemicals may improve their bioavailability and efficiency, hence reducing their application doses and rates and lowering the associated environmental risks of nano-enabled agrochemicals¹²⁹. The foliar application of nano-agrochemicals is expected to increase in the future¹²⁷. This enhances the opportunities for plants to get in contact with NPs through leave exposure. However, studies carried out so far with the foliar exposure pathway are quite rare, even though this information has important implications for the safety application and risk assessment of NPs. Additionally, studies also demonstrated that

plants respond differently to NPs under hydroponic and soil cultivation. TiO₂NPs had for instance no effects on the growth of tomatoes under hydroponic cultivation¹³⁰ but enhanced the growth of tomatoes upon soil cultivation¹³¹. Therefore, the differences in uptake, translocation and phytotoxicity of AgNPs in lettuce exposed via different pathways (foliar versus root exposure, hydroponics versus soil cultivation) were investigated in this thesis.

1.6.2 Effects of nanoparticles on soil microbial communities

Next to plants, research also demonstrated the impacts of NPs on soil microbes. Soil microbes play critical roles in maintaining the health of both soil and plants by governing soil biological processes including nutrient transformation and cycling, energy flow, and degradation/detoxification of contaminants. Investigating the NPs-microbe interactions is therefore critical for the risk assessment of NPs in the terrestrial ecosystem, given the increasing emissions of NPs into the soil. So far, the majority of the research investigating the impacts of NPs on soil microbes was conducted with single cultured species. Tripathi et al.¹¹⁴ documented the available data about the impacts of AgNPs on soil heterotrophic microbes and found that *Escherichia coli* was the most investigated species, followed by *Staphylococcus aureus*, *Pseudomonas*, *Salmonella typhi* and *Bacillus subtilis*. The effects of AgNPs on these bacteria include the inhibition of their growth, the decline of respiration, the disintegration of the plasma membrane, the damage of cell membranes, and even cell death¹¹⁴. Recently, two reviews summarized the nanotoxicity of metallic NPs to beneficial soil bacteria^{132,133}. The authors reported that exposure to TiO₂, CuO and ZnO NPs also induced bacterial toxicity, for example causing growth inhibition, inhibition of cell viability, inhibition of metabolism, inactivation of enzymes and proteins, mitochondrial dysfunction, cell membrane damage, and DNA damage^{132,133}. It also highlighted that the responses of microbial communities to NPs are poorly understood¹³³.

Investigating the impacts of NPs on the natural soil microbial community rather than on single cultures is needed as exposure of NPs to natural soil microbial communities represents a more environmentally realistic exposure scenario. To date, NPs have been reported to affect microbial growth⁹³, biomass¹³⁴, abundance¹³⁵, activity^{93,135} and

metabolic profiles¹³⁶ of soil microbial communities as well as the activity of soil enzymes^{93,134}. The impacts of NPs on soil microbial communities are highly dependent on the type of NPs tested, the exposure duration, and the actual dosage¹³⁵. For example, the application of a low concentration of 10 mg/kg of ZnO and CuONPs stimulated the enzymatic activity and population size of soil microbial community⁹³. In contrast, Xu et al.¹³⁴ found that CuONPs applied at concentrations of in between 100 and 1000 mg/kg inhibited the soil microbial biomass and the activity of soil enzymes, whereas the impacts induced by CuONPs were stronger than in the case of TiO₂NPs. Samarajeewa et al.¹³⁷ demonstrated that microbial growth and activity in a sandy loam soil were inhibited by AgNPs and the effects were more pronounced upon increasing exposure time. Recently, research regarding the impacts of NPs on soil microbial communities is developing from morphological and physiological endpoints (including microbial growth and biomass of soil microbial communities and soil enzyme activities) to the changes in structure and composition of the soil community (from domain to species levels) with the help of high-throughput sequencing technologies. For example, Cu(OH)₂ nanopesticides significantly shifted the composition of a soil bacterial community and the changes in the composition of the bacterial community varied over time¹³⁸. Meier et al.¹³⁹ found that AgNPs induced both taxonomic and functional changes in the structure of a soil bacterial community by 16S and metatranscriptomic sequencing. These authors also identified the specific taxa that were tolerant (eg. *Sphingomonas* and *Bradyrhizobium*) or sensitive (eg. *Terrimonas*) to AgNPs exposure¹³⁹. However, the above studies were performed with bare soil and the information about how the effects of NPs on soil microbial communities are affected by the cultivation of plants is limited.

