FATE AND EFFECTS OF ENGINEERED NANOPARTICLES IN THE ENVIRONMENT AND NEW APPROACHES FOR THEIR RISK ASSESSMENT

Ph.D. THESIS

Emel TOPUZ

JUNE 2015

VRİJE UNIVERSITEIT

FATE AND EFFECTS OF ENGINEERED NANOPARTICLES IN THE ENVIRONMENT AND NEW APPROACHES FOR THEIR RISK ASSESSMENT

Ph.D. THESIS

Emel TOPUZ

Promotor: Prof. Dr. Nico van Straalen
Copromotor: Dr. Cornelis A.M. van Gestel

JUNE 2015

Table of Contents

“The most genuine mentor in life is science.”

Mustafa Kemal Atatürk

CHAPTER 1
INTRODUCTION

The needs for modern life of human beings are resulting in demands for rapid technology and product development strategies for industries. The numbers of the products those are highly promising with advanced functionalization to satisfy the demands of consumers, have been increasing dramatically since the last decade. The term “emerging contaminants” has been introduced to the environmental literature in order to indicate new potentially hazardous pollutants. An emerging contaminant is defined as a chemical or material that is characterized by a perceived, potential, or real threat to human health or the environment or a lack of published health standards (EPA, 1999). A contaminant may also be defined as “emerging” because a new source or a new pathway leading to human exposure has been discovered or a new detection method or treatment technology has been developed (DoD, 2009). Among these emerging pollutants, health and personal care products (PPCP), pharmaceuticals and endocrine disrupters, perfluorinated compounds, disinfection byproducts and nanomaterials have gained most attention in the literature. Nanomaterials differ from other conventional pollutants as well as emerging pollutants due to their physicochemical characteristics depending on their nanoscale and may need more thorough investigation than other pollutants.

Nanomaterials are materials or products that are scaled between 1 and 100 nm (EPA, 2007). As the knowledge on nanomaterials has been improving since 2007, the European Union suggested the following more detailed definition: “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 nm-100 nm” (EU 2011). Engineered nanoparticles (ENPs) are new for the environment, since they are not natural and manufactured with different production technologies to add different functions to various kinds of products and their usage in products and so occurrence in the environment started almost a decade ago.

1.1 Definition of the Problem

Physicochemical properties of ENPs, such as reactivity, strength, electrical properties, and optical characteristics, may differ from those of micro/macro sized particles because of their nanoscale that provides high surface area and quantum effects (Farre et al., 2011). Because these properties make them highly attractive and suitable for many different new technological applications, the use of ENPs in nanotechnology has increased all over the world. However, the ENP product inventory database is limited. Although there are some local inventories, well-organized databases are lacking because of the absence of regulations for ENP declaration in the products. There are two premier databases (www.nanowerk.com and www.nanoproject.org) for the amount and type of ENPs and their usage data based on product categories. Therefore, it is difficult to make an analysis in terms of environmental release (Holden et al., 2014). Moreover, analytical measurements of ENPs in the natural environment are very scarce due to technical difficulties in detecting and identifying nanoparticles in complex environmental matrices like surface waters, soils and sediments (Gondikas et al., 2014).

Every year, a huge number of ENP products are being released to the market and most of the them are in the health and fitness category (about 50%) including personal care products, clothing, cosmetics, and sunscreens, which enter municipal wastewater streams directly as well as industrial wastewater streams (Nanotechproject, 2013). The most widely used ENPs in terms of number of products are Ag and TiO_2. AgNPs are mostly used in textiles (30-50%) and TiO_2 NPs in cosmetics (70-80%). AgNPs show a 20 fold increase in the number of the products they are used in (from 2006 up to 2014) (Royce et al., 2014). In addition, various kinds of coating materials are used for AgNP production, which can lead to completely different characteristics. Citrate, sodium borohydride ($NaBH_4$), and polyvinylpyrrolidone (PVP) are the most frequently used capping agents for AgNPs (El Badawy et al., 2010). More than 10,000 tons/year of TiO_2 NPs are used in products (Piccinno et al. 2012). As the number of products that contain ENPs have been increasing dramatically since 2006 (about 521%), the need to understand the release of ENPs from products to the environment and conducting Environmental Risk Assessment (ERA) has become an urgent issue. Various information and data including release from products, their fate, as well as, their effects in the environment are required to apply ERA. However, all of these processes are highly complicated and depend on various factors.

ENP-containing products may lead to release of nanomaterials to the environment during and after use and depending on their physicochemical characteristics. Hence, the sources of ENPs in the environment might be increasing accordingly depending on the treatment efficiency achieved in municipal wastewater treatment systems and landfilling facilities. Due to their characteristics specific to their nanoscale, the size distribution or shape of ENPs entering the environment may differ from their original ones. Therefore, product specifications will not be sufficient for the characterization of the ENPs that are released to the environment from products. As a consequence, the sources, release pathways, fate and transport before entering the environment need to be understood to be able to perform an environmental ERA of ENPs.

Quantification of the emission of ENPs will not be sufficient for assessing their hazards and the possible exposure and effect pathways for human and ecosystem, since their fate in environment will be affected by numerous characteristics, such as particle size, size distribution, particle shape and structure (Farre et al., 2011). The physicochemical properties of ENPs are important in order to evaluate their fate and transport in the environment. Chemical composition, mass, particle number and concentration, surface area concentration, size distribution, specific surface area, surface charge/zeta potential, stability, solubility, and nature of ENPs are universally agreed properties that are essential for a proper assessment of ENPs (Klaine et al., 2008).

In conclusion, there is a need to implement ERA for ENPs. Proposed ERA approaches should apply tools that decrease uncertainties about release, fate and effects and compensate for the gap in data and information about various aspects of ENPs. Since the current knowledge of ENPs enables the usage of retrospective ERA, studies focusing on the possible relations between key release/fate processes or effects and ENP or environmental characteristics should be conducted for precise predictions.

1.2 Environmental Risk Assessment

The environmental risk assessment of conventional chemicals is regulated at national and international levels. However, the need for environmental risk assessment of ENPs is of relatively recent concern due to their short history in product manufacturing. Usage of ENPs has been increasing dramatically in recent years and their potential hazards are not known yet at all (Bleeker et al., 2013). In addition, risk communication, integrated with risk management (Kuhnel, et al., 2014), through regulators, managers and the public, is troublesome due to the scarcity in quantitative risk assessments (Klaine et al., 2012). Basically, ERA consists of 4

steps including hazard identification, exposure assessment, effect assessment and risk characterization (Van Leeuwen and Vermeire, 2007). The Predicted environmental concentration (PEC) and the Predicted No-Effect Concentration (PNEC) are determined by exposure and effect assessment, respectively. Risk is characterized usually with risk quotients which are derived as the ratio of PEC and PNEC. If PEC/PNEC is higher than 1, it is assumed there is risk. This PEC/PNEC approach requires data and information about the release, environmental fate and effects of ENPs. Risk classification, which is the transition step between ERA and risk management (Van Leeuwen and Vermeire, 2007), however, is not possible since the risk quotients only inform about the presence of a risk.

Such PEC/PNEC based risk assessment is not applicable yet due to the high variation in outcomes on ENP fate and effects among the studies. Moreover, bioavailable fractions and modes of action also influence the reliability of hazard assessments (Holden et al. 2014). Comprehensive environmental assessment, risk characterization methods and decision analysis techniques are suggested for use in risk assessment studies to decrease uncertainties (Miseljic and Olsen, 2014, Schauman et al., 2014, Gajewicz, et al., 2012, Gotschalk et al., 2011) and to identify urgent research needs (Linkov et al., 2009). ENPs with similar physicochemical properties or biological effects should be classified and managed with detailed decision trees (Hunt et al., 2013). Since the low concentrations of ENPs in the environment or the lack of adequate measurement methods prevent the usage of retrospective risk assessment, a framework for the prognostic risk assessment of ENPs is needed based on the new developments in scientific knowledge (Quik et al., 2015). An approach for environmental risk assessment of ENPs should include emission sources in the environment, possible effects of the usage of ENPs, flexibility for adapting recent information and data, feedback for research areas and product improvements (Wiessner and Bottero, 2011).

As an alternative to the PEC/PNEC, approach Grieger et al. (2011) and Sorenson et al. (2010) applied a worst case scenario approach. Grieger et al. (2011) defined protected units (PU) and causes of risks (CR) and the worst case scenario was obtained by combining a protection-specification sub model, a risk decomposition sub-model and a worst-case composition sub model. All possibilities for each ecosystem component and the mode of action of the ENPs were elaborated in PU-CR diagrams, however, including the factors about the release of ENPs from products in the worst case definition could provide a more holistic approach. In addition, risk communication could be supported by obtaining a risk magnitude based on the combination of all PU-CR diagrams. Money et al. (2012) established the baseline model FINE

with expert commentary, which has a Bayesian nature. Risk quotients were achieved by combining the no observed effect concentrations for sediment and water and factors for particle behavior, exposure potential, general organism hazard and risk in these compartments. In addition to including expert opinions for factor assignment, risk characterization might also be applied using expert opinions. Moreover, the release of ENPs could be included in the factors to incorporate the likelihood of their occurrence in the environment.

To develop a predictive risk assessment framework and to enrich the existing approaches, multiple criteria decision analysis tools such as analytical hierarchy process (AHP) might be applied for the definition of the compartments related with risk besides using worst case scenarios and Bayesian tools. AHP may offer the solution for complicated problems in a systematic manner, which cannot be obtained with modelling (Saaty, 1988). AHP is based on 3 main principles including decomposition of the problem, comparison of the factors in the hierarchy and analyzing the priorities of these factors (Saaty, 1994). Therefore, AHP may identify the factors related with environmental risks of ENPs and define the relation between all factors. Impact characterization of ENPs including release, fate and toxic effects on ecosystems (Miseljic and Olsen, 2014) can be performed with AHP tools. Linkov et al. (2007) implemented an AHP approach by taking into account human health and environmental effects, social importance and stakeholder preference to select the best nanomaterial for usage in products. However, this method treats environmental risk assessment as a criteria to make selections, and does not provide detailed factors such as causes of the risk to be evaluated and to quantify the risk.

Since one of the main concerns for ERA of ENPs is the lack of data and information, expert judgement for the evaluation of the factors and combining the characterization components (Krueger et al., 2012), using fuzzy scales and fuzzy inference rules, respectively, can be incorporated into risk assessment frameworks. Fuzzy scales show to what extent a member (such as risk factor) belongs to a defined set (such as risk) (Zadeh, 1965), which can provide the conversion of linguistic expert judgements into fuzzy numbers with membership degrees and help decreasing uncertainty (Darbra et al., 2008). Membership degrees are scaled with specific functions like triangle or trapezoidal. Standard Trapeozidal Numbers (STFN) is optimistic with a wider membership functions (Gentile et al., 2003). Fuzzy inference may help developing a rule-based scheme with ‘if…then…’ clauses which include the main components of the desired quantification. The inputs of the rule base are fuzzified with membership functions such as STFNs and the relation between inputs and the desired output

is defined with rule-dased models (Musee et al., 2006). One of the most commonly used models is called as “min-max method”. Membership degree of the rule is selected as the minimum membership degree from the inputs. Maximum operator is used to combine all of the rules for desired output (Jang et al., 1997). A fuzzy model for risk assessment of organic pesticides in aquatic ecosystems (Liu rt al., 2013), a soil protection index mapping applied with fuzzy rule base (Oinam et al., 2013) and minimization environmental risk of landfilling site with the combination of fuzzy models (Aydi et al., 2013) are among recent studies where fuzzy models are applied for ERA. Rule based scheme can be applied successfully for ERA of ENPs. Risk magnitude may be estimated by developing a rule-based scheme of the main components selected for the assessment of the risk.

1.3 Release of Ag and TiO_2 NPs from products and their occurrence in the environment

Release of Ag and TiO_2 NPs from products

For ERA studies, knowledge on the releases of ENPs from products is crucial in order to determine the likelihood of their occurrence in the environment. However, there are uncertainties about the release processes because of the gaps in data on main release sources (manufacturing process, production process and usage period), on released forms of ENPs and for simulating realistic conditions (Gottschalk and Nowack, 2011). In addition to the release process, the form of ENPs released also has high importance. Release processes can lead to alterations in ENP composition and properties (Gottschalk and Nowack, 2011), so for ERA the form of ENPs that ecosystem components could be exposed to has to be considered.

Since AgNPs and TiO_2 are widely used in daily consumer products such as personal care products, textiles etc., most studies described in the literature focused on the release from textiles to washing water solutions and sweat. These studies agree on the critical role of the manufacturing process in the variation in the amounts and size of released ENPs (Ben and Westerhoff, 2008; Geranio et al., 2009; Benn et al., 2010; Windler et al. 2012; Quadros et al., 2013). The total amount of Ag released from different fabrics to the washing solution showed high variation such as up to 1.36 mg Ag/g sock released in tap water (Ben and Westerhoff, 2008) or from 0.3 to 377 (1-45%) mg Ag/g fabric released in simulated washing water (Geranio et al., 2009). The release of TiO_2 from the product functionalized for antimicrobial purposes (5 mg/L) was much higher than from product functionalized for UV-protection (von Goetz et al., 2013; Windler et al., 2012). Also the size of the AgNPs released from products showed large variation, e.g. it was in ranges of 10-500 nm (Ben and Westerhoff, 2008),

mostly >450 nm (at least 50%) (Geranio et al., 2009), <450 nm or mostly <20 nm (Ben et al., 2010). The dissolved Ag fraction ranged from <0.01% (Benn et al., 2010) to 2% (Hedberg et al., 2014).

Conditions of the medium in which Ag release occur lead to alterations in form and amount of Ag released (Tejamaya et al. 2012, Gondikas et al. 2012, Kennedy et al. 2012). Released Ag in sweat was as much as 38% on a mass basis in the study of Quadros et al. (2013), while Hedberg et al. (2014) observed a decreased release of Ag in washing solution when AgNPs were added to model laundry detergent after they were exposed to artificial sweat. Excessive Ag release occurred because of catalysis of the dissolution by Cl^- (Quadros et al., 2013) and dissolved Ag forms mostly included Ag-chloro complexes (0.34 mg/L solubility) (von Goetz et al., 2013). In addition to Cl^-, the presence of bleaching agents (H_2O_2 or peracetic acid) in washing solution promotes the dissolution of Ag (Geranio et al., 2009). On the other hand, the presence of detergents with zeolites (Hedberg et al., 2014), amine or carboxyl groups in the washing solution, high pH and low dissolved oxygen conditions (Quadros et al., 2013) limit the dissolution of Ag. AgNP characteristics such as surface coating also play a role in Ag release. Electrostatically charged AgNPs could lead to higher Ag release during the first times of washing because of their weak bonds on the surface of Ag (Hedberg et al., 2014). In addition to these factors, usage habits may have an impact on Ag release from the products. Increased release of Ag from blankets after several washings, for instance, could be due to the tear of fibers during usage or UV exposure of the AgNPs (Quadros et al., 2013).

AgNPs and especially TiO_2 NPs are commonly used in building paints for anti-microbial or coloring purposes. Released TiO_2 and pristine TiO_2 NPs behave differently (Al-Kattan et al., 2014), so studies with pristine NPs cause uncertainties about the fate of these ENPs in the environment. Al-Kattan et al. (2013) applied 113 cycles of weathering, which included 3 hours of UV light, 0.5 h of irrigation and 2.5 h of drying. The released amount of TiO_2 was highest during the first cycles (up to 1.5 µg/L), and the concentration became constant at around 0.7 µg/L (0.007% of TiO_2 on a mass basis) after 10 cycles. The size of released TiO_2 was around 100 nm and the concentration of TiO_2 particles with a size of over 450 nm was below 0.2 µg/L. Release of TiO_2 was higher for plaster surfaces (6 µg/L) than for cement fibers (<2 µg/L). Although UV exposure increased the release of TiO_2 from plaster and cement surfaces due to the degradation of organic coatings with photo catalytic activity, it was not significant even after 1 year. According to the authors it is not possible to extrapolate this data to 20 years, which is the average time for renewal of paintings. So the possibility of paint

degradation and release of TiO_2 should be kept in mind for ERA. In their further study, Al-Kattan et al. (2014) showed that in the presence of 3 mM Ca^{2+}, TiO_2 was aggregated to particle with sizes of more than 450 nm and ended up in the sediment; however, the presence of Natural Organic Matter (NOM) did not affect their stability. Kaegi et al. (2008) analyzed the facade run off of TiO_2 from a building and urban runoff. The particles were around 150 nm on average. TiO_2 release was much higher from new façade (600 µg/L) than from 2 years aged facades (8 µg/L). Urban runoff was resulted in a TiO_2 concentration of 16 µg/L, with particles having a larger size distribution which shows the possible contribution of other road or façade coatings.

In conclusion, the amount, size distribution and form of released ENPs are crucial for their occurrence in the environment and highly dependent on the manufacturing way of product, conditions of the medium to which release occurs, characteristics of the ENPs and the usage habits for the product. In addition to the high variation in characteristics of ENPs, the variations in these factors also contribute to uncertainties for ERA.

Occurrence of Ag and TiO_2 NPs in the environment

Detection of ENPs in natural environmental media poses some difficulties and appropriate analytical methods still have to be developed (Praetorius et al., 2012). Therefore, most of the existing studies are based on modeling approaches that rely on predictions of the release of ENPs from products and their transformation/transportation on the way to the environment (Boldrin et al., 2014; Benn et al., 2010). Considering the common usage of ENPs in daily consumer products, they have a high potential to end up in Wastewater Treatment Plants (WWTPs) and/or Municipal Solid Waste Disposal Facilities (MSWDF) before entering the environment. Therefore, addressing the fate of ENPs in these facilities, including their possible transformation and partitioning between water and solid phases, may provide an idea about their occurrence in aquatic and soil media. This may help determining the possible amount and form ending up in the environment and can be contributory to ERA studies on ENPs.

The percentages of ENPs (used in personal care products) that will end up in WWTPs and landfills were estimated as 28-32% and 36-43%, respectively, based on consumer surveys considering use and disposal habits. The amounts of Ag and TiO_2 that are produced globally are 452 and 88000 tons/year, and 200 and 47700 tons/year, respectively end up in WWTPs (Keller et al., 2013). The studies about the fate of Ag and TiO_2 NPs agree on the partition of these ENPs to the sludge phase (>90%) in WWTPs (Barton et al., 2015; Kirkegaard et al,

2015; Hendren et al., 2013; Kaegi et al., 2011; Westerhoff et al., 2011; Gottschalk et al.,2009; Blaser et al., 2008; Boxall et al., 2007). Modeling studies predicted WWTP effluent concentrations in the range of 1.4-8 ng/L for AgNPs (Barton et al., 2015; Benn et al. 2008). However, the range of estimate was much wider in the study of Lazareva and Keller (2014), being 0.003-0.26 and 1.33-43.88 µg/L for AgNPs and TiO_2 NPs, respectively, in WWTP effluent. The same study predicted biosolids concentrations of 0.18-2.01 mg Ag/kg biosolid and 70-367 mg TiO_2/kg biosolid. AgNPs were mostly transformed to Ag_2S due to the reducing conditions present in the WWTP, which is less toxic (Hendren et al. 2013; Kaegi et al., 2011; Kim et al., 2011; Levard et al.,2011). Therefore, redox transformations are fairly effective for Ag, however, generation of core shell structure, dissolution and subsequent transformation is also possible (Dale et al. (2013). TiO_2 seems to be affected only by partitioning to the solid phase due to its high insolubility and undergoes negligible redox transformation (Barton et al., 2015).

Leachate seems to be most relevant pathway for the exposure of ecosystem components via landfill facilities (Marcoux et al., 2013). Reduction, dissolution/precipitation and adsorption/desorption processes can influence the release of ENPs from landfill areas to leachate (Boldrin et al, 2014). Most of the ENP fractions were aggregated and large enough to partition to the solid phase (63 and 71% of Ag and TiO_2, respectively) and the leachate solution consisted 7.9% and 8.7% of Ag and TiO_2, respectively. Dissolved Ag and TiO_2 were less than 1% in leachate solution. Ag was present in leachate in ionic form or bound to ammonia or chloride ($Ag(NH_3)_2^+$; $AgCl_2$ and $AgCl_{(aq)}$) when sulfide concentration was lower than 5 µg/L, otherwise Ag interacted with hydrogen sulfide or sulfide ($AgHS_{(aq)}$; AgS^-). Chloride was not as effective as sulfide and ammonia in affecting the speciation of Ag. Electrostatic adsorption and filtration can decrease the transport of ENPs through porous media, however, adsorption onto colloidal matters may promote mobility (Hennebert et al., 2013). Design, operation, composition, depth, and age of the landfill and precipitation rates can influence landfill leachate and cause temporal and spatial variations in the occurrence of ENPs in the environment (Bolyard et al., 2013). The fate of ENPs in leachates needs to be investigated in terms of agglomeration and interaction with other waste components especially under anaerobic conditions (Marcoux et al., 2013).

WWTPs, landfill facilities, and run-off are the main sources of the ENPs and their transformation products in the environment. Not only the release from products but also the fate of ENPs in waste treatment and disposal facilities are quite complicated process, making

it hard to predict the amount and form of ENPs that may occur in the environment and lead to uncertainties for ERA.

1.4 Fate of AgNPs and TiO_2 in the environment

The fate of AgNPs and TiO_2 NPs in the environment is closely related with their exposure potential and bioavailability. Exposure potential of organisms is determined by ENP behavior which is influenced by agglomeration, dissolution, advective transportation, sedimentation, and sediment resuspension (Garner et al., 2014; Praetorius et al., 2012). Unlike for organic chemicals, the interaction of the ENPs with the suspended material in the aquatic environment cannot be characterized by a using sorption coefficient (k_d) which is calculated from the octanol-water partition coefficient, because ENPs are not thermodynamically in equilibrium with the water and solid phase (Lowry et al., 2010). ENPs are just present as thermodynamically unstable suspensions in the water (Handy et al., 2008).

ENP specific characteristics, such as coating material, size, surface energy, and heteroaggregation, have an important role in fate processes (Praetorius et al., 2012). The high surface energy of ENPs leads to aggregation (Handy et al., 2008) and results in low exposure for pelagic aquatic species and a limited reactivity due to the reduced transportation and surface area. On the contrary, dissolution provokes mobilization and exposure of aquatic species (Garner et al., 2014). ENPs collide with suspended matter depending on their diffusion coefficients and surface properties and the properties of the suspended matter. So, their interaction can be called heteroaggregation (Quik et al., 2012). Heteroaggregation is expected to be more dominant in natural water (Quik et al., 2014), except for the discharge points where higher concentrations of ENPs may be observed (Garner et al., 2014). Attachment efficiency is used to evaluate homoaggregatıon of ENPs in natural water. Recent modeling approaches are using the heteroaggregation constant (k_{het}), which is the product of collision efficiency (K) and attachment efficiency (α). However, collision efficiency is not constant because of the variable natural colloid and natural water characteristics (Quik et al., 2015). ENP and other particle concentrations, pH, ionic strength and temperature have an impact on attachment efficiency (Arvidsson et al., 2011).

In addition to the ENP specific characteristics, water chemistry is highly effective for the fate and transport of ENPs in the aquatic environment (Behra et al., 2013). The pH, water temperature, concentration of different ions, and dissolved organic carbon (DOC), sulfide, dissolved oxygen and chloride concentration are important characteristics of the environment

affecting the behaviour of ENPs (Garner et al., 2014; Fabrega et al., 2011; Kennedy et al., 2010; Liu and Hurt, 2010; Chen and Elimlech, 2009; Cumberland and Lead, 2009; Fabrega et al., 2009).