Importantly, soil microbes always co-exist with plant roots in the rhizosphere and they can be connected by a number of pathways^{140,141}. Plant roots can release root exudates into the rhizosphere, which provide the primary carbon source for supporting the growth of soil microbes^{142,143}. Meanwhile, plants rely upon these soil microbes like plant growth-promoting bacteria (PGPB) for nutrient uptake and cycling, and for protection of the plants against environmental stressors^{141,144,145}. NPs with antimicrobial properties may ultimately disrupt the interactions between plants

and soil microbes, resulting in either detrimental or beneficial impacts on soil microbial communities and/or plant growth¹⁴⁶. On the other hand, plant roots may affect the transformations of NPs in soil and hence change the impacts of NPs on the rhizosphere bacterial community. However, very limited research conducted so far about the in-depth understanding of the NPs-microbe-plant interactions.

1.6.3 Terrestrial trophic transfer of nanoparticles

As mentioned in section 1.4.1, a large number of studies have evidenced the uptake, translocation and accumulation of NPs in plants. As plants are at the basis of many food-webs, there is a great potential for NPs of being transferred from plants to consumers. Although there has been some progress in understanding the trophic transfer of NPs in freshwater and marine food chains, limited information is available on the trophic transfer and bioaccumulation of NPs along terrestrial food chains. As far as I am aware, so far less than 15 publications have investigated the transfer and biomagnification of metallic nanoparticles with terrestrial food chains. Even though all of the studies confirmed the trophic transfer of NPs, 1) the extent of transfer and biomagnification of the NPs to the subsequent trophic level was inconsistent across the food chains and tested NPs; 2) the majority of the current studies was performed with the nonactive NPs, AuNPs and CeO₂NPs, only one paper reported the similar data on soluble NPs CuONPs and AgNPs; 3) no research described in the literature determined the combined effects of different NPs on their trophic transfer via food chains; 4) no research of the publications investigated the impacts of trophic transfer of nanoparticles on alterations of the behaviour of the consumers. To date, extensive research has already demonstrated the accumulation of NPs in animals via direct exposure to NPs, which can cause toxic impacts, including inhibition of growth, activity and reproduction, for a range of animals¹⁴. Therefore, it is reasonable to ask whether the food chain transfer of NPs can also result in unintended adverse effects on consumers. Although the interest is emerging, the trophic transfer of NPs along terrestrial food chains is still a poorly understood field of research and deserves careful investigations for a comprehensive understanding of the fate, behavior, and hazards of NPs in the environment.

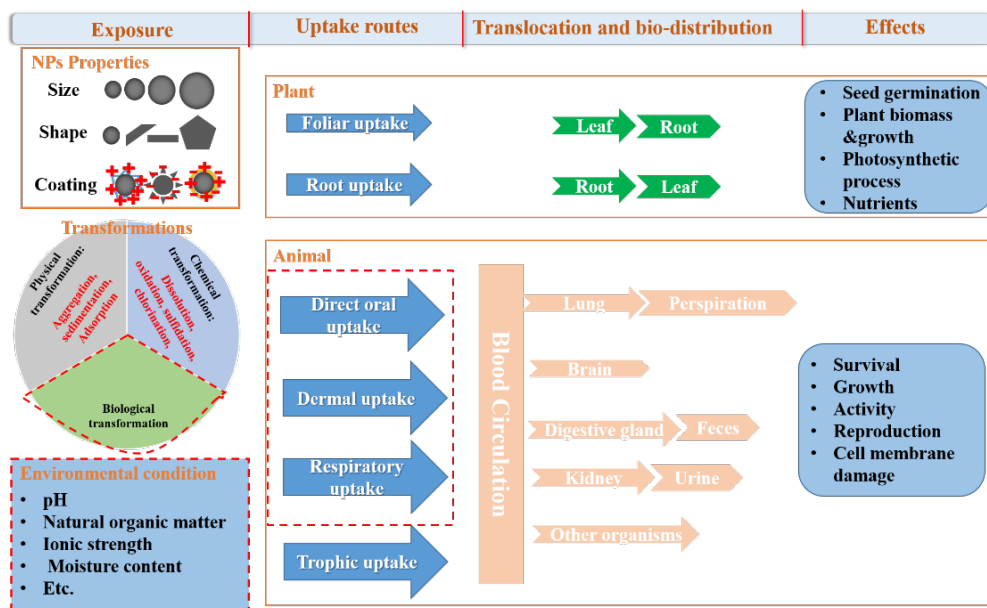


Figure 1.2 Schematic illustration of the interactions between NPs and organisms (modified from Vijver et al. Environ. Sci.: Nano, 2018, 5, 2473). The red dashed line indicates the factors that were not investigated in this thesis. The exposure scenarios of NPs to organisms were influenced by physico-chemical properties of NPs, the dynamic fate of NPs and the environmental conditions. At the nano-bio interface, the bioavailability of NPs depends on exposure route and tested organisms, and hence changes the subsequent translocation, biodistribution and effects of NPs in organisms.