When the ionic strength, especially Ca^{2+} and Mg^{2+} concentration, is higher; it leads to suppression of the electrostatic double layer which decreases the repulsive energy and results in ENP aggregation and sedimentation (French et al., 2009; Handy et al., 2008). Changing metal speciation, altering the surface charge of particles, sorption to mineral surfaces, interfering with mineral dissolution/precipitation reactions, and driving redox and photochemical reactions are the effects of DOC on the fate of substances in the environment (Aitken et al., 2006).The presence of DOC affects the behavior of ENPs by changing their surface charge (Zhang et al., 2009) or providing an adsorption surface (Keller et al., 2010). DOC binds to the ENPs and enhances their stability in the aquatic environment due to steric or electrostatic repulsion. (Chen and Elimelech, 2007; Chen et al., 2007; Chen et al., 2006). Humic acid macromolecules facilitate bridging in the presence of multivalent cations such as Ca^{2+}, resulting in enhanced aggregation (Chen et al., 2007). Different types and concentrations of DOC and cations may have different effects on the stability of ENPs, therefore on particle size distribution which changes their availability and mechanism of action for aquatic organisms.

The behavior of ENPs in soil may be more complicated because of the role of soil particles, which are negatively charged and may interact with ENPs for processes such as agglomeration or dissolution (Tourinho et al., 2013). The first thing to consider for the behaviour of ENPs in soil is to determine in which compartment (stationary phase and pore water) they will partition. Subsequently, the fate of ENPs can be evaluated in more detail in the most relevant phase to determine their bioavailability. Particle size is also very important for the mobility of ENPs in soil. Larger particles can be trapped in between soil particles depending on the pore size of the soil, smaller particles will be mobile pore water (Darlington et al., 2009). Soil related properties such as organic matter content, mineralogy, activity of sulfur species, pH, redox potential and water content are suggested to also be of importance for the transformation and fate of AgNPs in soil (Vandevert et al., 2014).

AgNPs

For AgNPs, aggregation, dissolution and metal-ligand binding modifications are critical transformation processes (Gondikas et al., 2012). These processes therefore also determine their exposure potential to organisms and are significantly influenced by ENP and

environmental characteristics. Coating materials are the major characteristics of AgNPs which mainly influence their fate and toxicity. Surface charge, aggregation and toxicity of the AgNPs are strongly affected by these coating materials (Levard et al., 2012). Citrate-coated AgNPs dissolved three times faster than PVP-coated AgNPs. After 48 hours, the dissolved Ag fraction was 36% and 47% for citrate and PVP-coated AgNPs, respectively (Gondikas et al., 2012). Dissolution is mostly affected by water chemistry (electrolyte composition, ionic strength, redox environment, pH, DOC). Oxidation is the major source of ionized Ag in the aquatic environment and dissolved oxygen (O_2) is the critical factor affecting this process (Kittler et al., 2010). However, complete dissolution of AgNPs is difficult due to the slow kinetics of the oxidation process (Ma et al., 2012; Liu et al., 2010; Nel et al., 2009). For AgNPs smaller than 20 nm, oxygen enhances surface oxidation and hence the dissolution process (Martinolich et al., 2012). Chloride rich environments increase the dissolution of AgNPs, as well (Kaegi et al., 2011; Levard et al., 2011; Liu et al., 2011 Li et al., 2010). Organic matter or ligands adsorb on AgNPs and change surface properties. Organic matter containing sulfhydryl bonds (thiols) may increase the solubility of AgNPs by competing with inorganic ligands (Adams and Kramer, 1999). Sulfhydryl bonds are usually found in low molecular weight organic matter such as humic acids and found in nanomolar or micromolar concentration levels in water and sediments depending on redox potential, presence of organisms that excrete thiol containing compounds and catalysts of thiol oxidation (Le Faucheur et al., 2005; Ciglenecki et al., 2000; Bell and Kramer, 1999). There are some studies about the dissolution of AgNPs in natural environmental samples. Chlorinated tap water causes almost complete dissolution in a few hours (Loosli et al., 2015). Smaller sized AgNPs usually dissolve slower than larger ones, in 24 hours, and there is no difference between different coating materials. Dissolution was higher in tap water than creek water and dissolution was slower after 24 hours (Mitrano et al., 2014). Solubility of AgNP-PVP was lower than 1% in natural water except for sea water (3%) (Angel et al., 2013). İncrease of chloride concentration results in the formation of AgCl(s) complexes which promotes agglomeration because of bridging effects. However, based on the equilibrium conditions, the most dominant form becomes $AgCl_x^{(x-1)}$-complexes which are less soluble in aquatic media than Ag ions (Chambers et al 2014). Considering transformation, Ag can be found in the form of Ag sulfide as clusters or complexes adsorbed on organic sulfides (cysteine and glutathione) that can bind Ag in the interstitial water of sediments (such as in River Rhine). Ag can also be found as Ag sulfides (Blaser et al., 2008).

Agglomeration (colloidal stability) is the major process affecting the fate as well as mobility and bioavailability of ENPs in the environment (Ottofuelling et al., 2011) due to their surface properties (Weinberg et al., 2011). Surface charge alterations of AgNPs due to ionic compounds influence their coagulation and settling kinetics (Gondikas et al., 2010; Mylon et al., 2004). Divalent cations compress the electrostatic double layer due to the screening of surface charge of citrate-coated AgNPs and lead to agglomeration (Chowdhury et al., 2012; Shih et al., 2012a; Ottofuelling et al., 2011; Domingos et al., 2010). AgNP-PVP is very stable owing to its coating material providing steric stabilization (Cumberland and Lead, 2009; El Badawy et al., 2010; Huynh and Chen, 2011; Kvitek et al., 2008; Thio et al., 2012; Zhang et al., 2012). However, dissolved organic carbon (DOC) may cause stabilization effects in natural water due to steric mechanisms (Chinnapongse et al., 2011; Delay et al., 2011; Gao et al., 2009; Thio et al., 2012). Although these studies report the effect of ions or NOM for different concentration ranges on the agglomeration of AgNPs, most of them are in synthetic media and more aimed at understanding mechanisms. For risk assessment, more concrete data on relations between ENP agglomeration and water chemistry would be beneficial. Moreover, verifying ENP agglomeration behavior and its relations in natural environments is required. Therefore, agglomeration studies need urgently be compared with dissolution studies which are relatively informative in terms of ERA for both synthetic and natural aquatic media.

TiO_2

When single TiO_2 NPs enter the aquatic environment, they tend to aggregate in order to decrease their interfacial energy which occurs due to their high surface energy (Mukherjee and Weaver, 2010; Yang et al., 2009). According to the modeling results of Praetorius et al. (2012), free TiO_2 concentrations in river water decrease over time, simultaneously its concentrations attached to suspended matter increase rapidly up to a maximum point and then decrease exponentially till the end of the river. TiO_2 concentrations in the sediment increase to several orders of magnitude higher than those in the river water.

Ion valance affects aggregation behaviour of TiO_2 NPs more significantly than pH. Large clusters of TiO_2 NPs were formed in the presence of Ca^{2+} ions (Chowdhury, et al., 2012). TiO_2 NPs are usually stable between pH 5 and 8, which are environmentally relevant values, however, pH is not effective in the agglomeration if it is higher than the point of zero charge (Garner and Keller et al., 2010). Humic acid affected ENP electrophoretic mobility more significantly in the presence of monovalent ion than divalent ions. Addition of humic acid decreased the ENP diameters at different ionic strengths. Aggregation could be irreversible

due to agitation and resuspension (Elzey and Grassian, 2010). Agglomeration of TiO_2 in freshwater and seawater was observed (>200 nm) after 0.2 and 50 hours, respectively, regardless of water chemistry. However, initial concentration was effective, with higher TiO_2 concentrations agglomerating more than lower concentrations, which should be considered as an uncertainty of scaling up for ERA studies. Concentrations of TiO_2 (0.01 and 0.1 mg/l) close to environmentally realistic conditions resulted in sedimentation (33.5-52.2%) (Brunelli et al., 2013).

The higher organic matter and clay content of the soil results in the higher adsorption of TiO_2 NPs, on the contrary, higher pH, zeta potential and ionic strength reduce adsorption (Kirkegaard et al., 2015; Vandevert et al. 2014; Fang et al., 2009). Acidic pH levels enhanced the transportation of TiO_2 particles in porous media in the presence of humic acid at concentrations as low as 0.5 mg/L. The mobility of TiO_2 NPs was reduced in the presence of >100 mM NaCl or 2 mM Ca^{2+} (Zhang et al 2015). However, NOM leads to the electrosteric stabilization by adsorbing onto ENPs (Fabrega et al., 2009) and may cause disagglomeration of TiO_2 NPs (Baalusha, 2009, Loosli, 2013).

Agglomeration is the major process for both AgNPs and TiO_2 NPs to be taken into account when predicting their speciation and bioavailability in the environment. However, existing studies are too diverse to define clearly which ENP and environmental characteristics are most representative for agglomeration predictions in natural environments. Both for AgNPs and TiO_2 NPs, a holistic and systematic evaluation, which relates the major environmental characteristics to agglomeration, would be useful to get an idea about their behavior and bioavailability in the environment. After making correlations of agglomeration with proper environmental characteristics, it would be possible to classify environmental bodies based on their water chemistry in terms of the agglomeration tendency of ENPs. Thus, feedbacks for ERA studies could be supplied for the major fate process of ENPs.

1.5 Toxicity of AgNPs and TiO_2 in the environment

Concentration levels that cause acute or chronic toxicity for single species or toxicity at the community level are essential for ERA studies to determine the strength of hazards. Toxicity of ENPs may vary depending on the species and endpoint (Riberio et al., 2014) and on the exposure environment. In addition to the magnitude of the toxic concentrations, estimation of environmental risks for ENPs is highly influenced by their intrinsic properties (size, coating, shape etc.) and environmental conditions (ionic strength, NOM etc.) which cause variations

and uncertainties for ERA. For example, the presence of other metals (Pb, Zn, Cd, Fe, Al, Ni and Cr) and NO_3^- reduced the toxicity of AgNPs and TiO_2 to zebrafish embryos, while Cl^- and PO_4 enhanced their toxicity (Pavagadhi et al., 2014).

AgNPs

Toxicity tests with AgNPs resulted in a large variation in L(E)C50 values (in mg/L) for various aquatic species such as crustaceans (0.01), algae (0.36), fish (1.36), nematodes (3.34), bacteria (7.10), yeast (7.90), mammalian cells (11.3), *Vibrio fischeri* (32), and protozoa (38) (Bondarenko et al., 2013). Acute toxicity of AgNPs, even with effects on survival, was observed at low concentration levels. The lowest LC50 for the toxicity of AgNPs to *Daphnia magna* was 1.1 µg/L (7 days) (Allen et al., 2010), the highest LC50 was 121 µg/L (<24 hours) (Volker et al., 2013). Bondarenko et al. (2013) collected aquatic toxicity data from the literature and determined the toxicity class of AgNPs according to EU-Directive 93/67/EEC (CEC, 1996), which uses the lowest median value for 3 key model species (crustaceans (*D. magna*), algae (*Pseudokirchneriella subcapitata*) and fish). Results showed that AgNPs are very toxic (<1 mg/L) to crustaceans. AgNPs are also toxic for the growth of *P. subcapitata* (chronic toxicity) at low concentrations (2-50 µg/L) (McLaughlin and Bonzongo, 2012; Wang et al., 2012). Ag is a unique ENP in terms of its toxicity due to the ionic form released in the aquatic environment. Ionic Ag affects functional groups in the cell membrane, like thiol (-SH), of higher aquatic life by interacting with proteins and enzymes and results in cell inactivation as the mode of toxic effect (Hiriart-Baer et al., 2006; Liau et al., 1997; Ratte, 1999; Wood et al., 1999).

Surface coatings of AgNPs lead to variations in toxicity (Croteau et al., 2011; Silva et al., 2014; Pavagadhi et al., 2014; Ahn et al., 2014). There are debates about the effect of size on the toxicity of AgNPs. While some studies report increasing toxicity with decreasing size of AgNPs (Cupi et al., 2015; Seitz et al., 2015; Ahn et al.,2014; Angelsfort et al., 2014; Allen et al., 2010), other studies did not observe any size-related effect (Gaiser et al., 2011; Kennedy et al., 2010). Azurin metalloprotein activity is affected by AgNPs because of dissolution of Cu(II) from the enzyme surface, which is affected by aqueous Ag salts and the dissolution product $Ag(I)_{(aq)}$.

Ag speciation is important in order to determine to which Ag forms aquatic organisms will be exposed, since dissolution of AgNPs results in the release of ionic Ag (Lee et al., 2004; Fortin and Campbell, 2000). There are still uncertainties about whether AgNPs themselves are toxic to bacteria or that ionic Ag released in the water causes the toxic effects (Shahverdi et al.,

2007; Panacek et al., 2006; Morones et al., 2005; Sondi and Salopek-Sondi, 2004). While (Navarro et al., 2008b) supports the idea that AgNP toxicity results from the ionic Ag form released from the ENPs, (Fabrega et al., 2009a) showed evidence that AgNPs have specific effect on toxicity. Yin et al. (2011) also supported this idea by showing that gum arabic coated AgNPs have greater effects on *Lolium multiflorum* than $AgNO_3$.

There are two mechanisms for the toxicity of AgNPs to bacteria. One of them is oxidative stress generated by the formation of reactive oxygen species (ROS) such as oxygen superoxide (O^{2-}) that can potentially be formed at the surface of the AgNPs (Choi and Hu, 2008; Hwang et al., 2008; Kim et al., 2007). The second one is the interaction of Ag^{+} ions with thiol groups of vital enzymes and proteins, affecting cellular respiration and transport of ions across membranes, ultimately leading to cell death (Bottero et al., 2011; Ratte, 1999). Toxicity of AgNPs to Gram-positive *Bacillus* species was affected by surface charge, showing that low zeta potential leads to an increase in bacterial growth inhibition. Negatively charged AgNPs attached to cell walls of the bacteria (El Badawy et al., 2010). Arabic gum and citrate-coated AgNPs inhibited *Nitrosomonas europaea* at 2 mg/L in nitrogen removal in activated sludge systems due to colloidal stability and Ag^{+} formation. PVP-coated AgNPs showed no effect on nitrification (Arnaout and Gunsch, 2012).

Toxicity can be decreased in the presence of NOM, which provides stabilization of the AgNPs. Therefore, the presence of NOM should be considered in terms of fate and toxicity of AgNPs (Seitz et al., 2015; McLaughlin and Bonzongo, 2012). Aggregation, dissolution and toxicity can be affected by the presence of anions such as citrate, sulfide and thiosulfate. Ag_2S was formed on the surface of AgNPs, resulting in the inhibition of surface oxidation and therefore inhibiting dissolution. In addition, released Ag ions can be bound by sulfides (Angel et al., 2013). However, the effect of dissolution is another issue of debate for predicting the toxicity of AgNPs. The existing studies do not agree at all whether the toxicity is due to dissolved Ag ions (Kim et al., 2011; Zhao and Wang, 2011, Kennedy et al., 2010) or nano-specific properties of the AgNPs (Das et al., 2013; Griffitt et al., 2008).

Jung et al. (2015) applied a high throughput study with algae and with the nematode *Caenorhabditis elegans*, including as endpoints food consumption at the population level and body length, locomotion speed and lifespan at the organism level. AgNPs were toxic and TiO_2 was safe for *C. elegans*. Algae were the mostly affected group; they are the primary producers of the ecosystem and food source for higher trophic levels. Therefore, direct structural changes in the ecosystem or indirect effects due to the change in water quality could be

observed (Bondarenko et al., 2013). Van der Ploeg observed reduced population growth of earthworms (*Lumbricus rubellus*) in the presence of AgNPs at concentrations up to 154 mg/kg dry soil. Carbone et al. (2014) found that AgNPs (10 mg Ag/kg dry soil) induced the microbial biomass and changed the microbial community in a forest soil after 60 days exposure.

Soil toxicity of ENPs is limited compared to aquatic toxicity for which various kind of organisms such as *Lemna minor* (Gubbins et al., 2011; Navarro et al., 2008b), algae and fungi (Navarro et al., 2008a), vertebrates (zebra fish) (Asharani et al., 2008; Chio et al., 2012), invertebrates (*C. elegans*) (Meyer et al., 2010; Roh et al., 2009), bacteria (*Escherichia coli)* (Dror-Ehre et al., 2009; Sondi and Salopek-Sondi, 2004); *Pseudomonas putida* (Fabrega et al., 2009b) and human cells (skin keratinocytes, lung fibroblast cells, and glioblastoma cells) (AshaRani et al., 2009; Lu et al., 2010) were studied. Chronic toxicity (reproduction and growth) is more pronounced than acute (survival) toxicity. LC50 values were reported as >2000 (*Eisenia andrei*), and >1000 (*Eisenia fetida*) mg Ag/kg dry soil by Kwak et al., (2014) and Heckman et al., (2011), respectively. Reproduction was much more sensitive than survival with EC50 values of 225 (*Enchytraeus albidus*) and 146 (*E. fetida*) mg Ag/kg dry soil (Gomes et al., 2013, Schlic et al., 2013). However, avoidance toxicity was observed at very low concentrations (5.75 mg Ag/kg dry soil) for *E. fetida* exposed to AgNPs in the study of Shoults-Wilson et al. (2011), which shows that behavior of soil organisms could be significantly affected in the presence of AgNPs. TiO_2 NPs also reduced the reproduction of *E. fetida* at 1000 mg/kg dry soil (Heckman et al., 2011). No effects of AgNPs on survival and reproduction were observed for the soil arthropod *Folsomia candida* at concentrations up to 673 mg Ag/kg dry soil (Waalewijn-Kool et al., 2014). Longer exposure times are needed for more accurate evaluations since complexation or oxidation reactions in soil can be delaying the release of Ag ions and so, the toxicity (Gomes et al., 2013). Apart from these organisms, Shah et al. (2014) observed alterations in soil bacterial community in 120 days dıe to the presence of AgNPs and TiO_2 NPs.

AgNPs are estimated to have marginal risk (Som et al., 2011) and toxic effects have to be understood well for ERA. Although aquatic toxicity data and knowledge seem to be fairly satisfactory for predictive risk assessment studies, especially at the single species level, toxicity for soil organisms and the relation between toxicity and major environmental characteristics need further investigation for ERA studies. Since the data is limited mostly to earthworms, testing other species of soil organism would be useful to understand the species

sensitivity distribution for soil toxicity of AgNPs. *E. crypticus* are ecologically relevant soil organisms owing to their crucial role in decomposition and bioturbation in soils (Castro-Ferreira et al., 2011) and are commonly preferred for toxicity testing due to their sensitivity to the wide range of stressors (Didden and Römbke, 2011). Santorufo et al. (2013) showed that metal bioaccumulation in *E. crypticus* was higher than in *E. andrei* and *F. candida*. Therefore, testing AgNP toxicity to *E. crypticus* would be beneficial for ERA studies and fill a gap in the literature. Considering the significance of organic matter for the speciation of AgNPs in the soil, correlating toxicity with organic matter content of the soil would add more value for the prediction of toxicity for different conditions for the ERA studies. Moreover, it is also required to understand toxicokinetics and toxicodynamics of AgNPs. Most of the studies did not focus on the uptake of AgNPs over time, which is also essential to understand the appropriateness of the standardized toxicity test method.

TiO_2

TiO_2 NPs are known to be toxic to eukaryotic and prokaryotic cells (Trouiller et al., 2009; Adams et al., 2006; Long et al., 2006). Aquatic organisms such as microbes, algae, invertebrate and fish were affected adversely by TiO_2 NPs (Scown et al., 2010). However, TiO_2 NPs usually have much higher L(E)C50 than AgNPs. EC50s for the toxicity to *Daphnia magna* (immobilization) were 51 µg/L and 14 mg/L for Ag and TiO_2 NPs, respectively (Cupi et al., 2015). Chronic exposure of *D. magna* caused low mortality, however, it decreased reproduction (Fouqueray et al., 2012). Jcobash et al. (2014) reported that reproduction rate of daphnids was reduced over multiple *D. magna* generations for all TiO_2 NP concentrations (1.19-6 mg/L), and population collapsed after 5 generations of exposure to 1.78 mg/L TiO_2 NPs. Reproduction of *C. elegans*, which could be more suspicious to TiO_2 due to their tendency for sedimentation, was affected by TiO_2 NPs at 10 mg/L (Angelstorf et al., 2014). An increased effect of light on the toxicity of TiO_2 NPs was mentioned due to the production of ROS (Angelsfort et al., 2014). Photo-stable TiO_2 NPs have no adverse effects up to g/L levels (Aruoja , et al., 2008). In addition, genotoxic and cytotoxic effects of TiO_2 NPs on fish cells were determined (Handy et al., 2008; Vevers and Jha, 2008). Cellular change may occur due to the accumulated degraded nanoparticles in the cells (Lewinski et al., 2008).

Like in water, TiO_2 NPs are much less toxic to soil organisms than AgNPs. Reproduction toxicity was observed at 1000 mg/kg dry soil for *E. fetida* (Heckman et al., 2011, McShane et al., 2012, Canas et al., 2011).

1.6 Aim and scope of the thesis

Considering the needs for the ERA of ENPs, the main aim of this PhD study is to propose an ERA approach for ENPs that enables evaluating all representative factors for the risk, provides tools for reducing uncertainties and points out further research needs and risk management strategies with quantitative risk communication. AgNPs and TiO_2 NPs were chosen as model ENPs. Two different AgNPs, with citrate and PVP coating, were used to compare the effects of coating material. In the context of this main aim, the following objectives were studied to generate data or knowledge for the proposed ERA:

1) To determine in a systematic manner the most relevant water chemistry parameters for the agglomeration of model ENPs, which is the key fate process for the fate of ENPs?
2) To classify the aquatic environment based on the relevant water chemistry parameters for agglomeration by considering the possible temporal changes according to the results of ENP agglomeration behavior in natural water samples.
3) To evaluate the toxicokinetics and toxicodynamics of AgNPs in *E. crypticus* by investigating the relation between uptake and effects.
4) To determine the survival and reproductive toxicity of AgNPs to *E. crypticus* and the relation between toxicity and soil organic matter content and pH of the soil.

In order to achieve these aims, the following research questions were formulated:

1) Can we provide a systematic evaluation procedure with multi-criteria decision making tools and incorporation of expert judgements to both the evaluations of risk factors and risk characterization? Is the proposed approach applicable to the model ENPs?
2) What are the specific roles of commonly observed ions and natural/synthetic organic matter for the agglomeration of model ENPs in natural surface waters? Which parameters can be used for the prediction of the change in size over time of the model ENPs?
3) What is the correlation between the agglomeration of the model ENPs and selected water quality parameters? Is it possible to classify aquatic sources based on these parameters and does the classification change with time?

4) What is bioavailability of AgNPs? Does *E. crypticus* accumulate AgNPs over time? Is the effect of AgNPs on survival best explained from exposure concentration or from Ag body concentration? Does toxicity change with time of exposure?

5) At what soil concentrations do AgNPs affect survival and reproduction of *E. crypticus*? Is toxicity related with organic matter content and/or pH of the soil?

The objectives of the thesis are elaborated in the following chapters:

Chapter 1: After a brief introduction about ENPs, the problems triggered to determine the aim of this thesis are defined. The needs for a proper ERA approach for ENPs are discussed based on the conclusions and suggestions of existing studies. Existing knowledge on the release, fate and effects of ENPs was reviewed in order to determine data gaps and formulate the research questions for this thesis.

Chapter 2: A study was performed on the tools that are applied within the ERA approach that is proposed in this thesis. The study demonstrates the applicability of these tools.

Chapter 3: Selected anions, cations, natural organic matter (humic acid and fulvic acid) and synthetic organic compounds (sodium dodecyl sulphate and ethoxylates) that were commonly observed in surface water were tested for their specific effects on ENP agglomeration under environmentally realistic conditions. ENP agglomeration was characterized using the change in size as measured using dynamic light scattering and Nanosight Nanotrack. Then, the combinations of ions, natural and synthetic organic matter were tested in terms of ENP agglomeration behavior to compare with the specific effects of each parameter. Based on these comparisons, the parameters most related with the agglomeration of selected compounds were determined to be Ca^{2+}/Mg^{2+} and dissolved organic matter concentrations in the aquatic medium .All agglomeration studies were conducted for 1 hour, 1 day and 1 week to assess ENP agglomeration changes over time depending on the type of ENP. A surface water simulated media in terms of agglomeration was proposed which was validated by comparison with natural surface water samples.

Chapter 4: Based on the results of Chapter 3, six surface waters and three wastewater treatment plants were selected with different Ca^{2+}/Mg^{2+} and dissolved organic matter concentrations to determine its effect on the change in ENP size. ENP agglomeration experiments were conducted in unfiltered and filtered samples to have environmentally realistic conditions. Agglomeration characterization was performed using the same methods as in Chapter 3. Agglomeration of citrate-coated AgNPs correlated well with Ca^{2+}

concentration. However, dissolved organic carbon led to deviations at a certain concentration range. PVP-coated AgNPs were stable at their original size regardless of water chemistry. TiO_2 NPs agglomerated up to micrometer scale in most water samples after 1 week. Correlation of their agglomeration behavior with Ca^{2+} concentration was weaker than that of citrate-coated AgNPs. However, the correlation improved when dissolved organic carbon content was higher than 2 mg/L. The effect of dissolved organic carbon on the stabilization of TiO_2 NPs was more pronounced after 1 day. Fractionation of the samples based on molecular weight of the organic matter using ultrafiltration showed that agglomeration was much more pronounced for the fraction below 10 kDa than for unfiltered samples. Based on the correlations found, a classification scheme for the agglomeration of model ENPs in water over time was proposed.

Chapter 5: The uptake of AgNPs and $AgNO_3$ in *E. crypticus* was followed for 10 days. A background solution with essential elements was spiked to inert quartz sand to prepare the exposure medium. *E. crypticus* were exposed to AgNPs at different dose levels for different times (2,3,5,7,10 days). Survival of *E. crypticus* was determined, and sand, filtered sand solution and *E. crypticus* were analyzed for total Ag concentrations. Ag mostly adsorbed to the sand, with strongest sorption found for ionic Ag and PVP-coated AgNPs. Citrate-coated AgNPs gave much higher Ag concentrations in the solution than the other two Ag compounds. However, the LC50 was also higher for citrate-coated AgNPs, so it was less toxic. The other Ag compounds had similar toxicity. Accumulation of Ag was observed depending on time and external concentration. For all compounds, the LC50 decreased with time and reached steady state after 7 days of exposure. However, LC50 values calculated based on internal Ag concentrations in the enchytraeids were constant over time and could be considered more representative of toxicity regardless of time.

Chapter 6: Survival and reproduction toxicity of AgNPs and $AgNO_3$ to *E. crypticus* were determined in three different standard soils, namely Lufa 2.2, Lufa 2.3 and Lufa 5M, having different organic matter contents and different pH. The standard ISO (2004) guideline 16387 was used for the toxicity tests. Effects on enchytraeid survival were observed at concentrations higher than 500 mg Ag/kg dry soil for the AgNPs, while $AgNO_3$ was more toxic. Reproduction was more sensitive than survival and there was no significant differences in toxicity between AgNPs and $AgNO_3$. Toxicity decreased with increasing soil organic matter content, but was not affected by soil pH.

Chapter 7: An ERA approach for ENPs was proposed by using analytical hierarchy process (AHP) and fuzzy inference tools. Risk of ENPs were based on the occurrence likelihood (OL), exposure potential (EP) and toxic effects (TE). According to the principles of AHP, sub-factors that are related with OL, EP and TE were determined systematically and a hierarchical structure was developed considering the placing of comparable factors at the same level. A fuzzy scale was proposed to score the factors in the hierarchy using expert judgement. Sub-factors were scored based on their contribution to risk and compared in terms of their importance for the risk to obtain priority weights for the factors. Then overall scores were calculated with a weight-average method and converted to standard trapezoidal numbers. Fuzzy sets corresponding to the overall scores were determined using the proposed scoring scale. OL, EP and TE were combined with a fuzzy inference rule base using expert judgement to obtain the risk magnitude and the risk class based on the scale proposed. Three case studies were analyzed to demonstrate the applicability of the method. The case studies showed that this approach can provide more informative results since it gives the risk class which helps identifying the required risk management strategy. Moreover, the priority weights of the factors may give a clue about research needs or may help identifying which factor should be focused on to reduce the risk .

Chapter 8: The overall conclusions and suggestions for further studies will be summarized.

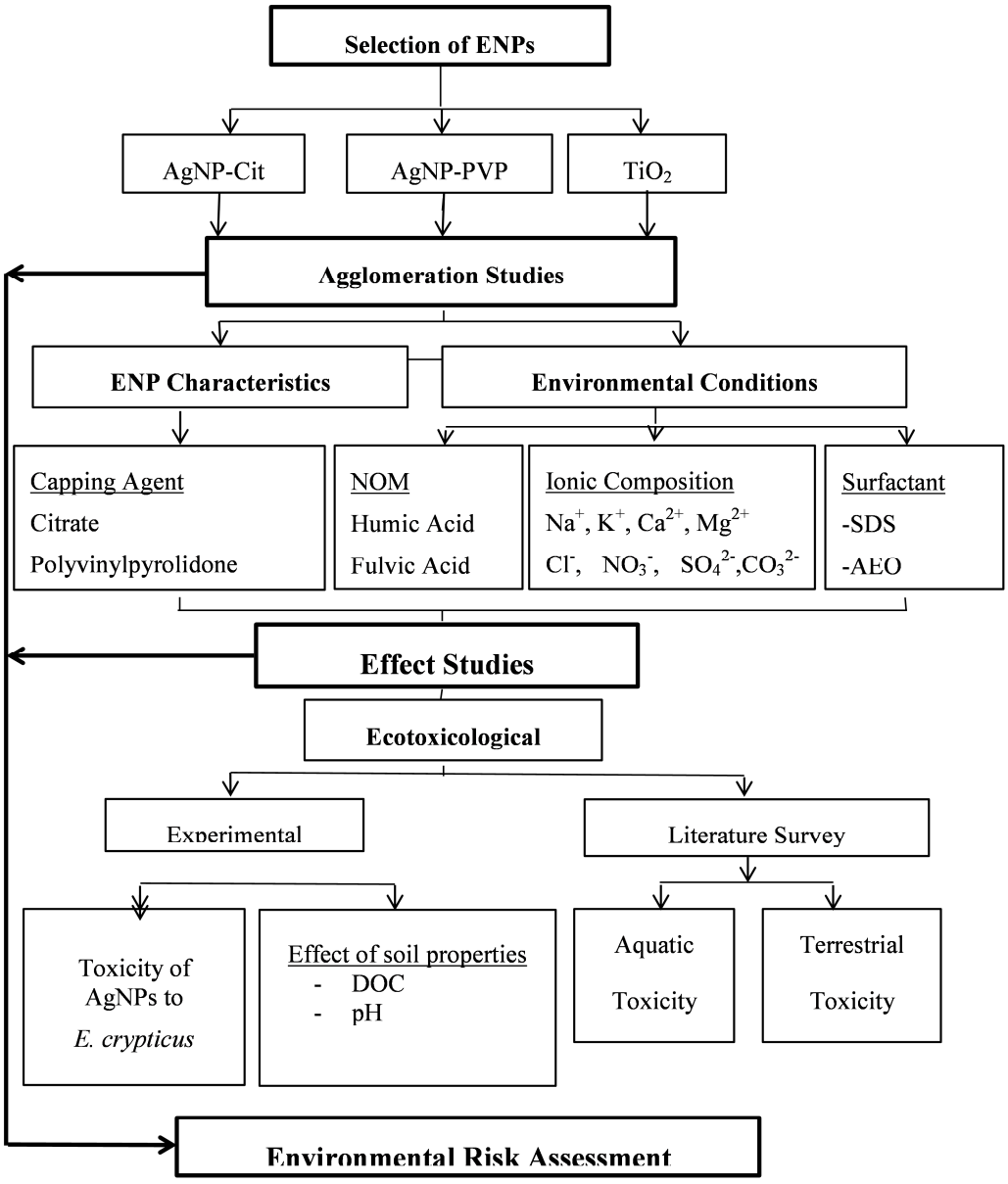


Figure 1. 1: A schematic view of the scope of the thesis.

CHAPTER 2

INTEGRATION OF ENVIRONMENTAL AND HUMAN HEALTH RISK ASSESSMENT FOR INDUSTRIES USING HAZARDOUS MATERIALS: A QUANTITATIVE MULTI CRITERIA APPROACH FOR ENVIRONMENTAL DECISION MAKERS

ABSTRACT

Environmental management, for which environmental and human health risk assessment is the first stage, is a requirement for industries both before construction and during operation in order to sustain improved quality of life in the ecosystem. Therefore, the aim of that study is to propose an approach that integrates environmental and human health risk assessment for industries using hazardous materials in order to support environmental decision makers with quantitative and directive results. Analytic hierarchy process and fuzzy logic are used as tools to handle problems caused by complexity of environment and uncertain data. When the proposed approach is implemented to a scenario, it was concluded that it is possible to define risk sources with their risk classes and related membership degrees in that classes which enables to decision maker decide which risk source has priority. In addition, they can easily point out and rank the factors contributing those risk sources owing to priority weights of them. As a result, environmental decision makers can use this approach while they are developing management alternatives for unfounded and on-going industrial plants using hazardous materials.

Emel Topuz, Ilhan Talinli, Egemen Aydin 2011, Integration of environmental and human health risk assessment for industries using hazardous materials: A quantitative multi criteria approach for environmental decision makers, Environment International, 37(2), 393-403.

2.1 Introduction

70,000 synthetic chemicals, most of which are hazardous materials, are used in industrial production processes, products and household goods and still, there is need for studies about environmental and human health risks assessments which are developed specifically for industrial activities (Moore, 2007). Also, European Union (EU) impose obligation of preparing Environmental Risk Assessment (ERA) for new and existing chemicals with REACH directive (EC, 2006).

Industries prepare their own environmental management systems (EMS) since Rio Declaration in 1992 (UNEP) which revealed the need of environmental management due to intensive industrial pollution. EMS are continuous loops which industries perform their activities based on Deming cycle (PDCA) in order to be able to fulfill environmental requirements related with them. As a result, environmental performances of those organizations are ennced by examining their selves continuously and improving their adverse conditions (Stapleton et al., 2001). Therefore, there are two main decisions that must be given in the context of EMS for industries. First one is the determination of environmental and human health hazards which are provided by ERA and human health risk assessment (HHRA) process and the second one is to specify the content of the management alternatives for industries by considering the results of ERA and HHRA process. Thus, they are basic tools that are essential and simplify the job of environmental decision makers for industrial organizations. Although ERA can be established as it will also contain factors that are necessary to evaluate HHRA to nearby public; it is needed to evaluate worker health risk because there are some specific factors affecting worker health. Clarifying factors that contribute environmental and human health risks systematically and having quantitative results in risk assessment process are the key points in order to determine environmental hazards and simplify the decision making process for developing management strategies. However, existing approaches do not exactly present an integrated risk assessment that considers all of the possible factors for environmental and human health risks of hazardous materials originating from industrial usage. Also, these approaches do not give quantitative results that can be used for decision making tools. Previous risk assessment studies focused on assessing risks either originating from routine discharges (planned emissions (PE)) (Di Marzioa, 2005) or accidents (accidental emissions (AE)) (Stam et al., 2000; Khan and Abbasi, 2001; Gunasekera and Edwards, 2003; Fabiano, 2005; Brown and Dunn, 2007; Andersson et al., 2007; Darbra et al., 2008; Wessberg et al., 2008; Verma, 2009; Clark and Besterfield-

Sacre, 2009) either in a non-quantitative manner or with simple predictive quantification (eg. PEC/PNEC) to a certain aspect of the environment such as water bodies (Stam et al., 2000; Holt et al., 2000; Hansen, 2007; Blaser et al., 2008), air media (Gunasekera and Edwards, 2006), soil (Khan and Abbasi, 2001; Andersson et al., 2007; Darbra et al., 2008, Wolf and Feijtel, 1998; Arunraj and Maititi, 2008; Li et. al, 2008) or groundwater (Li et al., 2007). In addition, there are studies that only focused on occupational safety and health for process industries (Papadakis and Chalkidou, 2008; Hassim and Edwards, 2006;). Also, most of the studies assessed the environmental risks based on processes in order to achieve most safety process design (Vassiliadis and Pistikopoulos, 2000; Gunasekera and Edwards, 2003; Sadiq et al., 2005; Khan et al., 2002); proposed approach in this study based on the risk source which enabled more accurate evaluation. Although Achour et al. (2005) developed a detailed risk assessment procedure for chemical industries; they did not use fuzzy numbers and inference system which enables reducing uncertainty and expert evaluation. Therefore, the aim of this study is to develop an integrated ERA and HHRA approach consisting risk factors related with all of the possible risk sources in an industry using hazardous materials and the environmental aspects, which has a holistic view and contains all of PE, AE, and indoor air emissions (IAE) in order to provide quantitative data for EMS tools which helps decision makers as well as achieve an integrated environmental concern. Identification, estimation, and hierarchisation phases are necessary for composing risk level as a result of a risk analysis methodology (Tixier, 2002). Tixier et al. (2006) used expert judgment and hierarchical structure in order to develop vulnerability map for industrial sites. Nevertheless, there is no approach that gives quantitative risk magnitude (RM) and risk class result for each of the risk sources directly specific to a full scale industry that is in planning stage or already established as well as capable of assessing the risks by considering both the ecosystem that industry is placed, hazardous materials that are used and human health.

2.2 Environmental and Human Health Risk Assessment

Risk must be defined in order to determine which components constitute concept of risk magnitude. Risk is the combination of the possibility of occurrence of an event and the likelihood and strength of undesirable effects of that event (Wessberg, 2008; Sonnemann et al., 2004; Dick et al., 1999). The relation between them must be analyzed systematically in order to perform beneficial and realistic risk assessment (Li et al., 2007). While theory of possibility is used in order to measure occurrence of hazards which are originated from the

combination of specific factors (Gentile et al., 2003), risk likelihood (RL) corresponds to probability of occurrence of a hazard. Risk strength (RS) means the significance of the results which will arise in the case of occurrence of the hazard. As a result, Risk Magnitude (RM) represents the likelihood, possibility and strength of the risk. Environmental risk (ER) can be defined as a risk whose results affect human health, life conditions, environment, soil, surface water, ground water, air, climate, flora/fauna, biodiversity, structure of community, buildings, view of city, cultural heritage and the relationships between those components (Wessberg et al., 2008). As industries are responsible for minimizing all of the environmental and human health risks they cause; proposed risk assessment approach, consisting of identification of the events causing hazard and risk, obtaining the magnitude of effects arising from risks and estimation the possibility of occurrence of the events (Darbra et al., 2008; Duffus and Worth, 1996), must present a holistic approach by considering all of the environmental aspects.

ERA and HHRA identify hazards and priorities, increases public awareness of potential problems, and provides guidance for corrective measures and legal controls by organizing, configuring and arranging scientific information. They can also be used for determination and measuring of efficiency of precautions (Sonnemann, 2004). However, problems may occur during implementation of ERA and HHRA process. Firstly, environmental and human health issues are too complex both to formulate risk magnitudes and consider all of the factors affecting risk possibility during evaluation. Also, expert evaluation is needed in order to compose risk components, quantification of which for risk formulation is very difficult. In addition, lack of data, vagueness and uncertainty in environmental information (Arunraj and Maititi, 2008) bring the need of expert opinions and using fuzzy inputs for evaluation process proposed in this study.

2.3 Proposed Integrated Risk Assessment Approach

Complex structure of environmental and human health risk assessment process, that are affected by multiple factors, lead to using Analytical Hierarchy Process (AHP) in order to define and assess factors in a systematic manner. Priority weights of risk factors were determined by using pair wise comparisons and so, a more realistic risk magnitude (RM) has been inferred. Fuzzy modeling provides evaluating uncertain, imprecise and vague data. Indicator risk factors are evaluated with linguistic variables such as “high contribution to risk” which is needed for non-quantifiable factors and limits of that factors are flexed with fuzzy membership functions providing a more reliable transition between risk categories. Experts

may easily define their professional judgments on complex environmental and human health factors and relationships using either linguistic expressions or fuzzy numbers or both of them. Main components of the RM that are defined in order to assess integrated risks were modeled with a group of “if-then” rules. Consequently, complex and uncertain structure of the system were related with qualitative human thinking instead of formulation and then RM was produced.

Zeng et al. (2007) has developed a new risk assessment approach for the delay risk of construction projects. The method was considered as proper to cope with the problems which may occur during implementation of integrated ERA and HHRA. Proposed approach is shown in Figure 2.1.

There are some modifications made to adapt the model proposed by Zeng et al. (2007) to integrated ERA and HHRA process. In preliminary step of the model, instead of evaluating opinions of multiple experts individually, a risk assessment group which contains experts from different disciplines is established. This group discusses, defines, determines and possibly rules out risk criteria in order to get achieve an agreed judgment which can be defined as ‘*differential designation’*. As environmental systems encompass multiple factors such as water, air, flora, fauna etc., experts on these factors must get their opinions together in order to conclude an ultimate result. Thus, it is possible to reach a more representative evaluation through discussing all of the factors with a holistic approach. Linguistic variables of factor index (FI), which represent risk possibility, have been changed in order to adapt to integrated approach as well as explanations for scoring of FI and RS. RM is represented by FI, RL and RS. Therefore, those components were not changed for calculation of RM. Fuzzy numbers are also not changed as they can also be representative for this approach.

Finally, hierarchy that will be used for scoring FI was constructed by assigning the aim as integrated ERA and HHRA for industrial hazardous materials.

2.3.1 Steps of Proposed Approach

Firstly, a risk assessment group which must include chemists and biologists that can evaluate the characteristics of substances; ecologists, water scientists, agricultural engineers, meteorologists, urban scientists that can evaluate the characteristics of ecosystem; industrial engineers that know the processes in industry and production flow; and environmental engineers that can evaluate all of that components from environmental point of view and draw a conclusion. People that worked for same kind of industry, which is being evaluated, for a

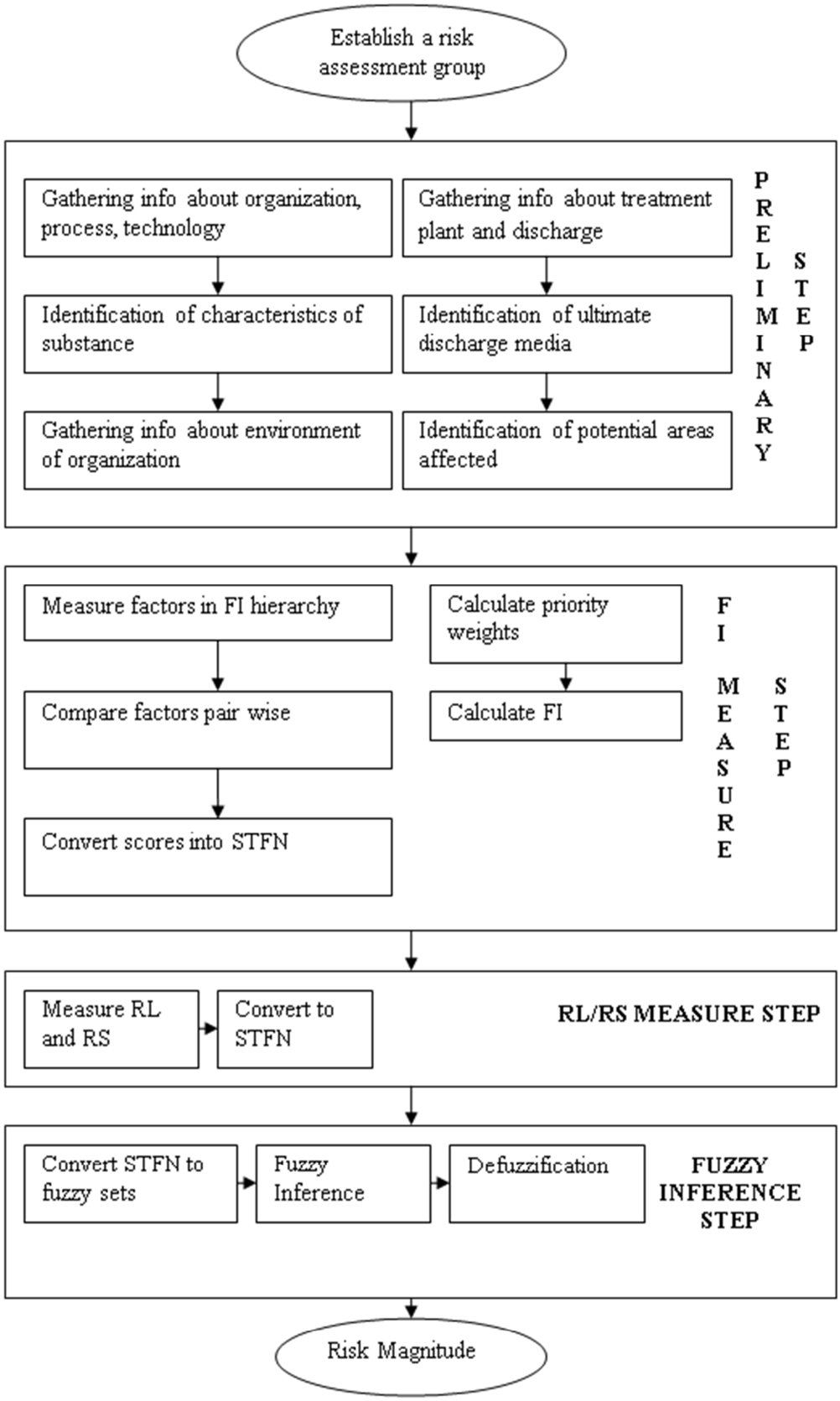


Figure 2.1: Proposed integrated risk assessment approach for industrial hazardous materials

long time and local people that know region very well can be included in the group as experienced people. Risk assessment group evaluates information/data that is related with risks, implements integrated procedure after controlling risk factors whether they are relevant with the case study or not, and assigns new risk factors if necessary.

Preliminary Step

When the production process in an industry is accepted as a system, risk factors will be objects or environment of it. As a result, enough information/data about organization, production process, technology that is used, handling conditions, substance characteristics, environment characteristics that industry is laid out, treatment and discharge system of industry and influenced area depending on substance and environment characteristics must be collected in order to perform hazard identification, exposure and dose-response assessment and risk characterization in the content of integrated approach. Possible influenced area can be determined solely after all of that information is gathered.

FI Measurement Step

Measure factors in FI hierarchy

It is needed to develop a hierarchy that consists of risk factors providing measurement of risk possibility beside measurement of risk likelihood. The purpose of preparing FI hierarchy is elaboration of risk factors and assessing the risks efficiently.

The overall structure of hierarchy in which main sections were provided with capital letters is shown in Figure 2.2. All of them will be explained level by level in detail below.

At the first level in the hierarchy, risk assessment of industrial hazardous materials is assigned as the purpose of the analysis. Emissions that are discharged routinely from industry defined as PE, emissions that occur due to accidents defined as AE and emissions that occur during production indoor defined as IAE are the main sources of environmental and human health risks for an industrial organization having different risk characteristics, so they are placed at the second level.