1.6.4 Selected species in this thesis

This thesis focuses on investigating the impacts of metallic NPs on the terrestrial ecosystem. Therefore, plants and soil bacterial communities were chosen as the test organisms. Lettuce is a worldwide cultivated leafy vegetable crop with an estimated annual production of around 27 million tons globally and an annual consumption of 25 pounds per person¹⁴⁷. It is a typical leafy vegetable that is suited for evaluating the ecotoxicity of chemicals and soil amendments to higher terrestrial plants, as recommended by various regulations (ASTM (American Society for Testing and Materials), 2003, US EPA (1996), ISO (International Organization for Standardization), 2005, OECD/OCDE, 2006a, OECD/OCDE, 2006b). Additionally, lettuce is an easily available plant that can comfortably be cultivated hydroponically.

Further, previous studies demonstrated that lettuce was well able to take up NPs. Therefore, lettuce (*Lactuca sativa*) was chosen as the model species in this thesis for investigating the uptake, translocation, accumulation and toxicity of metallic NPs in plants.

The rhizosphere is a highly biologically active zone where soil, plant roots and microbes interact.¹⁴⁸ These interactions are critical for ecosystem functioning and nutrient cycling in terrestrial ecosystems^{148,149}. Also, rhizosphere bacteria provide many functions in supporting the growth of host plants, including enhancing the tolerance to abiotic stress and protection against pathogens^{148,149}. It should be highlighted that the rhizosphere is also a critical place where the adsorption/absorption of toxic substances to/from plant roots occurs¹⁴⁸. The nano-bio interface inevitably influences the composition and structure of the rhizosphere bacterial community, which may disrupt the associated microbial functions and hence affect the growth of plants. Therefore, in this thesis the rhizosphere bacterial community was chosen as another terrestrial biota to better assess the impacts of NPs on the terrestrial ecosystem.

Gastropods have widely been used to monitor environmental pollution¹⁵⁰. Among them, terrestrial snails are recognized as excellent ecological and biological indicators for assessing the ecotoxicity of NPs^{150,151}. This is because of the ease of collection and sampling, their global distribution, short life cycle, small size, high reproductivity, high adaption to various environmental conditions, and ease of culture under laboratory conditions^{150,152}. They are also susceptible to stress and able to accumulate diverse pollutants including metallic trace elements^{150,152}. Further, snails can serve as a link for supplying food and energy between primary producers (e.g. plants) and secondary consumers (e.g. birds). Therefore, the land snail *Cornu aspersum* was selected as the primary consumer in this thesis to explore the potential trophic transfer and the consequent toxicity of AgNPs and TiO₂NPs along with the lettuce-snail food chain.

1.7 Objectives and research aims of this thesis

The research described in this thesis was performed with the objective to enhance the knowledge on the fate, accumulation and toxicity of NPs in terrestrial plant and food

chains (**Figure 1.3**), and is subsequently aimed at providing suggestions for improvements of the ecotoxicological risk assessment of NPs in terrestrial systems and more specifically for the application of NPs in agriculture. This thesis aimed to:

1. investigate the uptake, translocation and phytotoxicity of AgNPs (a typical metallic NP) in lettuce upon different exposure scenarios;
2. investigate the dynamics of metal ions dissolving from the NPs and their impacts on metal accumulation in plants and effects on a soil rhizosphere bacterial community;
3. investigate the trophic transfer, biomagnification and toxicity of metallic NPs in a terrestrial food chain.

The key research questions of this thesis can be summarized as follows:

1. How does the exposure pathway affect the uptake, translocation, and phytotoxicity of AgNPs in plants? (Chapter 2 and 4)
2. How do the shape, size and coating of NPs affect the uptake, translocation, and phytotoxicity of AgNPs? (Chapter 3)
3. What is the relative contribution of the nanoparticulate and the released ionic form to the overall toxicity of suspensions of NPs and on metal accumulation in plants? (Chapter 2 and 3)
4. How and to what magnitude does the dynamic dissolution of AgNPs in soil affect their bioavailability to plants? (Chapter 4)
5. How does the soil rhizosphere bacterial community respond to exposure to AgNPs, and does this response change over time? (Chapter 4)
6. How does a mixture of AgNPs and TiO₂NPs affect the transfer of the individual NPs along a terrestrial food chain of lettuce-snails and the associated impacts on the consumer? (Chapter 5)