Characteristics of production, treatment processes and discharge amount and time are known for PE. Risk posed by PE to the environment depends on characteristics of substance and ecosystem which are considered as the sub factors of PE. As hazard definition for all of the main risk sources are related with substance characteristics, they are vital for all of the main risk sources. While hazard characteristics of substances usually define potential hazard

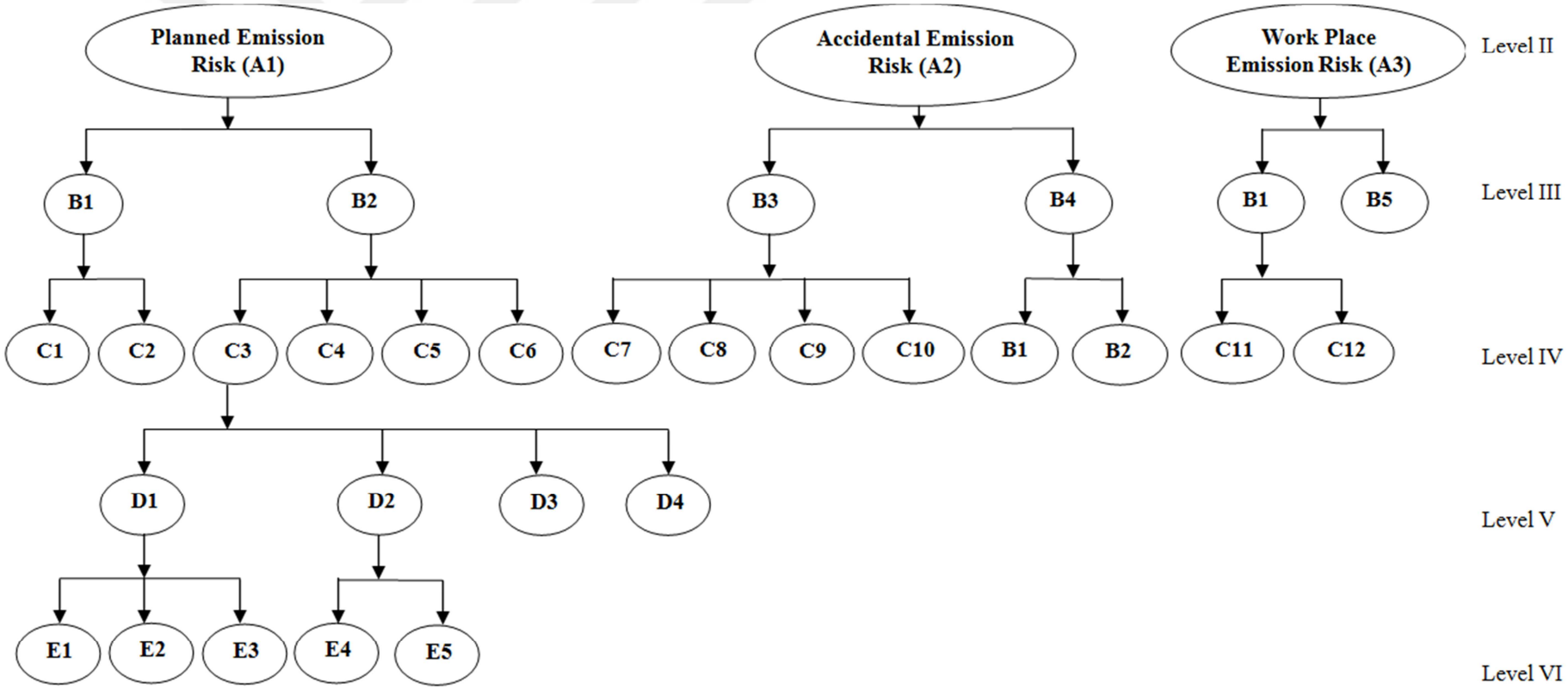


Figure 2.2 An overall structure of integrated risk assessment hierarchy for industrial hazardous materials

resulted by usage of them, characteristics related with exposure do not directly address potential hazard, but provide information to evaluate transport and fate of substance. Some reference scores can be used for factors, which are known widely and have limited uncertainty, in order to achieve more homogenous judgments. Flammability, reactivity, explosivity, corrosivity and toxicity are the main characteristics for defining hazard of the substance.

There are different characteristics of ecosystem related with risk. Therefore, spatial components become essential for such an ERA approach and there are studies about sustainable industrial area locations such as Ruiz Puente et al. (2007) and De Juan Luna et al. (2005). Some of them can be grouped under factors related with fate and transport of substance in the environmental components. Water, air, soil and groundwater compartments have different physical or chemical characteristics that affect the transport and fate of substance. Possible characteristics to be considered during integrated risk assessment approach process are provided in Figure 2.3. Each case has its own particular situation and consequently characteristics related with exposure should be considered case by case.

“Borders of region” is another important factor because it represents extent of affected area.

Life, activity and usage purposes of region are needed in order to assess damage or hazard to beneficial uses of regions including industrial, agricultural, touristic and/or residential as well as strategic importance.

Environmental sources refer to contribution of environmental components in the region to ER. Water bodies and watersheds, terrestrial ecosystems, forests, flora/fauna and population density are assigned as sub factors in order to define possible exposed groups and assess effects related with ecosystem.

AEs are considered in two sub factors as “factors related with occurrence of accident” and “post-accident factors” (Figure 2.4). While post-accident factors provides the assessment of risk factors directly related with substance and ecosystem characteristics, factors related with the occurrence of accident mostly represent the possibility of accident which is the couple of environmental risk, since accidents cause environmental emissions.

Transportation of the substance to the industry and in the industrial plant, storage and handling during industrial production and production processes has the potential of causing accident. Transportation modes of substances (marine, air, roadway, pipe transportation) to the industry are assigned to the lowest level of AEs originating from transportation accidents.

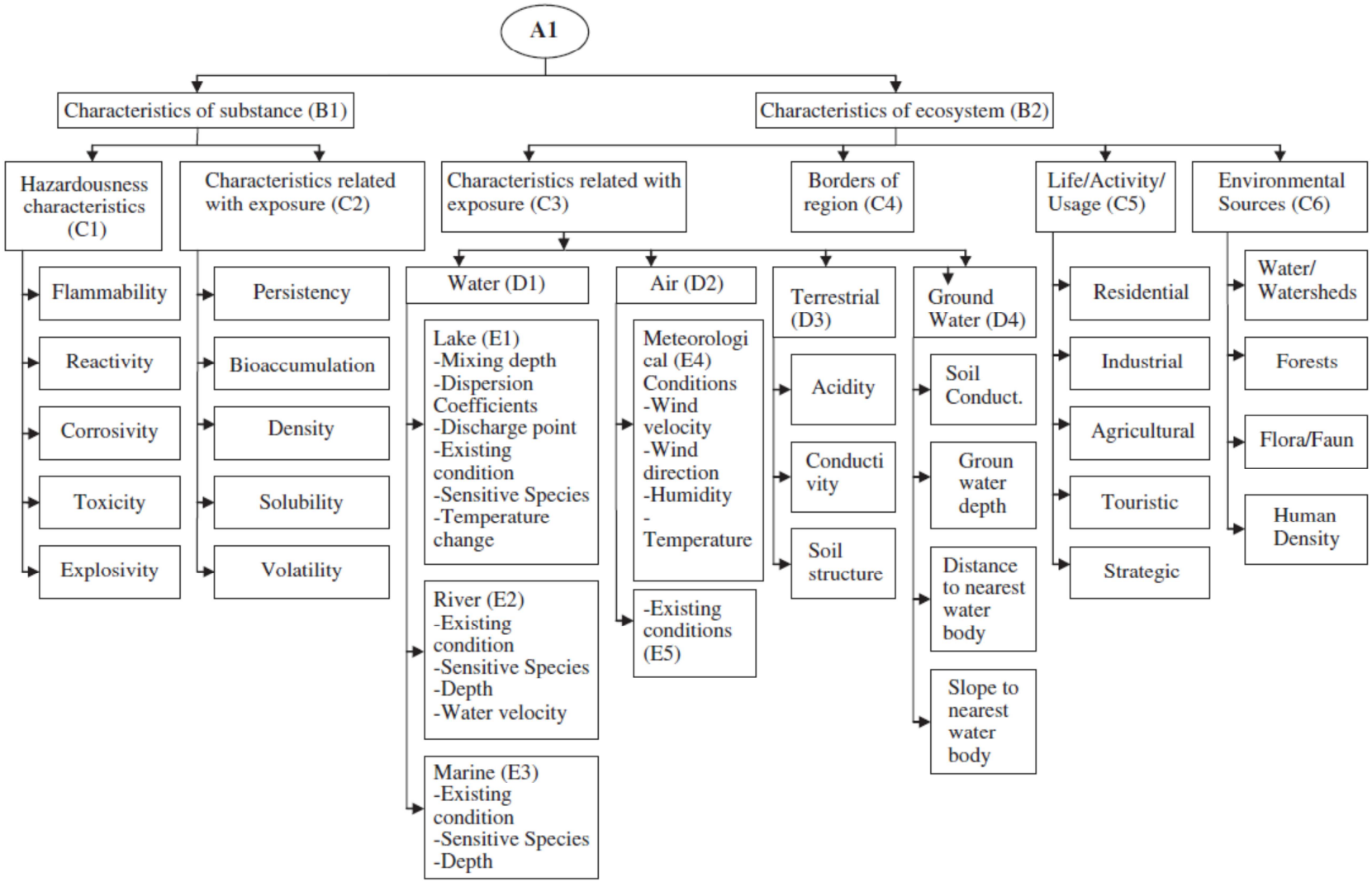


Figure 2.3: Sub factors of planned emissions

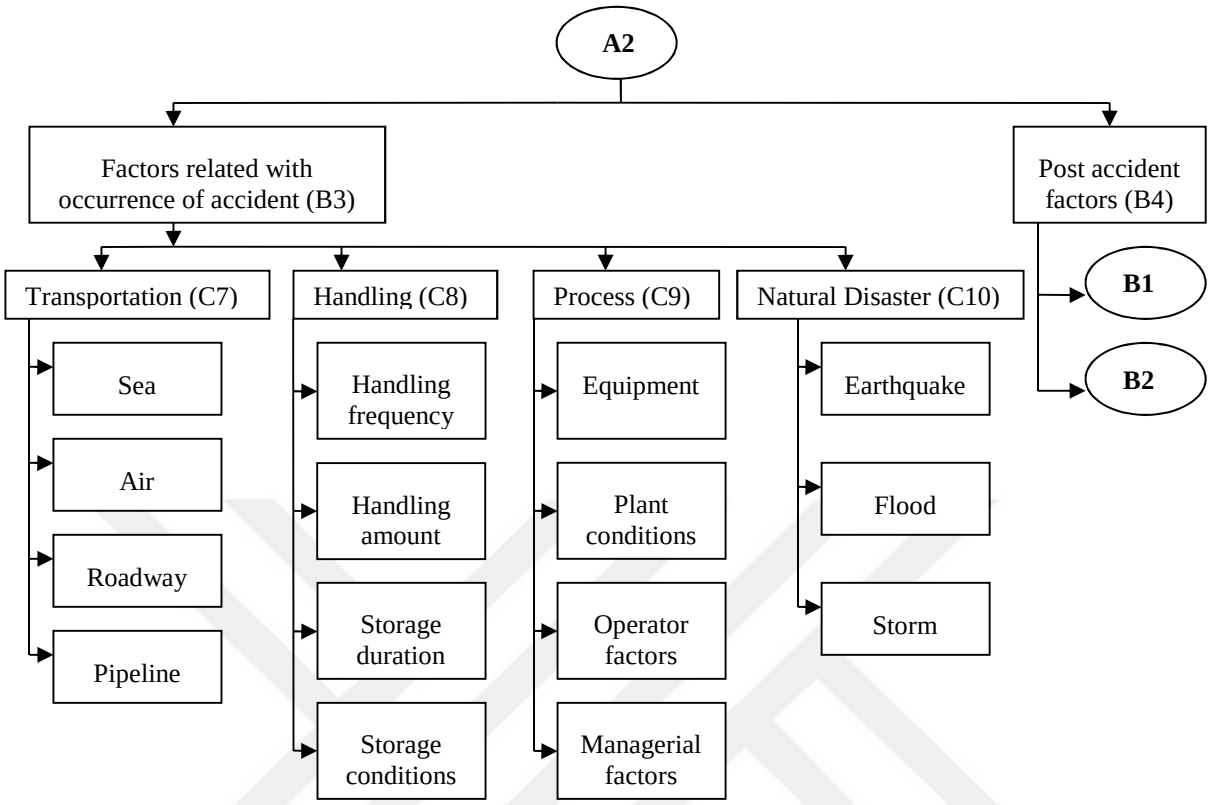


Figure 2.4: Sub factors of accidental emissions

As their RL and RS have particular aspects, it is needed to characterize risks and calculate RMs individually. Frequency and amount of handling, warehouse conditions and duration of storage constitute potential of handling accidents.

Failures of components of a manufacturing system increase the possibility of accidents. Experience and ability of workers, physical conditions of workplace (temp., humidity, messiness etc.), equipment that are not maintained, incompatible with process and/or substance, unsuccessful managerial measures are the factors to be considered by experts.

Accidents such as destructions, explosions, cracking etc. especially in storage areas can be caused by earthquakes, floods and hurricanes and cause severe damages. For IAEs, physical factors are critical for risk assessment besides characteristics of substance as shown in Figure 2.5. Work place conditions and factors related with workers are two different factor groups that represent physical factors in the work place. Because, workers are the only group of people that can be exposed to substance in the work place and work place conditions influence the exposure of workers. Age and health condition of operators must be taken into account as

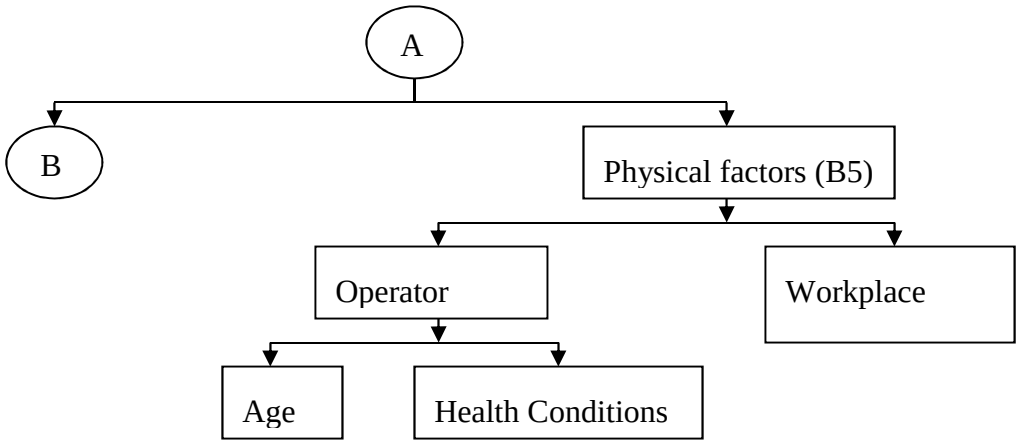


Figure 2.5: Sub factors for evaluating ER of indoor air emissions

the sub factors related with operators, because health condition and age of people specifies how people would be affected from an exposure. FI is composed by scoring the bottom level risk factors in the hierarchy. Expert group designates scores according to explanations given in Table 2.1. Their decisions about scores are based on their knowledge and experiences in the case of absent data.

Table 2.1: Explanations about FI, RL, RS, RM components

Definition of FI	**Explanation**	**FI Fuzzy Number**
very high (VH)	Very high contribution to environmental risk	(0.0,0.0,2.5)
high (H)	Significant contribution to environmental risk	(0.0,2.5,5.0)
medium (M)	No critical contribution to environmental risk	(2.5,5.0,7.5)
low (L)	No contribution to environmental risk	(5.0,7.5,10.0)
very low (VL)	Exactly no contribution to environmental risk	(7.5,10.0,10.0)
Definition of RL	**Explanation**	**RL Fuzzy Number**
very low (VL)	Very low probability	(0.0,0.0,2.5)
low (L)	Low probability	(0.0,2.5,5.0)
medium (M)	There is possibility	(2.5,5.0,7.5)
high (H)	There is high possibility	(5.0,7.5,10.0)
very high (VH)	Inevitable to occur	(7.5,10.0,10.0)
Definition of RS	**Explanation**	**RS Fuzzy Number**
very low (VL)	No acute effect, uncertain chronic effect	(0.0,0.0,2.5)
low (L)	Acute and chronic effect	(0.0,2.5,5.0)
medium (M)	High acute and chronic effect	(2.5,5.0,7.5)
high (H)	Intensive acute and high chronic effect	(5.0,7.5,10.0)
very high (VH)	Intensive acute and chronic effect	(7.5,10.0,10.0)
Definition of RM	**Explanation**	**RM Fuzzy Number**
negligible (N)	Risk can be accepted	(0.0,0.0,1.0,3.0)
minor (Mi)	Risk can be tolerated but precautions must be taken	(1.0,3.0,4.0,6.0)
major (Ma)	Risk must be reduced	(4.0,6.0,7.0,9.0)
critical (C)	Risk cannot be accepted	(7.0,9.0,10.0,10.0)

Expert group can give their scores with crisp numbers, fuzzy numbers, as a quantitative range or linguistic terms which are transferred standardized trapezoidal fuzzy number (STFN) then. In order to express FI, five linguistic variables were used as shown in Table 2.1. They were assigned from “Very High” to “Very Low” considering the degree of their contribution to risk.

Compare factors pair wise

Each member in a level is compared with other factors in the same level under the same group based on their relative contribution to ER. Chang’s 1-9 scale is used for pair wise comparison. According to that scale, the scale of 1 is given for factors that have equal importance. 3,5,7 and 9 denote weakly, strongly, very strongly and absolutely more important, respectively. If there are slight differences between factors, even scales (2, 4, 6, 8) are used (Saaty, 2001). Experts can give their scores in fuzzy scale if it is needed.

Convert scores into STFN

Since the scores for FI measurement and pair wise comparisons are in different formats, it is needed to convert them into a common form in order to precede calculations. Standard trapezoidal fuzzy numbers (STFN), which is also used in study of Zeng et al. (2007) and its conversion equation is shown, is preferred for that study. A trapezoidal membership function can be expressed as $A=(a^l, a^m, a^n, a^u)$. For example, triangular fuzzy numbers are converted into STFN as $a^m=a^n$, a numerical range corresponds to $a^l= a^m$ and $a^n= a^u$.

Calculate priority weights

Arithmetic averaging method given in equation 1 is employed in order to calculate priority weights of factors in comparison matrix. a_{ij} is the defuzzified form of score that is given for the comparison of F_i and F_j factor in the same level in which there are n factors. If total STFN is shown as $a_{ij}=(a^l_{ij} , a^m_{ij} , a^n_{ij} , a^u_{ij})$, the crisp value of a_{ij} can be calculated by using defuzzification equation 2.

$$w_i=\frac{1}{n}\sum_{j=1}^{n}\frac{a_{ij}}{\sum_{k=1}^{n}a_{kj}} \qquad i,j= 1,2,\ldots., n \qquad (2.1)$$

$$a_{ij} = \frac{a^1_{ij} + 2*(a^m_{ij} + a^n_{ij}) + a^u_{ij}}{6} \qquad (2.2)$$

While w_i is the weight of F_i in its own level, w'_i which is given in equation 2.3 shows the weight of F_i in the hierarchy. $w^{(i)}_{section}$ indicates the priority weight of i. section that is above F_i in the case of being t level above it.

$$w'_i = w_i \times \prod_{i=1}^{t} w^{(i)}\,\mathrm{sec}\,tion \qquad (2.3)$$

Calculate FI

FI can be calculated by using equation 2.4 where P_i is the STFN form of the score that is given by experts to the bottom level risk factors in the hierarchy. n indicates the number of bottom level risk factors in the hierarchy which belongs to a specific main risk source (A1, C7, C8, C9, C10).

$$FI = \sum_{i=1}^{n} P^*_i \times w'_i \qquad i = 1,2,\ldots,n \qquad (2.4)$$

RL and RS Measurement Step

RL and RS scores for the main risk sources that are explained in Section 2.3.1.2 must be given by experts according to the scale in Table 2.1. RS depends on kind of effect whether it is acute or chronic. RL represents the probability of the risk which is mainly determined according to the frequencies of events. Those given scores must be converted to STFN.

Fuzzy Inference

This step provides user to achieve ultimate risk magnitude by using fuzzy sets of risk components with the fuzzy inference engine.

Convert STFNs to fuzzy sets

It is needed to convert FI, RL and RS scores to fuzzy sets to be able to use in fuzzy inference system in which linguistic variables are used during fuzzy rule construction. Intersection between STFN forms of FI, RL and RS scores and their respective membership functions give the membership degrees of those factors in corresponding fuzzy set which is shown in Figure 2.6 for illustration of scenario implementation.

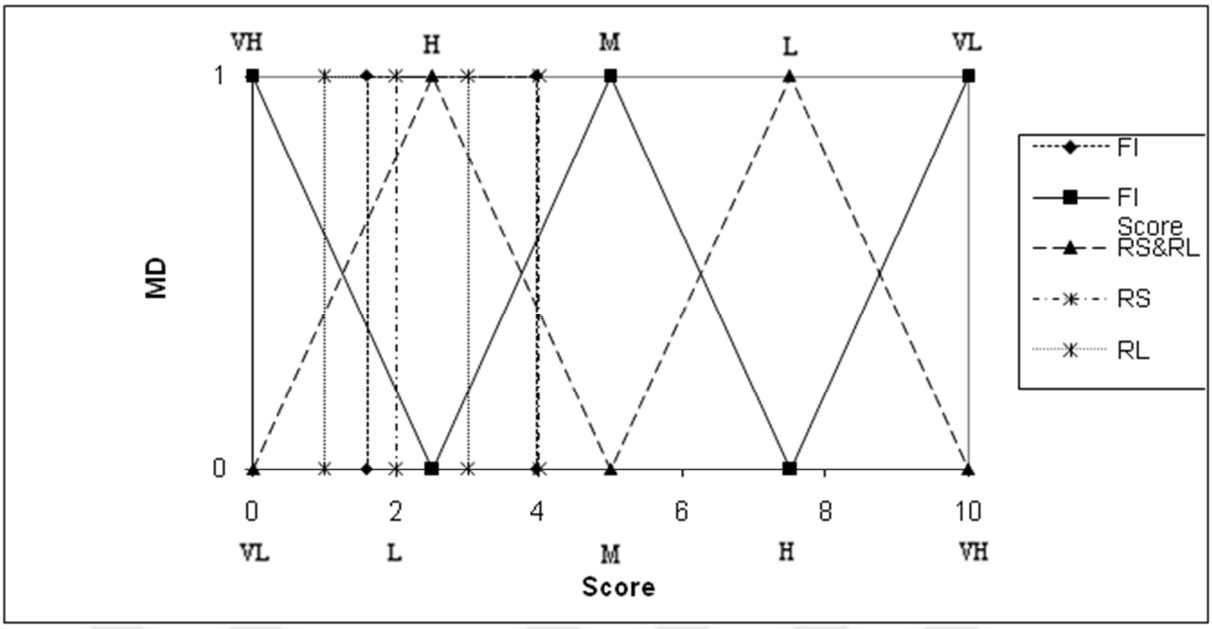


Figure 2.6: Determination of classes and membership degrees of FI, RL and RS for PE

Fuzzy inference

If-then rules are used in order to achieve RM by combining FI, RL and RS components. Fuzzy intersection (minimum) operation provides combining the FI, RL and RS parameters with "and" operator which leads to getting truncated fuzzy RM results. Therefore, fuzzy union (maximum) operation is used for getting a single fuzzy membership function.

Defuzzification

RM can be expressed in terms of a numerical value that matches fuzzy result achieved in fuzzy inference system. For that purpose, centre-average method is used as shown in equation 2.5 where Y_i indicates the center of the i^{th} fuzzy set of RM and $\mu_{RM}(y_i)$ is the membership function of the i^{th} fuzzy set of RM

$$RM=\frac{\sum_{i=1}^{q} Y_i \mu_{RM*}(y_i)}{(\sum_{i=1}^{q} \mu_{RM*}(y_i)} \qquad i=1,2,..,q \qquad (2.5)$$

2.4 Implementation of the proposed approach

2.4.1 Case Study 1: An industrial plant at the planning stage

A scenario was developed in which a PVC manufacturing factory will be built in Tuzla, Istanbul, Turkey and use vinyl chloride (VC) as raw material. Based on the results of that

scenario, proposed approach re-implemented for the case of Malkara, Tekirdağ, Turkey as an alternative to Tuzla.

Preliminary Step

Content of the necessary information gathered in preliminary step by risk assessment group that is established for that scenario are summarized in Table 2.2.

FI Measurement Step

Experts decided that there is no need to add more risk factors to the hierarchy for that scenario. In the light of the information they gathered, there were no chance for substance to enter water, soil and groundwater media and pose threat. Therefore, ecosystem characteristics related with those medias were not taken into account during assessment procedure. They gave scores for other bottom level risk factors by considering Table 2.1. In order to give examples for calculations, scores given by experts for planned emissions, their corresponding STFN values, priority weights in the hierarchy and FI values of them were summarized in Table 2.3.

An example comparison matrix for the second level of planned emissions were shown in Table 2.4 which includes fuzzy comparison scores and their corresponding STFN values and Table 2.5 which includes crisp values of the fuzzy scores. The scale of 1-1/2 indicates that substance and ecosystem characteristics may have equal importance or ecosystem characteristics may be slightly important than substance characteristics. Comparisons were made between row and column, so if column is more important than row, reciprocal of the scale such as 1/2 is used. Pair wise comparison matrixes are symmetric because of using reciprocals of scales. After scoring comparisons, fuzzy score is defuzzified as shown in Equation 2.6.

$$a_{21} = \frac{1+2*(1+2)+2}{6} = 1.5 \qquad (2.6)$$

Equation 2.7 and equation 2.8 demonstrate the calculation of weights of factors at their level and in the hierarchy, respectively by using the data of second level of PEs. For example, there are two more levels above flammability, so weight of flammability in its level was multiplied by the weights of hazard characteristics and substance characteristics at their own levels.

Table 2.2: Summary of gathered information in preliminary step

General Information		
Capacity	40000 ton VC/year	
Transportation frequency	Monthly	
Transportation mode	Roadway	
Transportation distance	100 km	
Process type	Polymerization	
Receiving environment	Air	
Substance Characteristics		
	Vinyl Chloride	**Acetonitrile**
Physical State	Liquid	Liquid
Color	Colorless	Colorless
Odor	Sweet	Pungent
Vapor Pressure (mmHg)	2580 (20 ^{0}C)	83 (20 ^{0}C)
Vapor Density (Air=1)	2.2	1.8
Boiling Point (^{0}C)	-13.9	77
Melting Point (^{0}C)	-153.7	-83
Solubility in water (g/L)	1.1	
Specific weight	0.9106	0.806
Molecular formula	C_2H_3Cl	C_3H_3N
Molecular weight (g/mol)	62.5	53.06
Flash point (^{0}C)	-61	0
Explosivity limit (%)	3.6-33	3-17
Auto ignition temperature	473 ^{0}C	480^{0}C
Bioaccumulation potential	No	N/A
Acute toxicity	Drowsiness, somnolence, mortality	Oral(LD50):27mg/kg(mouse) Dermal (LD50):63mg/kg(rab Vapor (LC50):333 ppm rat
Chronic toxicity	Cancer, chronic lung disorder, numbness	TWA: 2ppm 2B(Possible carcinogenic) Mutagenic for mammalian Possible teratogenic May be toxic to blood, nervous system, liver
Ecosystem Characteristics		
	Tuzla	**Yalova**
Population density	>1000 person/km^2	
Wind direction	Dominant in the direction of residential area	Dominant in the direction of residential area
Wind velocity	High	Medium
Climate	Terrestrial in winter, Mediterranean in summer	Terrestrial in winter, Mediterranean in summer
Vegetation	Maquis	Forestry
Natural disaster potential	Earthquake sec. seismic zon.	Earthquake first seismic zone
Touristic potential	Low	High
Strategic importance	High	Medium
Agricultural potential	Very Low	High
Borders of region	Very High	Medium
Industrial potential	Very High	High

Table 2.3: Scores and FI values for bottom level factors of substance and ecosystem characteristics (PE)

			STFN				W'	FI			
FACTORS	**SCORE**		**A**	**B**	**C**	**D**	**E**	**A*E**	**B*E**	**C*E**	**D*E**
Flammability	0	2	**0**	0	2	2	**0.11**	0.00	0.00	0.22	0.22
Reactivity	4	6	4	4	6	6	0.03	0.12	0.12	0.18	0.18
Explosivity	5	7	5	5	7	7	0.05	0.25	0.25	0.35	0.35
Corrosivity	6	7	6	6	7	7	0.01	0.06	0.06	0.07	0.07
Toxicity	0	2	0	0	2	2	**0.15**	0.00	0.00	0.30	0.30
Persistency	7	8	7	7	8	8	0.004	0.03	0.03	0.03	0.03
Bioaccumulation	8	10	8	8	10	10	0.004	0.03	0.03	0.04	0.04
Density	2	4	2	2	4	4	0.022	0.04	0.04	0.09	0.09
Solubility	6	8	6	6	8	8	0.004	0.02	0.02	0.03	0.03
Volatility	1	3	1	1	3	3	0.037	0.04	0.04	0.11	0.11
Wind Velocity	3	5	3	3	5	5	0.005	0.02	0.02	0.03	0.03
Wind Direction	2	5	2	2	5	5	0.011	0.02	0.02	0.06	0.06
Humidity	7	9	7	7	9	9	0.001	0.01	0.01	0.01	0.01
Temperature	4	7	4	4	7	7	0.011	0.04	0.04	0.08	0.08
Existing Conditions	4	6	4	4	6	6	0.005	0.02	0.02	0.03	0.03
Boarders of Region	2	5	2	2	5	5	**0.11**	0.22	0.22	0.55	0.55
Residential	0	2	0	0	2	2	**0.08**	0.00	0.00	0.16	0.16
Industrial	0	3	0	0	3	3	**0.16**	0.00	0.00	0.48	0.48
Agricultural	8	10	8	8	10	10	0.01	0.08	0.08	0.10	0.10
Touristic	6	8	6	6	8	8	0.03	0.18	0.18	0.24	0.24
Strategic	3	6	3	3	6	6	0.06	0.18	0.18	0.36	0.36
Water and Watersheds	6	7	6	6	7	7	0.031	0.19	0.19	0.22	0.22
Forests	5	8	5	5	8	8	0.004	0.02	0.02	0.03	0.03
Flora-Fauna	6	8	6	6	8	8	0.01	0.06	0.06	0.08	0.08
Population Density	0	3	0	0	3	3	**0.059**	0.00	0.00	0.18	0.18
	FI for PE							**1.60**	**1.60**	**3.97**	**3.97**

Table 2.4: Pair wise comparison matrix for the second level of A1 – Fuzzy Scores

	Substance Characteristics		Ecosystem Characteristics	
	Score	STFN	Score	STFN
Substance Characteristics	(1, 1)	(1, 1, 1, 1)	(1, ½)	(1, 1, ½, ½)
Ecosystem Characteristics	(1, 2)	(1, 1, 2, 2)	(1, 1)	(1, 1, 1, 1)

Table 2.5: Pair wise comparison matrix for the second level of A1 –Crisp Scores

FACTORS	Substance Characteristics	Ecosystem Characteristics
Substance Characteristics	1	0.75
Ecosystem Characteristics	1.5	1

As it can be seen in Table 2.2, FI is calculated by multiplying scores and priority weights in hierarchy, and overall FI for a main risk source such as PE is achieved by summing up FIs of all of the related sub factors.

$$w_{substancecharacteristics} = \frac{\frac{1}{1.5+1} + \frac{0.75}{0.75+1}}{2} = 0.41 \quad (2.7)$$

$$w'_{flammability} = w_{flammability} * w_{hazard\ characteristics} * w_{substancecharacteristics} = 0.31*0.83*0.41 = 0.11 \quad (2.8)$$

Measurement of RL and RS

RL and RS for six main risk factors were scored according to the scale given in Table 2.1 and shown in Table 2.6.

Table 2.6: RL and RS scores in the form of STFN for main risk sources of scenario

FACTORS	RL	RS
ER sourcing from planned emissions	(1, 1, 3, 3)	(2, 2, 4, 4)
ER sourcing from transportation accidents	(0, 0, 3, 3)	(6, 6, 8, 8)
ER sourcing from handling accidents	(2, 2, 4, 4)	(5, 5, 6, 6)
ER sourcing from process accidents	(1, 1, 2, 2)	(3, 3, 4, 4)
ER sourcing from natural disaster accidents	(1, 1, 2, 2)	(7, 7, 9, 9)
ER sourcing from indoor emissions	(4, 4, 6, 6)	(6, 6, 7, 7)

Convert STFNs to fuzzy sets

FI, RL and RS scores must be converted into fuzzy sets. For this purpose, fuzzy membership functions of risk criteria shown in Table 2.1 were drawn and intersection points of FI, RL and RS scores with fuzzy membership function were found in order to achieve classes and membership degrees of FI, RL and RS as shown in Figure 2.7 in which FI and RL&RS membership function were shown. For example, STFN of FI=(1.6;1.6;3.97;3.97) for planned

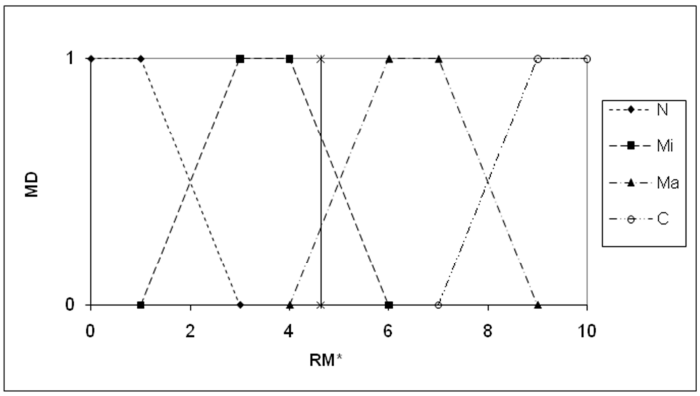


Figure 2.7: Determination of classes and membership degrees of RM for PE

emissions intersects the very high (0.37), high (0.65) and medium (0.59) classes. Therefore, corresponding fuzzy set for FI is;

FI={(very high, 0.37), (high, 0.65), (medium 0.59)}

Fuzzy Inference System

Table 2.7 is given as an example of fuzzy inference step for planned emissions. Fuzzy rule base was prepared by using fuzzy classes of factors for all of the combinations of them. For instance, expert group decided that if FI is very high (0.37), RS is medium (0.6) and RL is very low (0.61), then RM is Major (0.37). FI, RL and RS criteria were composed by using "and" operator in order to achieve RM. Membership degree of that major risk magnitude is 0.37 which corresponds to the minimum membership degree among FI, RL and RS that were combined. Membership degrees of RM is inferred by using fuzzy union (maximum) operator and shown in bold type in Table 2.7. The maximum membership degree for major group in the rule base is 0.6, so membership degree of major risk magnitude is 0.6. Membership degrees for other risk classes (minor, negligible, critic) were obtained in a similar way.

Defuzzification

After obtaining membership degrees for risk magnitude, they were defuzzified as shown in equation 2.9. Defuzzified risk magnitude (4.63) was drawn on fuzzy membership function of RM in order to achieve actual class and membership degree of RM.

Table 2.7: Fuzzy inference of RM for PE

FI	**RS**	**RL**		
		VL (0.61)	L (0.81)	M (0.21)
VH(0.37)	M (0.6)	Ma (0.37)	Ma (0.37)	**C (0.21)**
	L (0.82)	Mi (0.37)	Ma (0.37)	C (0.21)
	VL (0.18)	N (0.18)	N (0.18)	Mi (0.18)
H (0.65)	M (0.6)	Ma (0.6)	**Ma (0.6)**	Ma (0.21)
	L (0.82)	Mi (0.61)	**Mi (0.65)**	Ma (0.21)
	VL (0.18)	N (0.18)	N (0.18)	Mi (0.18)
M (0.59)	M (0.6)	Ma (0.59)	Ma (0.59)	Ma (0.21)
	L (0.82)	**N (0.59)**	Mi (0.59)	Mi (0.21)
	VL (0.18)	N (0.18)	N (0.18)	Mi (0.18)

$$RM^* = \frac{0.59*2 + 0.65*4 + 0.6*7 + 0.21*10}{0.59 + 0.65 + 0.6 + 0.21} = 4{,}63 \qquad (2.9)$$

As it is shown in Figure 2.7, RM intersects RM membership function on the point of 0.7 for Minor and 0.3 for Major class, which means planned emission risk belongs to major class with a degree of 0.7. An overall summary of results are given in Table 2.8.

Table 2.8: Summary of risk class, membership degrees and high score factors for main risk sources

FACTORS	Ma	Mi	High Score Factors
Risk of planned emissions	0.3	0.7	T(0.15). F(0.11). Border of re. (0.11) Ind (0.16). Res (0.08)
Risk of transportation accidents	1	0	Pre-Acc (0.1). Acc (0.9)
Risk of handling accidents	0.21	0.79	Pre-Acc (0.21). Acc (0.79)
Risk of process accidents	0	1	Pre-Acc(0.21). Acc (0.79)
Risk of natural disaster accidents	0.71	0.29	Pre-Acc (0.63). Acc (0.37)
Risk of indoor emissions	1	0	PF (0.14). SC (0.86)

2.4.2 Case Study 2: An existing industrial plant

Validation of the proposed approach was aimed with the second case study. When a model is applied to a case which already happened and effects are clear, consistency between results of the model and actual effects show the validity of the model (Leeuwen ve Vermeire ,2007; Papadikis and Chalkidou, 2008). Therefore, second case study was implemented for an already existing industry which had an accident because of Marmara Earthquake in 1999. That industry is located in Yalova (Table 2.2), use acrylonitrile (ACN) as raw material and produces about 250,000 tone/year synthetic acrylic fibers for textile industries. Raw material storage tank (in volume of 10,000 tons) of this industry cracked because of earthquake and about 6,500 tons of ACN (Table 2.2) released to land, water and atmospheric environment by infiltration, spilling and volatilization, respectively. Acute environmental and human health effects of that accident were observed at nearby location but chronic effects were not declared yet due to being relatively a new accident. Proposed approach was applied by considering the conditions (for industry and ecosystem) before earthquake and after earthquake, respectively.

2.5 Results

2.5.1 Case Study 1

Risk class and membership degree in that class were achieved for all of the major environmental and human health risk sources that are possible to occur in the industry given in scenario by using proposed approach. According to the results, there is no critical risk for the industry. However, all of the environmental risk sources except process accidents are in

the major risk class. Owing to the getting results with their membership degrees of risk classes, it was possible to rank them according to their risk magnitudes. Risk arising from work place emissions and roadway transportations were the most major ones, because they belong to major risk class with a membership degree of 1. It is necessary to know the priority of factors that constitute work place risk in order to make strategic plans for reducing it. Characteristics of substance have the biggest contribution to work place emission risk relative to physical factors. If the volatility of substance is limited with preventive cautions and exposure of worker was reduced, toxicity of substance would get a lower score by experts, priority of substance characteristics would be reduced and membership degree of risk in major class would be reduced to 0.25. Consequently, work place emission risk will be reduced by 75% in major class.

Emissions of transportation accidents cause the second major environmental risk. There are uncertainties about where, when, why and what kind of accident will occur. Contribution of post-accident factors to roadway transportation (AE) is as dominant as 90%. Results in Table 8 point out that it is needed to change transportation route in order to minimize ecosystem scores and reduce RS.

For earthquake related accidents, place must be selected according to seismic zone degree of region. However, ecosystem values have high contribution to risk regardless of earthquake potential. If effects of factors related with occurrence of earthquake were reduced by measures, risk possibility would be decreased.

Ecosystem characteristics are also dominant factor for planned emissions. In particular, life/activity/usage characteristics of ecosystem bear high risk for industry. Since Tuzla is an industrial and residential area, those factors highly contribute to risk. In addition to these, toxic nature of VC and wideness of region had also great contribution to risk. Although, efficient measures were planned for regular emissions, poor air quality of Tuzla due to other industrial activities in the region prevents reducing risk any more.

Emissions arising from process accidents are in minor risk group. As there are not so much problem with factors related with occurrence of accident, and RS and RL is low due to controlled conditions relative to transportation accidents during handling and process, minor environmental risk depends on post-accident factors. However, handling accidents were in major risk class due to its more complicated pre accident factors.

Except work place emissions, all main environmental risk sources have a common factor that makes high contribution to risk. In order to conclude how much risk will be reduced, if ecological characteristics were changed by selecting a different site for factory, Malkara has been selected as a new candidate for factory sitting. Implementation of approach for Malkara was not explained in details; instead related results were reported in comparison with Tuzla scenario. Detailed scores and results of Malkara Scenario are provided in Appendix A. As it is shown in Figure 2.8, ER is reduced due to changes in ecosystem characteristics, since Malkara is not an industrial and residential area.

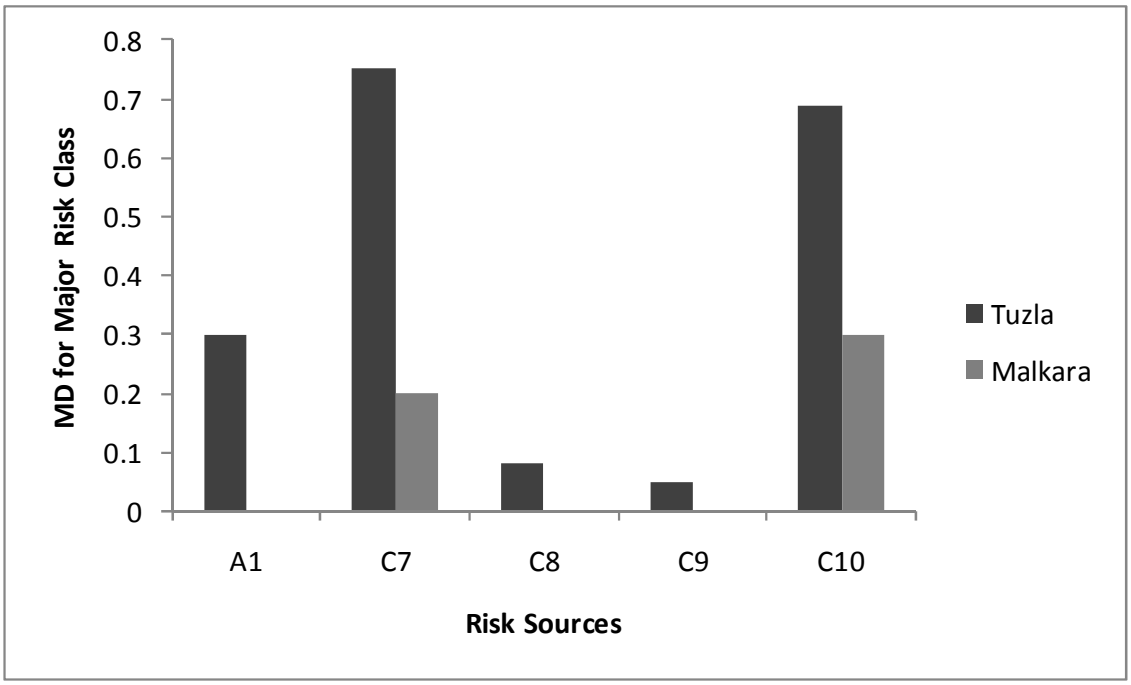


Figure 2.8: Comparision of RMs in case of using VC in Tuzla and Malkara

Handling and process accidents have been reduced completely from major risk to minor, as the ecosystem characteristics were exactly changed. Malkara is less prone to earthquake than Tuzla, so membership degree of earthquake accident in Major environmental risk class was reduced more than half. Risk arising from roadway accidents was reduced dramatically owing to shortened distance of transportation and less critical ecosystem values along by route. A non-industrialized and residential region, low population density and relatively limited borders of region caused reduction also in the risk possibility and risk strength. On the other hand, forests in the region, rich flora/fauna and being agricultural area caused an increase in both risk possibility and strength (Figure 2. 9).

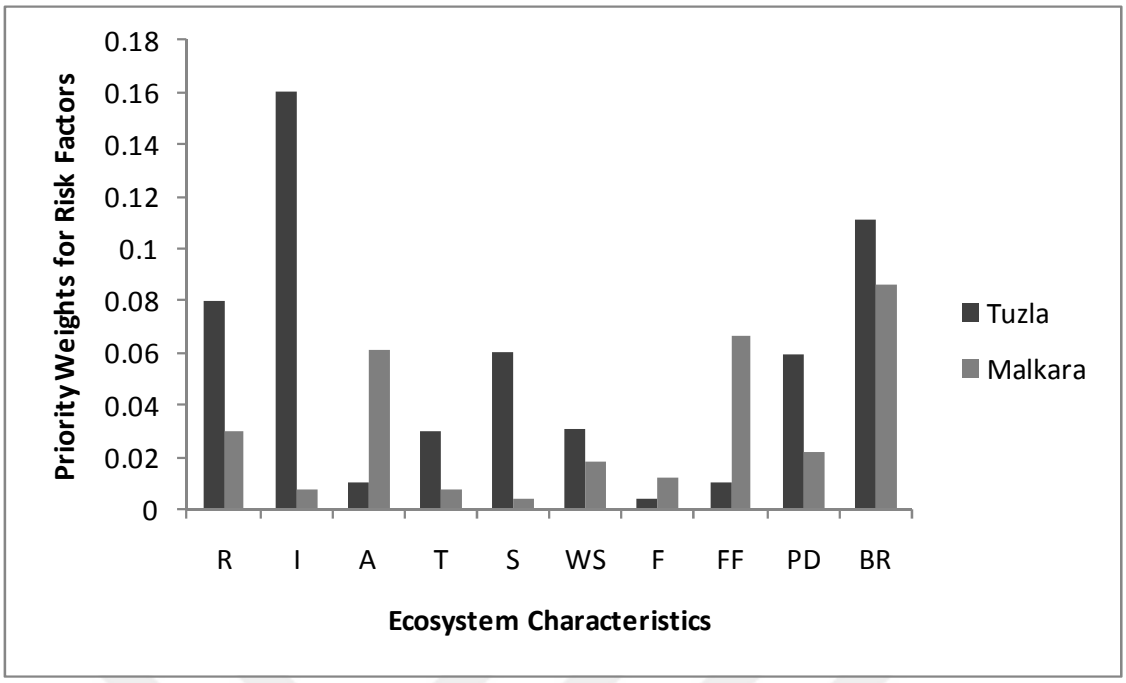


Figure 2.9: Contribution of ecosystem characteristics to ER for AE sourcing from transportation accidents

2.5.2 Case Study 2

Implementation of the proposed approach to second case study by considering the conditions before earthquake showed that natural disaster accidents has the highest membership degree (MD) for major risk (Table 2.9). There were clearly major risk (MD=1) for the case of earthquake due to high contribution of pre-accident factors in addition to post accident factors which shows ecosystem were affected intensively because of ecosystem and substance characteristics given in Table 2.2. Handling accidents had the second priority risk degree in major class (MD=0.15), since no precaution was taken for raw material in the storage tank (i.e. acrylonitrile) not to be emitted to the environment.