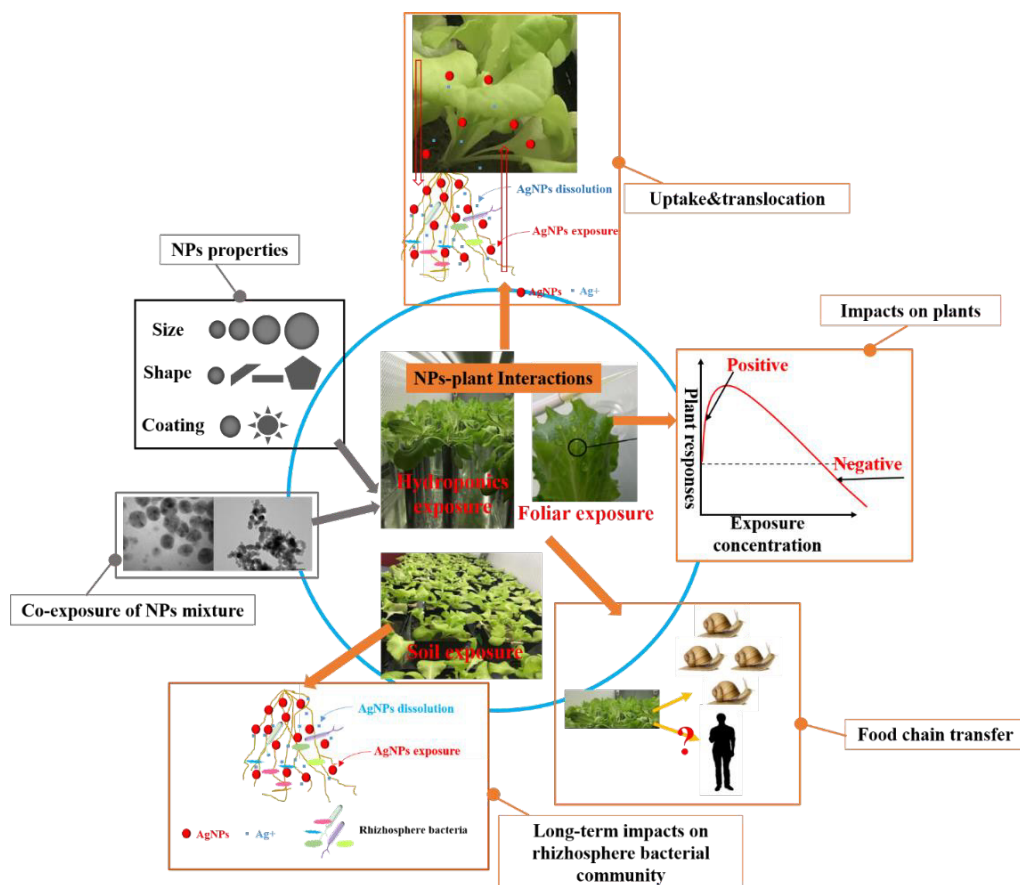


Figure 1.3 An overall graphic illustration of the experimental set-up and the main focuses of this thesis.

1.8 Outline of this thesis

Chapter 1. General introduction regarding metallic nanoparticles, their fate, uptake and effects on terrestrial species. Also, food chain transfer is discussed and a summary of the objectives of this thesis is provided.

Chapter 2. In this chapter we investigated how the exposure route of AgNPs affects their accumulation, toxicity and anti-oxidant responses in lettuce. In addition, we determined the relative contribution of ionic and particulate Ag to the observed toxicity and Ag accumulation in lettuce.

Chapter 3. In this chapter, we determined how the size-aspect ratio and coating of silver nanowires affect the contributions of dissolved and particulate Ag to the overall phytotoxicity of these particles on lettuce. Additionally, we quantified the toxicokinetic rate constants of particulate and ionic Ag in lettuce exposed to different types of silver nanowires.

Chapter 4. In this chapter, we determined the dissolution of AgNPs amended to soil at different exposure doses and at different exposure times in the presence and absence of plants. Concomitantly, the associated impacts on soil pH, Ag accumulation in plants, and the alterations of the rhizosphere bacterial community were investigated.

Chapter 5. In this chapter, we investigated the trophic transfer of single AgNPs and TiO₂NPs as well as the trophic transfer of a mixture of AgNPs and TiO₂-NP from lettuce to snails and their distribution in snails. Furthermore, the adverse effects of single NPs and the mixture of AgNPs and TiO₂NPs on snails associated with food chain transfer were compared over time.

Chapter 6. The main findings of the thesis are summarized; the challenges and future perspectives towards nanoecotoxicology and risk assessment are discussed.