Table 2.9: Summary of risk class and membership degrees for the second case study

	Before earthquake		After precautions taken	
FACTORS	Ma	Mi	Ma	Mi
Risk of planned emissions	0	1	0	1
Risk of transportation accidents	0.05	0.95	0.05	0.95
Risk of handling accidents	0.15	0.85	0	1
Risk of process accidents	0	1	0	1
Risk of natural disaster accidents	1	0	0	1
Risk of indoor emissions	0	1	0	1

Moreover, although process accidents, transportation accidents and planned emissions affect only sea and air in the case of earthquake accidents leakage of stored chemical after cracking of storage tank contaminates not only sea and air but also soil and groundwater. As the proposed approach is risk source based and have a flexible application procedure, differences in factors contributing to different risk sources were objectively reflected to the application as well as to the results. These results were compatible with the real case explained in Section 2.4.2. However, proposed approach needs more validation studies, this case study was applied for validation purpose and liable results were achieved. If proposed approach was applied before earthquake, it would be known that earthquake is the most serious risk and must be reduced by preventing leakage of chemical to environment with pre-accident measures. Therefore, it would be suggested that this industry must built an extra impervious storage tank, which has half capacity and just around of the existing one, as a precaution for leakage problem and cover the storage tank with foam to prevent volatilization. According to the results of proposed approach applied for the case of building an extra impervious storage tank and covering the tank with foam (Table 2.9), risk class of natural accidents would become minor (MD=1). This would also be an expected result due to precautions suggested. These two different cases also showed the robustness of the proposed approach which was applied different cases successfully and gave compatible results with realistic conditions.

2.6 Conclusion

Characterization of environmental and health risks for industries using hazardous materials is a requirement for EMSs. Content and implementation of management strategies must be determined according to the results of ERA. However, there were lots of factors that compose these risks; therefore it was needed to evaluate those factors systematically in order to achieve an exact assessment. While applying the proposed approach, it was seen that AHP is useful to reduce the complexity of assessment process. It was proven that risk sources for industries using hazardous materials are PE, AE and IAE, they are affected by different sub factors and have differences from RS and RL point of view such as differences in effected environmental media for AE and PE for the second case study. Hazard definition, effect and dose-response assessment, which represent risk possibility, were conducted by using factors in the hierarchy. Fuzzy rule based modeling provided to achieve a numerical RM for environmental and health risks by composing FI, RL and RS with expert opinions, which is not so possible to achieve with formulas due to complexity of system. Risk characterization was made by using fuzzy

membership functions and risk class and its degree in that class were concluded. As a result, risk sources could be ranked according to priorities of them. For example, in Yalova case it was clear that natural disaster accidents have the highest priority in major risk class. In addition to the priorities of risk sources, factors that make contribution to risks were also ranked according to priorities of them. Thus, risk could be reduced effectively, as in the case of Tuzla and Yalova in which all of major risks are reduced due to changing sitting area and taking precautions for pre-accident factors, respectively. Furthermore, selections of managerial alternatives for reducing ER such as substance, technology or site were simplified. Numerical RM values provide making strategic analysis for integrated risks. Industry can determine its strong and weak points from environmental point of view. Additionally, results of the approach can be used as benchmark for EMS, as the percentage of risk reduction can be concluded numerically. In conclusion, using that approach enable the usage of PDCA which is the base of EMS. It is also clear that proposed approach can be used to simplify EIA in which quantitative data and result is needed in order to achieve an objective decision. It is suggested to develop more detailed hierarchical methodologies for the factors that are in the middle levels at the hierarchical structure of this proposed approach in order to provide more accurate input data for this study.

CHAPTER 3

A SYSTEMATIC EVALUATION OF AGGLOMERATION OF AG AND TIO_2 NANOPARTICLES UNDER FRESHWATER RELEVANT CONDITIONS

ABSTRACT

This study aims to investigate effects of freshwater components in order to predict the agglomeration behavior of silver nanoparticles coated with citrate (AgNP-Cit), or polyvinylpyrrolidone (AgNP-PVP), and TiO_2 nanoparticles. Agglomeration studies were conducted in various media based on combinations of ions, natural organic matter (humic, fulvic acid) and surfactants (sodium dodecyl sulphate, alkyl ethoxylate), at a constant ionic strength of 10 mM. Agglomeration level of AgNP-Cit and TiO_2 was mostly dependent on the concentration of Ca^{2+} in media, and their size strongly increased to micrometer scale over 1 week. However, AgNP-Cit and TiO_2 were stabilized to particle size around 500 nm in the presence of NOM, surfactants and carbonate over 1 week. AgNP-PVP maintained their original size in all media except in the presence of Mg^{2+} ions which led to significant agglomeration. Behavior of these engineered nanoparticles was similar in a natural freshwater medium.

Emel Topuz, Laura Sigg, Ilhan Talinli, A systematic evaluation of agglomeration of Ag and TiO_2 nanoparticles under freshwater relevant conditions, 2014, Environmental Pollution, 193, 37-44.

3.1 Introduction

Engineered nanoparticle (ENP) production has dramatically increased in the recent years (Woodrow Wilson International Center for Scholars). Nanoparticles may be released into the environment due to their use in numerous consumer products, so that exposure of ecosystem components and humans to these nanoparticles might be expected (Bhatt and Tripathi, 2011; Geranio et al., 2009; Gottschalk et al. 2013). Therefore, their sources, release pathways, fate and transport in the environmental media, as well their possible effects need to be understood for environmental risk assessment (ERA) studies. While some of the studies are focused on the release of these ENPs from consumer products and waste water treatment plants (Kaegi et al., 2013; Gottschalk and Nowack, 2011), others focused on the fate and transport in the environmental media (Chinnapongse et al., 2011; Thio et al., 2012; Ottofuelling et al., 2011).

Agglomeration (colloidal stability) is one of the major processes affecting the fate as well as mobility and bioavailability of the ENPs in the environment (Ottofuelling et al., 2011). ENP characteristics affecting their agglomeration behavior include surface change, e.g. by surface reactions, and properties of capping agents used for stabilization (Hyunh and Chen, 2011). Citrate (Cit) and polyvinylpyrrolidone (PVP) are frequently used capping agents for silver nanoparticles (AgNPs) (El-Badawy et al., 2010), with citrate as a stabilizing agent by surface charge and PVP as a steric stabilizer. In addition to intrinsic properties of ENPs, presence of natural organic matter (NOM) (Keller et al, 2010), ionic strength (IS), pH (French et al., 2009; El Badawy et al., 2010) and the background electrolyte composition of the aquatic media (Chen and Elimelech, 2006) affect the behavior of ENPs. Also, some specific pollutants for aquatic environment such as surfactants are used as stabilizing agent for ENPs and might affect the agglomeration of ENPs due to the their adsorption on the surface of ENPs leading steric or electrostatic repulsion, or to a change in hydrophobic properties of ENPs (Kvitek et al., 2008) .

Although there are studies investigating the agglomeration of AgNPs or TiO_2 suspended in different synthetic media, they are not sufficient to evaluate their behavior in natural freshwater media. In most studies on agglomeration, only a limited selection of ions was used (Li et al., 2010; Cumberland and Lead, 2009; El-Badawy et al., 2010; Thio et al., 2012; Ottofuelling et al., 2011; Hyunh and Chen, 2011; Chinnapongse et al., 2011; Jin et al., 2010; Akaighe et al., 2011; Chowdhury et al., 2012; Shih et al., 2012a). However, other components of the natural freshwater media such as NOM or other organic compounds (surfactants etc.) should also be included in the media used for agglomeration studies and

should be combined with major ions, such as Ca^{2+}, Mg^{2+}, CO_3^{2-}. In addition, either humic acid (HA) or fulvic acid (FA) are used as representative of NOM whereas they co-exist at significant amounts in freshwater sources (Aiken, 2014). Hammes et al. (2013) used six different standard water media for the agglomeration studies of ENPs considering various freshwater components. However, stability was evaluated using theoretical calculations only for Au nanoparticles. Moreover, monitoring of agglomeration levels of the selected ENPs for a longer time period up to 1 week is lacking in many existing studies. It is critical to monitor agglomeration for a long time period such as 1 week for more reliable interpretations about fate of ENPs, e.g. for transport processes in rivers

Therefore, this study aims to investigate agglomeration of AgNP-Cit, AgNP-PVP and TiO_2 over 1 week in freshwater simulated media in which various types of ions, NOMs and surfactants are present as a mixture (freshwater simulated media). Common ions present in freshwater (Sigg and Stumm, 2011) and having potential to affect agglomeration (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}, CO_3^{2-}) were selected and used at relevant concentrations for freshwater. NOM (HA, FA) and surfactants (sodium dodecyl sulphate, alkyl ethoxylate) were used to represent organic matter components of freshwater. For a systematic evaluation, selected freshwater components were mixed with different combinations to investigate the role of each component for agglomeration. AgNP-Cit, AgNP-PVP and TiO_2 (without coating) were used as model ENPs in this study since they are most commonly used ENPs in daily life products (WWICS). Furthermore, the agglomeration behavior of these ENP is expected to differ, as citrate is stabilizing AgNP by surface charge, whereas PVP exerts a steric stabilization, and TiO_2 is an oxide which may be stabilized by surface charge, depending on pH and the presence of adsorbing cations and anions. This approach will provide insight about the relation of agglomeration levels (in terms of hydrodynamic diameter) of ENPs with various components of freshwaters. Comparison of agglomeration in freshwater simulated media and in natural freshwater shows that prediction of behavior in real freshwater based on the selected components might be possible.

3.2 Materials and Methods

3.2.1 Chemicals

AgNP-Cit and AgNP-PVP were purchased from NanoSys GmBH (Switzerland) at 1g/L concentration, TiO_2 P25 was obtained from Evonik Industries in powder form. Nominal size of AgNP-Cit, AgNP-PVP (1 g/L) and TiO_2 (0.1 mg/L) provided by the manufacturer were

around 25 nm. Daily prepared working suspensions of AgNP-Cit, AgNP-PVP and TiO_2 (5 mg/L) in nanopure water (NW) were used for agglomeration experiments. Stock suspension of TiO_2 (100 mg/L) was prepared from powder in NW and both stock and working suspensions of TiO_2 were sonicated in a bath sonicator for 2 hours. Ionic media solutions were prepared in NW by using NaCl, KCl, $CaCl_2$, $MgCl_2$, Na_2CO_3, $NaNO_3$, and Na_2SO_4 (Fluka). Their concentrations in different media are shown in Table S1. Sodium hydroxide (Sigma Aldrich) or nitric acid solutions (Merck) were used to adjust pH. Stock solutions of HA and FA (20 mg/L) (Standard Suwannee River I, SRHA and SRFA, International Humic Substances Society) were prepared in NW and their final pH was adjusted to 7.5. Sodium dodecyl-sulphate (SDS) and Lutensol, the nonionic surfactant alkyl ethoxylate (AEO), were obtained from Sigma Aldrich and Mifa company, respectively, and stock solutions prepared in NW (20 mg/L).

3.2.2 ENP Characterization

Zetasizer (Nano ZS, Malvern Instruments) equipped with a red laser (633 nm) was used for DLS measurements. Z-average size (based on intensity mean) was measured in triplicate with at least 12 sub-measurementsand zeta potential (ZP)was calculated from electrophoretic mobility using Henry equation. NTA using a NanoSight LM10 equipped with a LM14 temperature controller (NanoSight Ltd.) was used to get at least 3 different videos of ENPs in suspension which were evaluated by using NanoSight NTA 2.2 Analytical Software to get mode and mean size of the ENPs in suspension. UV-vis absorbance of AgNP suspensions was recorded between 200 and 800 nm using a spectrophotometer UVIKON 930 (Kontron Instruments).

3.2.3 Agglomeration Experiments in Synthetic Media

The ionic strength in each medium containing ions was 10 mM, which is a typical for freshwater. Hence, the effect of various ions was compared at constant IS in the media. SRHA andSRFAwere used to represent NOM present in freshwater and SDS and AEO were used to represent surfactants present in freshwater. These compounds were used as test medium at concentrations of 2, 10 and 15 mg/L. Although, surfactants are found at very low concentrations (ng/L or µg/L) in freshwater, these concentrations were used to observe whether these surfactants may provide stabilizing effects. Effects of the organic compounds were tested in water without added ions and in the presence of ionic media. Effect of pH in the range 2.4 – 12.4 was also examined in nanopure water medium with added HNO_3 and NaOH.

NaCl was mixed with KCl, $NaNO_3$, $CaCl_2$ or $MgCl_2$, respectively to investigate the possible interaction of monovalent and divalent ions. Apart from these mixtures, a special "ionic" medium was prepared to simulate a typical freshwater with moderate hardness in terms of IS (10 mM), composition of ions and their concentration levels (in Appendix A, Table A.1). Ionic medium was mixed with NOM (5 mg/L) and/or surfactants (1 mg/L) in different combinations in order to simulate freshwater conditions and investigate possible interactions of these components.

The concentrations of ENPs studied were selected based on their lower detection limits for DLS and NTA. Daily prepared working solutions of ENPs were spiked to prepared media in order to get 1 mg/L of AgNPs and TiO_2. Experiments were conducted in duplicate in polypropylene tubes in which adsorption of ENP to tube walls is minimal. Samples were shaken at 100 rpm to simulate mixing conditions in natural freshwaters, e.g. in streams. The pH was adjusted to 7.5 and 8.5 for the AgNPs and TiO_2 samples, respectively, to compare the media without considering pH differences.

Experiments were conducted in duplicate for DLS measurements. NTA measurements were made for one sample since NTA was only used for comparison. Time series measurements were done after 1 hour, 1 day and 1 week of agglomeration to investigate the change in size over time. Time intervals were selected based on the preliminary experiments. Student's t test was applied for 95% confidence level (assuming equal variances) by using Microsoft Excel software.

3.2.4 Agglomeration Experiments in Surface Water

A surface water sample was taken from Chriesbach (Dubendorf, Switzerland) river and filtered through 0.2 µm cellulose nitrate filters. This sample was also analyzed for ions,alkalinity and DOC (dissolved organic carbon) (Table A.2). Agglomeration experiments were conducted similar to the experiments with synthetic media.

3.3 Results

3.3.1 Comparison of DLS and NTA for size measurement

NTA and DLS results were very consistent with each other both in average size and trend of the change in size especially for the samples having Z-average size below 300 nm (Table A3-A8). However, for the larger sizes, they are only consistent about the trend of the change in

mean size. DLS and NTA have different shortcomings with respect to size measurement. DLS tends to give too high size results in the case of partial agglomeration, because the calculation of average size is biased towards larger sizes. In the case of NTA, individual particles are tracked. Larger size particles cannot be correctly quantified using NTA, because only a few large particles can be imaged. However, NTA is helpful to verify the partial agglomeration since the presence of different particle sizes can be observed directly. The use of these two methods is thus useful to evaluate whether substantial agglomeration has taken place. Size comparisons in various media and over time can be done using either DLS or NTA. However, the absolute size obtained with these two methods has to be considered with caution. UV-Vis measurements can be used for the qualitative evaluation of AgNP stability; maximum absorbance at 420 nm did not shift significantly for samples which were stable according to the DLS and NTA measurements (Figure A3-A4).

3.3.2 Agglomeration of selected ENPs in the presence of various cations

Z-average sizes of ENPs in NW medium were measured as 61±0.6 nm, 42±0.4 nm and 317±85 nm for AgNP-Cit, AgNP-PVP and TiO_2, respectively. AgNPs and TiO_2 were most stable around pH 7.5 and 8.5, respectively, which are within the typical pH levels of freshwaters. Since the isoelectric point of TiO_2 is around pH 6-7 (Jiang, 2009), hydrodynamic diameter became higher than 1 µm in this pH range. For natural freshwater media with pH 6-7, the effect of repulsive forces will become low and agglomeration level will increase for TiO_2, whereas at higher pH TiO_2 may be stabilized by negative surface carge. Z-average size of AgNP-PVP and AgNP-Cit was stable between pH 4-12 (Figure A1). Stabillity of AgNP-PVP, which was compatible with the study of El Badawy et al. (2010) and Thio et al. (2012),.might be attributed to the non-charged polymeric structure of PVP. Surface charge density is more related with the stability of the AgNP-Cit in this pH range (El-Badawy, et al., 2010).

Effects of monovalent (Na^+, K^+) and divalent cations (Ca^{2+}, Mg^{2+}) were compared by using solutions prepared with chloride as a counter-ion. Monovalent cations at these concentrations did not change the Z average size of all ENPs significantly over 1 week (Figure 3.1). However, Z-Average size of AgNP-Cit in the presence of K^+ were always slightly higher ($p<0,05$) than in the presence of Na^+ over 1 week (Figure 3.1.a and Figure 3.1.c), which might be due to the larger hydrated diameter of K^+ than Na^+ promoting agglomeration.

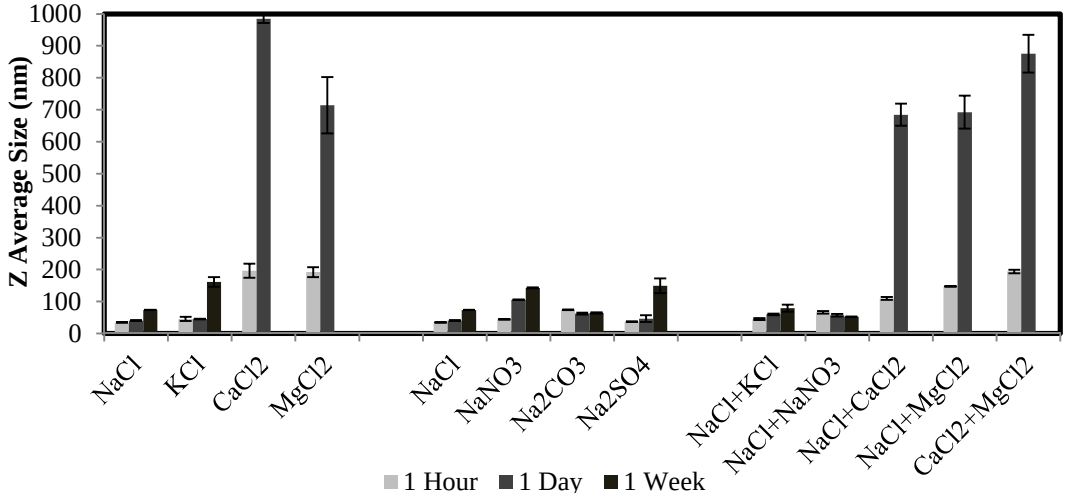


Figure 3. 1(a): Z Average Size of ENPs suspended in ionic media (Table S2) AgNP-Cit

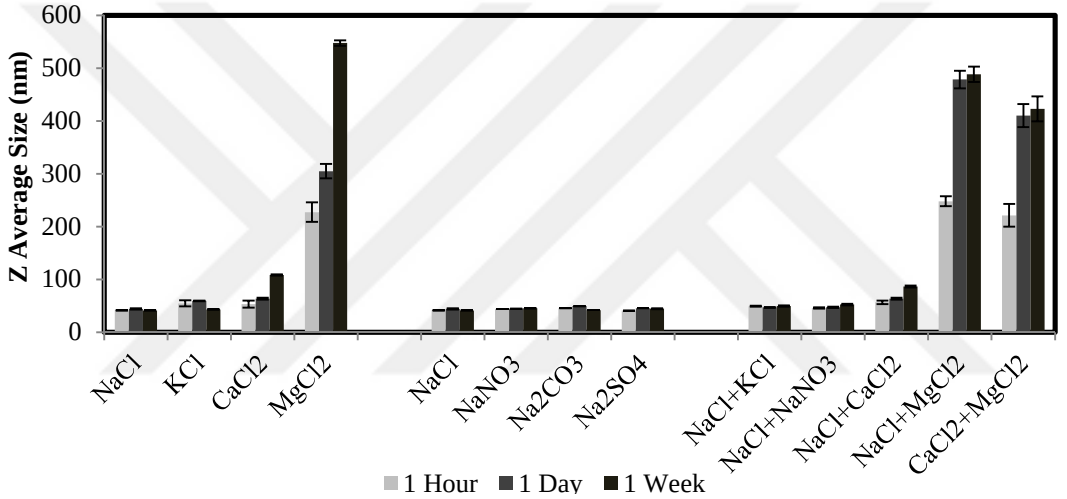


Figure 3. 1(b): Z Average Size of ENPs suspended in ionic media (Table S2) AgNP-PVP

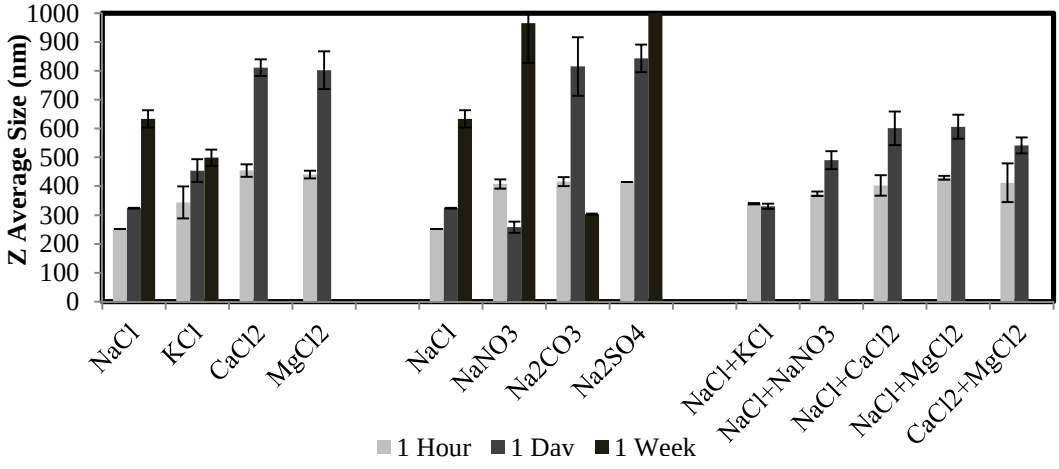


Figure 3. 1(c): Z Average Size of ENPs suspended in ionic media (Table S2) TiO_2 for the time points 1 hour, 1 day and 1 week.

Nevertheless, relation between agglomeration and hydrated diameter should be studied further since it could not be validated for other ions in this study. Moreover, agglomeration of AgNP-Cit to sizes over 100 nm in suspension of KCl in 1 week was not confirmed by NTA measurements (Table A3). AgNP-Cit in suspension agglomerated up to 200 nm in the presence of Ca^{2+} and Mg^{2+} within 1 hour (Figure 3.1.a), whereas TiO_2 agglomerated from 400 nm to 800 nm over 1 day (Figure 3.1.c). Since the Z-average sizes of these samples were higher than 1 µm after 1 week and polydispersity index was so high, the maximum size measured was accepted as 1 µm (Figure 3.1.c). Moreover, these results were also consistent with the ZPs of the samples which were much closer to zero surface charge levels than in suspensions with monovalent cations (Table A3, A7). These results support the effects of divalent cations in screening the surface charge of AgNP-Cit and TiO_2 and in compressing the EDL (Electrostatic Double Layer) leading to agglomeration (Ottofuelling et al., 2011; Mukherjee and Weaver, 2010; Domingos et al., 2010; Shih et al., 2012a; Keller et al., 2010; Chowdhury et al., 2012).

Agglomeration of AgNP-Cit was quite slow, since Z-Average sizes of AgNP-Cit only strongly increased from 200 nm to µm scale after more than 1 day (Figure 3.1.a). The effect of Ca^{2+} on the agglomeration of AgNP-Cit was more pronounced than that of Mg^{2+}, which can be attributed to the higher affinity of Ca^{2+} to complex with citrate, as reported by Hyunh et al., (2011), Chinnapongse et al., (2011), El Badawy et al., (2010) ; Akaighe et al., (2012). Moreover, both of the cations (Ca^{2+} and Mg^{2+}) have the same effect on the agglomeration of TiO_2 in contrast to AgNP-Cit which supports the specific interaction of Ca^{2+} with the citrate coating of AgNPs.

In contrast, the only critical cation for the agglomeration of AgNP-PVP was Mg^{2+} ions at concentrations higher than 2.5 mM (Table A1). AgNP-PVP in suspension agglomerated to 227 ± 18 nm over 1 hour and to 547 ± 5.1 nm over 1 week in the presence of Mg^{2+} (3.3 mM-Table A1), whereas Ca^{2+} led to Z-average size of only 108 ± 1.3 nm over 1 week (Figure 1.b). Moreover, ZP of AgNP-PVP suspension with Mg^{2+} was very close to that with Ca^{2+} and it did not change over time in spite of agglomeration (Table A5). Therefore, the effect of Mg^{2+} ions to AgNP-PVP appears to be specific, possibly by complexation of Mg^{2+} with PVP coating.

The results of the ion mixture experiments indicate the effects of various ions at constant ionic strength. Z-average sizes of AgNP-Cit and TiO_2 were significantly higher in the case of mixtures with divalent cations than in monovalent ion mixtures (Figure 3.1.a-1.c) and were lower than the Z- average sizes observed with individual divalent cations. For AgNP-PVP, in

the case of mixtures with Mg^{2+}, Z-average sizes were significantly higher than in other ion mixtures (Figure 3.1.b). Therefore, it was clear that agglomeration behavior of ENPs suspended in ionic mixture media was dominated by the most effective ion determined in individual experiments and changed based on its concentration. Higher effect of Ca^{2+} than of Mg^{2+}on the agglomeration of AgNP-Cit was also supported with ion mixture media. Results point out that agglomeration level of AgNP-Cit did not change in this range of Mg^{2+} concentration (3.3-1.7 mM), in contrast to the effect of Ca^{2+}, which is due to both the screening of surface charge and complexation with citrate.

3.3.3 Agglomeration of selected ENPs in the presence of various anions

Monovalent (Cl^-, NO_3^-) and divalent anions (CO_3^{2-}, SO_4^{2-}) were compared by using solutions prepared with sodium as a counter-ion. Although Z-Average size of AgNP-Cit in NO_3^- and SO_4^{2-} media revealed agglomeration over 100 nm in 1 week (Figure 3.1.a), NTA measurements with average sizes below 100 nm showed only an agglomeration trend (Table A3). Critical coagulation concentrations for AgNP-Cit were determined around 50 mM for $NaNO_3$ (El Badawy et al., 2010; Li et al., 2010; Gondikas et al., 2012) and Na_2SO_4 (Chinnapongse et al., 2011) over 1 day. It appears therefore as possible that, they could have agglomeration potential over 1 week at 10 mM concentration levels (Figure 3.1a). As CO_3^{2-} seems to be effective on stabilizing AgNP-Cit based on the results of NTA (Table A3), agglomeration experiments were carried out with $CaCO_3$ (2.5 mM of Ca^{2+}) and $CaCl_2$ (2 mM of Ca^{2+}) in order to investigate the change in size in the presence of Ca^{2+} with and without CO_3^{2-}. Z-average size of AgNP-Cit in $CaCO_3$ suspension was 53 ± 2.9 nm after 1 week supporting the stabilizing effect of CO_3^{2-}, whereas 2 mM of $CaCl_2$ lead to 620 ± 49 nm over 1 day (Figure 3.1.a). Carbonate is also used as a coating for AgNP and has thus a stabilizing effect, as also shown with carbonate coated AgNPs (Piccapietra et al., 2012).

Z-average size of original AgNP-PVP suspension did not change in the presence of any anions studied and was stable over 1 week (Figure 3.1.b). Stability of AgNP-PVP in the presence of $NaNO_3$, NaCl and $CaCl_2$ for 1 day was proven by Hyunh et al., (2011), El-Badawy et al., (2010), Gondikas et al., (2012) and Thio et al., (2012), respectively. PVP is an amphiphilic compound, which has a very high molecular weight and makes very strong N bonds with Ag, and should thus provide a strong steric stabilization even over 1 week (Cumberland and Lead, 2009; El Badawy et al., 2010; Huynh and Chen, 2011; Kvitek et al., 2008; Thio et al., 2012; Zhang et al., 2012).

Chloride might be effective for stabilizing TiO_2 when it is present with monovalent cations like Na^+ and K^+, in contrast to the results with $NaNO_3$ and Na_2SO_4 (Figure 3.1.c). ZPs which were closer to the zero surface charge were also indicating the agglomeration of TiO_2 in the presence of $NaNO_3$ and Na_2SO_4 (Table A8-Table A9). Shih et al., (2012b) also pointed out the higher critical coagulation concentration for Cl^- than SO_4^{2-}. TiO_2 was stabilized in the presence of CO_3^{2-} over 1 week due to its pH buffering effect, as shown also by Ottofuelling et al., (2011) for the stabilization effect of HCO_3^- on TiO_2 over 15 hours. Stabilization effect of CO_3^{2-} was supported with media of $CaCO_3$ and $Ca(NO_3)_2$. Mean size of TiO_2 after 1 week in $Ca(NO_3)_2$ medium was high (> 1µm) and similar to the $CaCl_2$ medium (> 1µm). However, the mean size in $CaCO_3$ medium (212±6 nm) was lower and similar to the Na_2CO_3 medium (303±2 nm). In addition, mean size of TiO_2 after 1 week in $NaNO_3$ and Na_2SO_4 was higher than 1µm. Therefore, both Cl^- and CO_3^{2-} may be expected to exert more specific interactions with the TiO_2 surface than NO_3^- and SO_4^{2-} (Abdullah et al., 1990; Brown et al. 1999), and TiO_2 is stabilized with electrostatic repulsion due to the increased negative surface charge

3.3.4 Agglomeration of selected ENPs in the presence of NOMs and surfactants

Effects of HA and FA without added ions on the agglomeration of ENPs in suspension differ for each ENP. AgNP-PVP was extremely stable in the presence of HA or FA up to 15 mg/L over 1 week (Figure 3.2.b). AgNP-Cit seems to be slightly agglomerated in the presence of HA depending on its concentration (Figure 3.2.a). HA (15 mg/L) caused a slight increase in average size of AgNP-Cit which was also observed with NTA images. FA was more effective for stabilizing AgNP-Cit.

Mean size values were very similar to the original AgNP-Cit suspension for all FA concentration levels and there was no shift in size distribution profiles of NTA and UV-spectrum of the samples (Figure A4). HA is more hydrophobic and has higher molecular weight than FA which might result in strong steric stabilization due to its higher affinity to bind surface of ENPs, but also in bridging effects between nanoparticles. Gao et al., (2012) also highlighted the agglomeration effect of HA when it is present at high concentrations due to the bridging effect between NPs. However, Chinnapongse et al., (2011) found slight agglomeration of TiO_2 in the presence of FA. Although the mean size of TiO_2 in suspensions with HA or FA fluctuated over 1 hour and 1 day, the ultimate mean size at the end of 1 week was always smaller than the mean size of original TiO_2 suspension after 1 week, pointing out the dispersing effect of NOM for TiO_2.

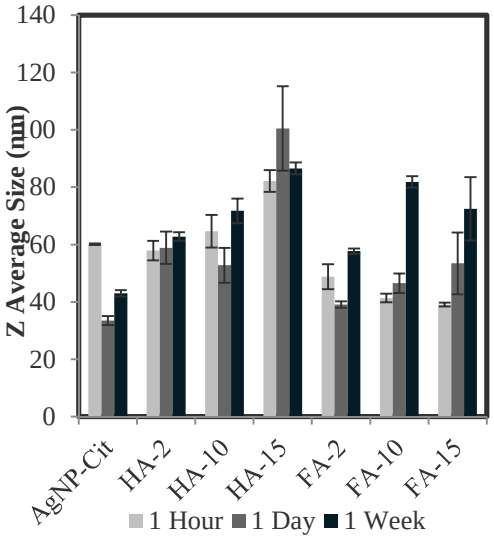


Figure 3. 2 (a): Z Average Size of ENPs suspended in NOM media- AgNP-Cit

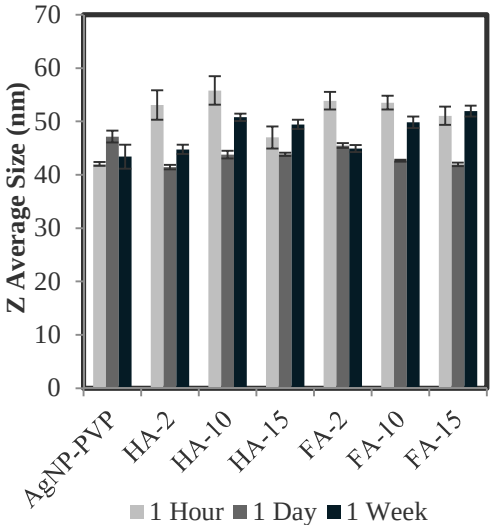


Figure 3. 2 (b): Z Average Size of ENPs suspended in NOM media- AgNP-PVP

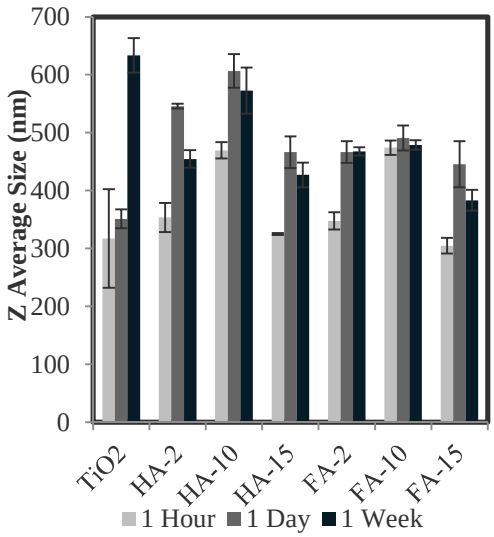


Figure 3. 2 (c): Z Average Size of ENPs suspended in NOM media- TiO_2 for thee time points 1 hour, 1 day and 1 week (HA and FA: 2, 5 and 15 mg/L)

Results obtained for SDS and AEO media were usually very similar to the results for HA and FA. Mean size of AgNP-PVP did not change in SDS or AEO suspension up to 15 mg/L over one week (Figure A5.b). Although Z-average sizes of TiO_2 in the suspension of SDS and AEO were fluctuating slightly for 1 hour and 1 day, they were always lower than the Z-average size of original TiO_2 suspension after 1 week, which shows the dispersing effect of surfactants (Figure A5.c). Zhang et al. (2008) found similar results for SDS media and showed that a size

of 500 nm was irreversible for TiO_2 and that TiO_2 could not be dispersed anymore, totally in agreement with our study. On the other hand, SDS and AEO lead to lower Z-average size of AgNP-Cit over 1 week (Figure A5.a). These effects may be attributed to steric stabilization (Kvitek et al., 2008).

3.3.5 Agglomeration of selected ENPs in the presence of freshwater simulated media (ionic medium mixed with NOM or/and surfactants)

Ionic medium was the background of all of the combinations, with a composition close to the conditions in freshwaters with moderate hardness (Table A1). Different combinations were prepared as a candidate to be a freshwater simulated medium to investigate the optimum one. Z-average sizes of AgNP-Cit in ionic medium (Figure 3.3.a) were lower than Z-average size of AgNP-Cit in $NaCl+CaCl_2$ medium over 1 hour (Figure 1.a) due to the lower concentration of Ca^{2+} in ionic medium (Table A1). Addition of HA, FA (5 mg/L) and SDS, AEO (1 mg/L) to the ionic media did not lead to any agglomeration effect (Figure 3.3.a), in agreement with the media including only HA or FA (Figure 3.2.a). In contrast to effects within 1 hour, AEO promoted agglomeration especially over 1 day which could be due to the fact that AEO is a nonionic surfactant and adsorption of AEO to AgNP-Cit might lead to bridging effects. Kvitek et al., (2008) also reported low stabilization effect for non-ionic surfactants (Brij group) due to steric effects. Further studies should be conducted to investigate the possible mechanisms for the agglomeration effect of non-ionic surfactants. Similar to the ionic medium combined with AEO, simulated fresh water combinations including ions, NOMs and AEO lead to significant agglomeration over 1 week supporting the effect of AEO on agglomeration of AgNP-Cit. Therefore, agglomeration of AgNP-Cit in long term like 1 week would be affected by several components in freshwater simulated media.

Z-Average sizes of AgNP-PVP were below 100 nm over 1 week in all media which indicates high stability of AgNP-PVP (Figure 3.3.b). However, mixture of ionic medium with NOMs and/or surfactants resulted in very slight agglomeration ($p<0.05$) from 60 nm to 80 nm which (Figure 3.3.b) can be attributed the effect of Mg^{2+} (0.4 mM) which was at a lower concentration than in individual Mg^{2+} media (3.3 mM). AgNP-Cit was more vulnerable to agglomeration than AgNP-PVP as it is expected. Neutralization of the negative charges of citrate coating with the cations present in media could lead to the agglomeration for AgNP-Cit while AgNP-PVP was stable due to the effect of steric stabilization. (Huynh and Chen, 2009; Chinnapongse et al., 2011; Cumberland and Lead, 2009; El Badawy et al, 2010; Mukherjee, 2010; Thio et al, 2012).

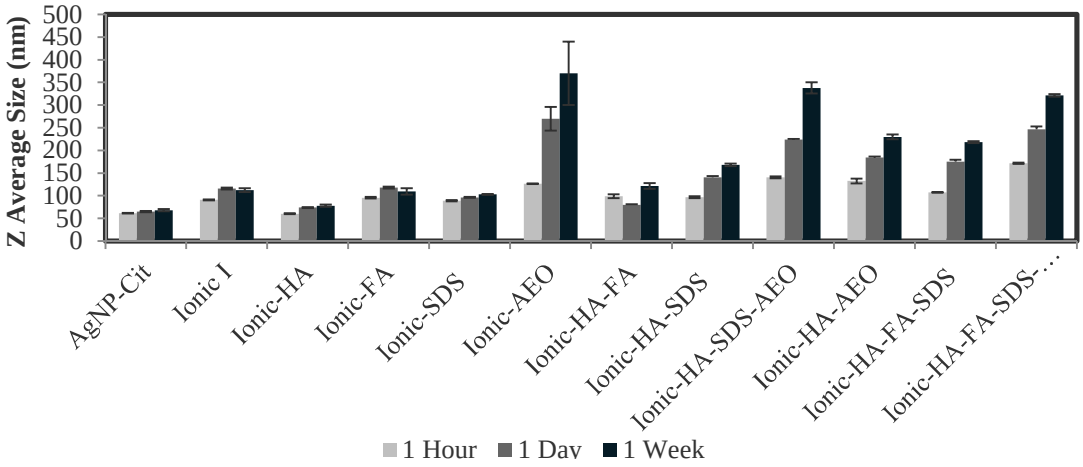


Figure 3. 3 (a): Z Average Size of ENPs suspended in freshwater simulated media- AgNP-Cit.

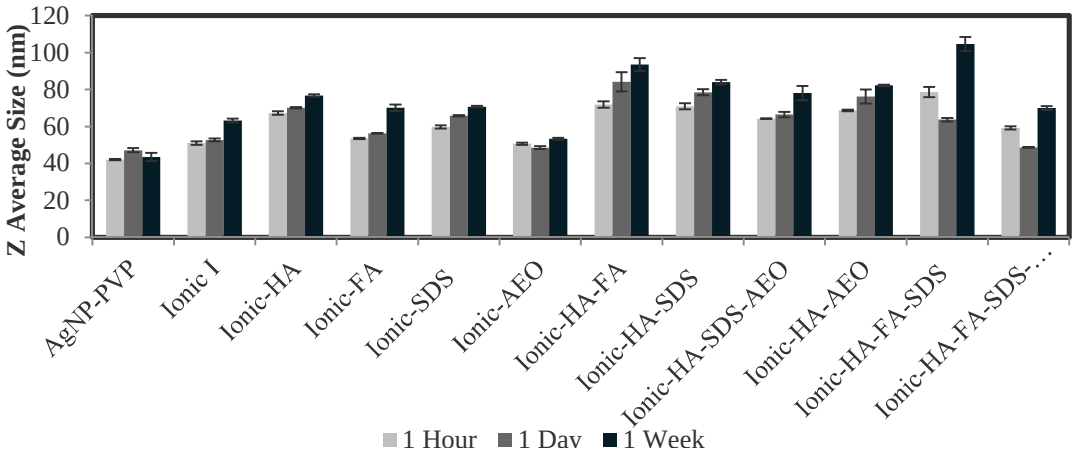


Figure 3. 3(b): Z Average Size of ENPs suspended in freshwater simulated media- AgNP-PVP.

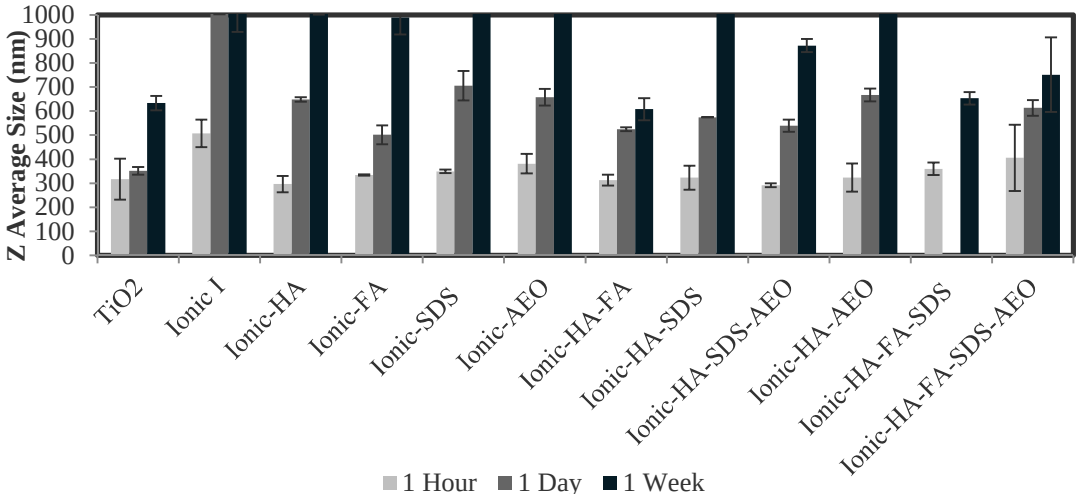


Figure 3. 3 (c): Z Average Size of ENPs suspended in freshwater simulated media- TiO_2 for the time points 1 hour, 1 day and 1 week (Ionic media were given in Table S2, HA and FA: 5 mg/L, SDS and AEO: 1 mg/L).

Highly stabilized nature of AgNP-PVP might provide the flexibility of using only ionic media to predict the agglomeration levels in freshwater media. Figure 3.3.c shows that mostly the presence of ions determines the size of TiO_2 in suspension. The scale of Figure 3.3.c is limited at 1000 nm since the higher hydrodynamic diameters measured with DLS are not so reliable. Average size for ionic medium was very close to average sizes for divalent cations and their mixtures with NOMs and/or surfactants, supporting effects of these compounds on agglomeration. Average sizes for 1 hour and 1 day samples of TiO_2 suspended in ionic media mixed with NOM or surfactants suggest that NOM and surfactants may act as stabilizer for a short time. However, results after 1 week were very similar to the results for ionic medium itself which shows that agglomeration up to 1 µm might be inevitable over 1 week in spite of presence of NOMs or surfactants (Figure 3.3.c). Chowdhury et al., (2012) stated that HA is only effective for dispersing in the presence of monovalent cations. In agreement with this study, freshwater simulated media showed that the dispersant effect of HA was not observed after 1 day over 1 week. TiO_2 agglomerated more in the co-presence of organic matter and divalent cations (Figure 3.3.c). Domingos et al., (2010) also reported a similar effect for Ca^{2+} in the presence of FA. However, FA seems to be effective for stabilizing TiO_2 at a size around 600 nm even for 1 week when it is present in fresh water simulated medium with other NOM or surfactants (Figure 2.3.c). The combinations without FA resulted in higher average sizes. Dispersing effect of FA (more than 5 mg/L) was also pointed by Domingos et al., (2010) when it is present at high concentrations by adsorbing on the surface and leading to thick steric barriers. In conclusion, HA, SDS and AEO may not stabilize TiO_2 in freshwater as effectively as when they are present alone over 1 week, in contrast to FA which seems to be also effective in freshwater simulated media. Nevertheless, several components in freshwater media might be effective for agglomeration of TiO_2.

3.3.6 Agglomeration of selected ENPs in a natural surface water sample

Whereas AgNP-Cit and TiO_2 agglomerated in a natural water sample with the composition shown in Table A2, AgNP-PVP was still very stable over 1 week (Figure 2.4). Z-average size of AgNP-PVP, which was 52±6.4 nm over 1 week (Figure 3.4), was very similar to the expected result for water media containing 0.7 mM Mg^{2+}.

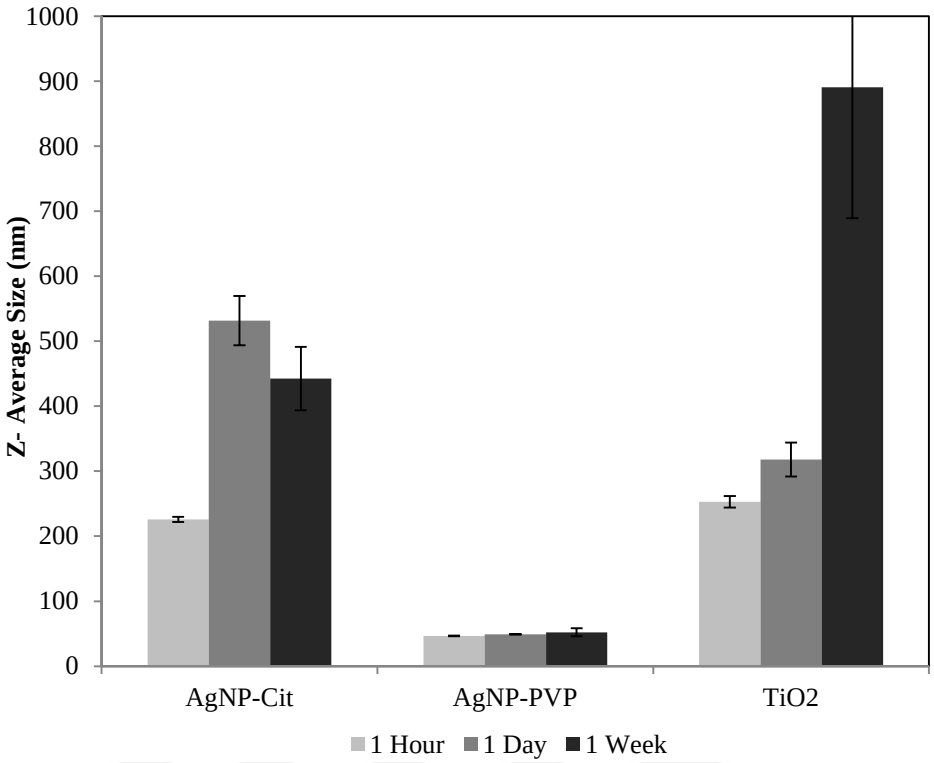


Figure 3. 4: Z Average Size of ENPs in Chriesbach river water sample for the time points 1 hour, 1 day and 1 week.

Concentration of Ca^{2+} in Chriesbach (2.8 mM) was high enough to lead to agglomeration of AgNP-Cit over 1 hour, in a similar way as in a medium with 3.3 mM $CaCl_2$ (Figure 3.1.a). However, AgNP-Cit only agglomerated up to 500 nm over 1 day (Figure 3.4), in contrast to the $CaCl_2$ medium which led within one day to agglomerate size of 900 nm (Figure 3.1.a), and this size was stable over 1 week. Therefore, stability of AgNP-Cit after 1 day could also be attributed to the effect of alkalinity (CO_3^{2-}) or NOM present in water, as in freshwater simulated media.

Z-average size of TiO_2 was 344±19 nm over 1 day and agglomerated up to 891±201 nm over 1 week (Figure 3.4). For this surface water sample, high concentration of Ca^{2+} promoted rapid agglomeration of AgNP-Cit and TiO_2. However, presence of NOM or surfactants (around 2 mg/L) might play a stabilizing effect and keep a constant size after 1 day over 1 week for AgNP-Cit. On the other hand, stabilization of TiO_2 over 1 week could not be sustained which was also observed in freshwater simulated media without FA. In conclusion, similar trends were observed for all ENPs in Chriesbach water sample as in freshwater simulated media (including ions, NOMs and surfactants). Nevertheless, a significant number of natural water samples, which have different concentration levels of Ca^{2+}, Mg^{2+}, DOC, pHand alkalinity, should be studied since they are dominant factors to predict the behavior of ENPs over long

time in the aquatic systems. Moreover, agglomeration or stabilization of the NPs might be promoted depending on the type of organic matter. Therefore, agglomeration of ENPs depending on the structure of organic matter should be investigated with fractionation of water samples, such as in terms of molecular weight or hydrophobicity.

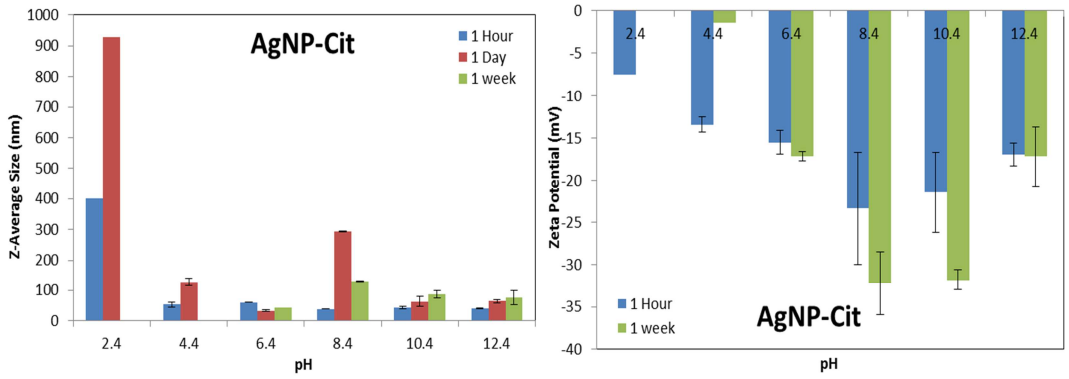
3.4 Conclusion

Comparison of size distribution profiles given by DLS and NTA helped in the interpretation about the potential changes in size distribution.

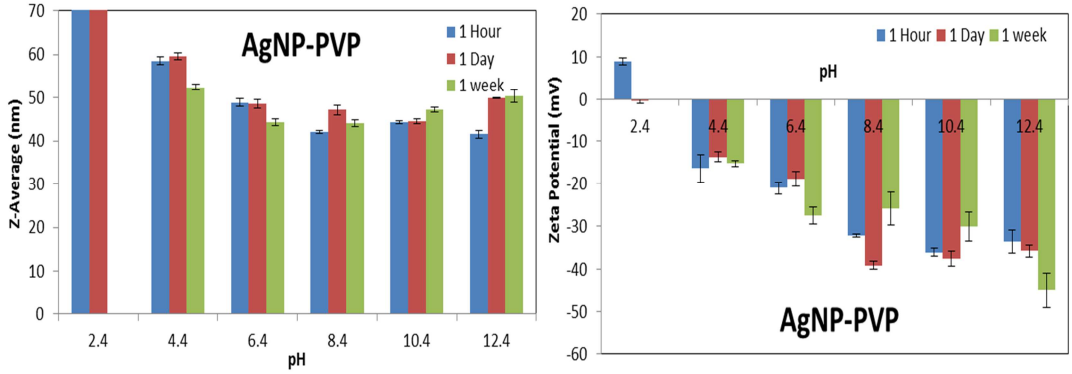
Freshwater simulated media suggested in this study, which include major components (ions, NOM and surfactants) and give similar results to natural freshwater, might be useful for the prediction of behavior of ENPs in freshwater based on the selected components. In addition, various combinations of the freshwater simulated media evaluated in this study can be used in future experimental studies investigating the fate and toxicity of ENPs and/or provide insight for the composition of media, which both simulate the freshwater media and are compatible with studies of ENP effects. Evaluation of agglomeration over time course of 1 week in freshwater simulated media provided valuable data for fate models dealing with ENPs and ERA studies. If agglomeration of AgNP-Cit and TiO_2 was only monitored for hours in a very simple medium including ions and/or only one type of NOM, results may be misleading with a possible over- or underestimation of agglomeration.

From the present results, it seems likely that AgNP-Cit and TiO_2 will rapidly settle down in natural freshwater media depending on the concentration of divalent cations and alkalinity, due to high agglomeration levels. It would be therefore of interest to investigate what fraction of ENPs has the potential to sediment from water column and which kinds of benthic organisms could be exposed to ENPs. Moreover, these critical factors promoting agglomeration must be considered in the toxicity studies in terms of uptake and accumulation where size dependency is investigated and ionic media are used. All of these behavior predictions in freshwater simulated media could be the key points for the hazard and exposure assessment steps of ERA studies. In addition, they might give an idea about the uncertainty level which are the typical limitations of these studies and should be considered through the evaluation procedure for a proper ERA.

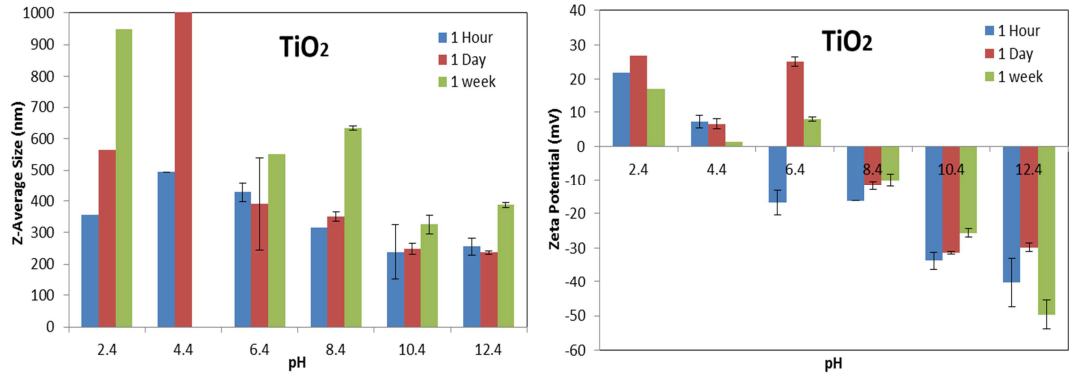
APPENDIX A: Chapter 3



a)



b)



c)

Figure A.1: Effect of pH on the agglomeration of ENPs a) AgNP-Cit b) AgNP-PVP c) TiO_2

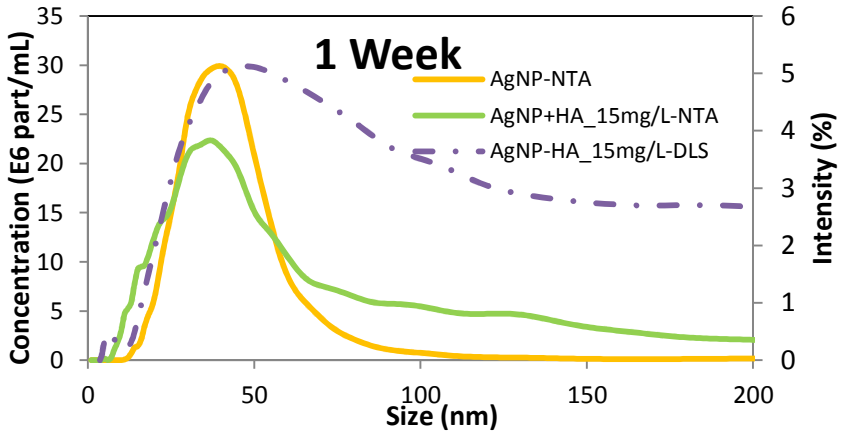


Figure A.2: Comparision of size distribution profiles of DLS and NTA for HA (15 mg/L).

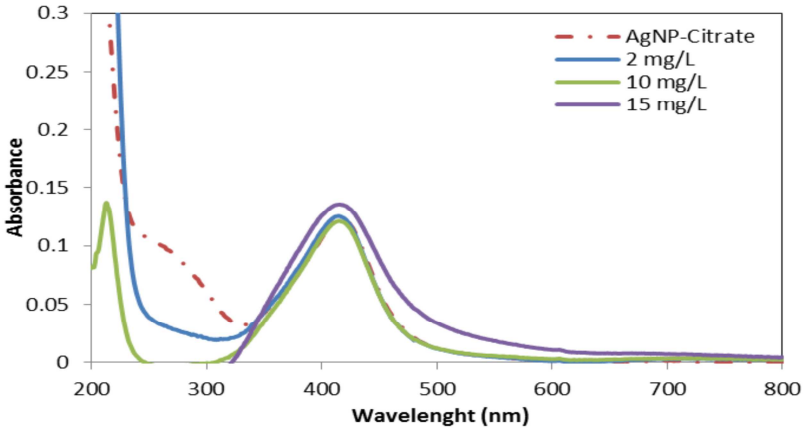


Figure A.3: Absorbance profile of AgNP-Cit suspended in HA media at different concentrations

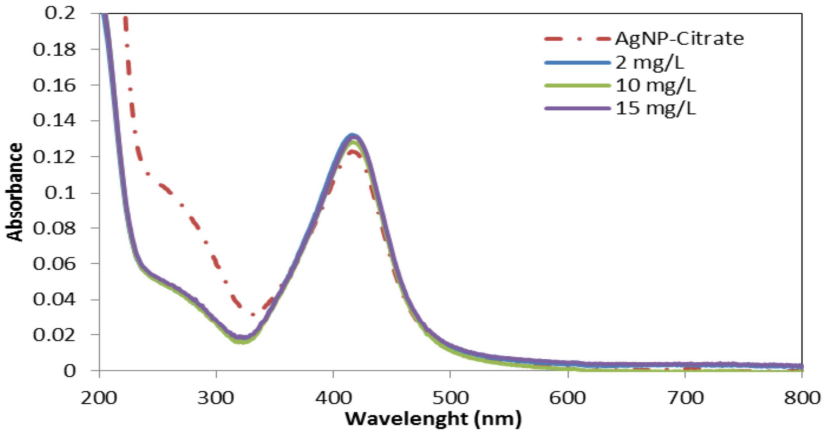
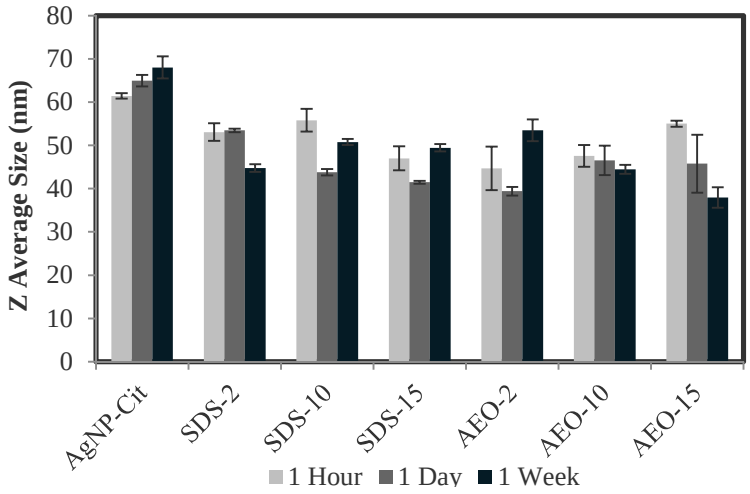
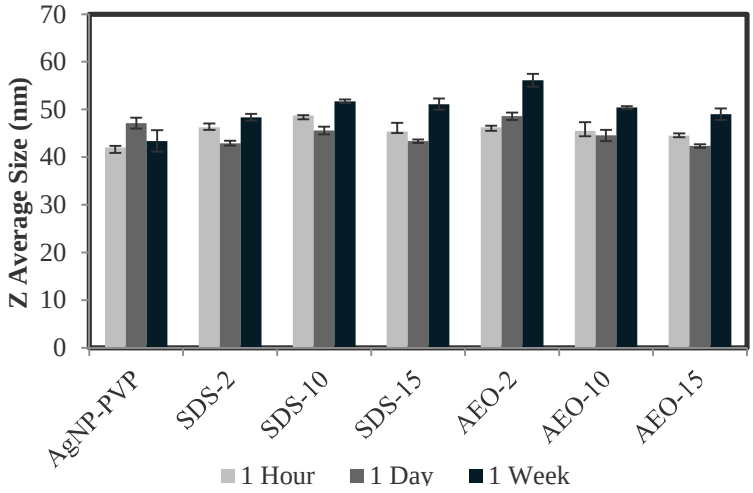


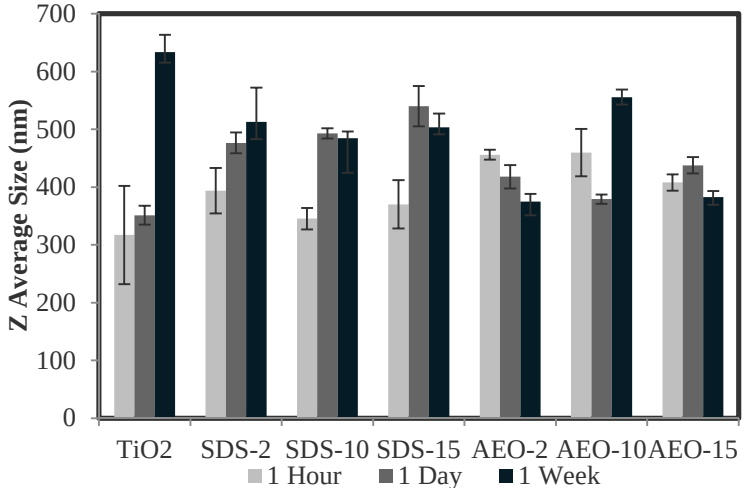
Figure A.4: Absorbance profile of AgNP-Cit suspended in FA media at different concentrations



a)



b)



c)

Figure A.5: Z-Average Size of ENPs suspended in different surfactant media

Table A.1: Concentrations of ions in each synthetic media

Media	NaCl (mM)	KCl (mM)	$CaCl_2$ (mM)	$MgCl_2$ (mM)	$NaNO_3$ (mM)	Na_2CO_3 (mM)	Na_2SO_4 (mM)
Single	10	10	3.3	3.3	10	3.3	3.3
NaCl+KCl	5	5	-	-	-	-	-
NaCl+$NaNO_3$	5	-	-	-	5	-	-
NaCl+$CaCl_2$	2.5	-	2.5	-	-	-	-
NaCl+$MgCl_2$	2.5	-	-	2.5	-	-	-
$CaCl_2$+ $MgCl_2$	-	-	1.7	1.7	-	-	-
Ionic	-	0.5	2	0.4	-	0.8	-

Table A.2: Water quality characterization for Chriesbach river

Parameter	**Chriesbach**
pH	8.23
DOC (mg C/L)	2.0
Alkalinity (mM)	6.5
Hardness (mM)	3.86
Na^+ (mM)	0.97
K^+ (mM)	0.1
Ca^{++} (mM)	2.9
Mg^{++} (mM)	0.8
Cl^- (mM)	1
NO_3^- (mM)	0.1
SO_4^- (mM)	0.27
Ionic Strength (mM)	9

Table A.3: Z-Average Size (DLS), Average Size (NTA) and Zeta Potential of AgNP-Cit in different single synthetic media

Media	1 Hour						1 Day						1 Week					
	Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)	
	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
NaCl	35	0.7	46	1.9	-15	2.4	41	0.7	48	0.4	-12	2.2	74	0.6	50	2.0	-4	0.9
KCl	45	6.6	60	0.9	-16	2.3	45	1.1	52	5.6	-	-	162	14.9	49	2.1	-2	0.4
CaCl2	196	22.4	191	10.2	-12	0.7	984	12.7	-	-	-13	0.2	-	-	-	-	-	-
MgCl2	192	15.3	207	5.8	-12	0.1	641	140.2	-	-	-13	1.0	-	-	-	-	-	-
NaNO3	44	1.2	46	1.9	-13	1.7	106	0.5	55	3.2	-15	2.0	143	1.3	69	4.1	-11	0.6
Na2CO3	74	1.2	72	9.2	-23	3.6	62	2.8	65	8.8	-18	1.4	64	1.2	105	7.3	-16	2.3
Na2SO4	37	1.0	57	3.2	-18	1.3	47	10.5	63	0.5	-13	0.6	150	22.8	86	7.2	-20	1.7
NaCl+KCl	46	2.9	40	2.9	-6	0.1	60	2.3	65	8.2	-7	1.6	79	11.2	151	7.2	-6	2.4
NaCl+NaNO3	54	4.0	67	7.1	-6	0.6	57	4.0	67	4.4	-	-	52	0.8	103	3.5	-9	1.7
NaCl+CaCl2	164	4.4	139	10.1	-15	0.8	685	34.9	-	-	-15	0.3	-	-	-	-	-	-
NaCl+MgCl2	148	1.3	162	18.0	-14	1.4	693	51.9	-	-	-15	1.1	-	-	-	-	-	-
CaCl2+MgCl2	194	5.7	-	-	-13	0.6	875	59.3	-	-	-13	0.9	-	-	-	-	-	-
HA-15mg/L	82	3.7	42	1.1	-20	2.1	87	2.0	49	3.8	-17	0.5	100	14.7	49	13.4	-17	0.5
HA-10mg/L	65	5.7	53	5.2	-24	4.8	72	4.3	46	1.3	-18	1.5	53	6.1	78	15.7	-18	1.5
HA-2mg/L	58	3.4	49	4.4	-24	1.8	63	1.5	49	0.5	-28	2.2	59	5.6	47	2.8	-28	2.2
FA-15mg/L	39	0.6	41	1.4	-23	1.8	53	10.8	49	3.8	-23	4.3	72	11.0	55	8.9	-22	-
FA-10mg/L	41	1.5	44	3.1	-21	2.8	47	3.4	43	1.2	-23	1.8	82	2.0	52	3.4	-24	2.1
FA-2mg/L	49	4.4	43	2.5	-26	1.8	39	1.1	43	1.2	-24	2.1	58	1.0	49	6.4	-23	-
SDS-15mg/L	47	2.0	42	1.1	-20	2.1	44	0.3	49	3.8	-20	3.1	49	0.9	49	13.4	-17	0.5
SDS-10mg/L	56	2.7	53	5.2	-24	4.8	44	0.7	46	1.3	-20	2.9	51	0.7	78	15.7	-18	1.5
SDS-2mg/L	53	2.8	49	4.4	-24	1.8	41	0.4	49	0.5	-18	1.5	45	0.9	47	2.8	-28	2.2
AEO-15mg/L	45	5.0	68	0.7	-22	0.2	39	1.0	68	4.3	-24	2.0	38	2.3	61	4.2	-24	2.0
AEO-10mg/L	48	2.5	50	3.2	-16	3.6	47	3.4	61	4.8	-17	-	44	1.0	64	3.1	-17	-
AEO-2mg/L	55	0.7	105	3.6	-20	7.2	46	6.7	55	5.1	-22	0.6	53	2.5	48	0.7	-22	0.6

*Std Dev of Z-Average Size and Zeta Potential are calculated with at least 12 sub-measurements of two replicates. Std Dev of Average size is calculated with 3 video images of one sample. “-“ means no data avaliable

Table A.4: Z-Average Size (DLS), Average Size (NTA) and Zeta Potential of AgNP-Cit in different mixture of synthetic media

Media	1 Hour						1 Day						1 Week					
	Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)	
	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
Ionic	91	1.1	95.4	4.3	-14	0.0	116	2.2	144	5.8	-14	1.1	112	4.3	105	2.6	-12	1.0
Ionic+HA	60	0.9	93.0	15.6	-14	1.9	74	1.1	106	12.0	-15	0.3	78	2.5	115	3.9	-16	0.5
Ionic+FA	96	1.7	87.7	19.4	-15	4.3	118	2.0	94	4.0	-15	1.5	109	7.1	91	7.1	-10	3.5
Ionic+SDS	89	1.5	91.9	47.2	-15	1.4	96	1.1	106	4.2	-12	1.6	103	0.4	84	1.3	-14	0.3
Ionic+AEO	166	2.2	119.7	30.0	-14	0.0	270	25.9	-	-	-14	0.4	370	69.9	-	-	-	-
Ionic+HA+FA	99	4.0	103.3	15.1	-13	0.8	80	1.0	86	3.4	-9	1.2	122	6.5	105	2.6	-6	1.6
Ionic+HA+SDS	97	2.0	132.5	8.0	-12	0.6	141	2.4	-	-	-14	0.6	168	2.6	162	2.8	-13	0.7
Ionic+HA+SDS+AEO	141	1.9	146.8	20.3	-13	0.4	224	1.5	198	8.1	-14	0.7	338	12.7	237	38.0	-14	0.7
Ionic+HA+AEO	132	5.3	144.9	3.7	-14	0.1	184	2.2	164	3.3	-14	0.4	230	5.3	206	5.2	-14	0.5
Ionic+HA+FA+SDS	107	0.7	107.7	7.3	-14	0.2	175	4.2	174	16.6	-	-	218	2.2	153	8.8	-13	0.0
Ionic+HA+FA+SDS+AEO	172	1.2	161.3	4.0	-13	0.4	247	6.5	216	8.2	-14	0.4	321	2.6	275	32.0	-20	0.1

*Std Dev of Z-Average Size and Zeta Potential are calculated with at least 12 sub-measurements of two replicates. Std Dev of Average size is calculated with 3 video images of one sample. “-“ means no data avaliable

Table A.5: Z-Average Size (DLS), Average Size (NTA) and Zeta Potential of AgNP-PVP in different single synthetic media

Media	1 Hour						1 Day						1 Week					
	Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)	
	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
NaCl	41	1.3	45	3.0	-23	2.1	44	1.2	44	-	-25	1.9	41	0.1	45	4.0	-17	1.0
KCl	55	5.6	54	1.5	-12	1.1	59	0.6	60	4.4	-15	3.4	43	0.3	51	3.5	-13	3.3
CaCl2	53	6.5	93	2.8	-10	1.9	63	1.8	123	12.4	-10	0.3	108	1.3	109	10.0	-12	1.3
MgCl2	227	18.4	166	12.1	-8	0.3	305	13.8	-	-	-13	0.4	547	5.1	-	-	0	0.0
NaNO3	44	0.5	48	3.3	-23	2.0	45	0.2	49	3.4	-24	3.6	45	0.8	54	1.4	-12	1.4
Na2CO3	46	0.5	47	1.6	-33	1.7	49	0.2	41	1.6	-31	0.4	42	0.2	41	1.6	-29	1.8
Na2SO4	41	0.2	50	0.5	-33	0.4	42	0.7	45	0.1	-15	0.6	44	0.7	71	7.4	-20	0.6
NaCl+KCl	49	0.8	46	2.0	-12	1.3	47	0.2	5	0.6	-20	2.2	50	1.3	49	1.7	-11	1.7
NaCl+NaNO3	41	1.1	46	2.1	-15	0.2	47	1.1	11	9.2	-17	0.0	52	1.7	50	1.1	-13	2.2
NaCl+CaCl2	57	3.4	71	4.9	-11	0.3	63	1.8	-	-	-10	0.3	86	1.8	-	-	-10	0.9
NaCl+MgCl2	248	9.2	230	6.8	-15	0.2	478	16.5	-	-	-16	1.3	488	14.6	-	-	-17	0.6
CaCl2+MgCl2	221	21.5	-	-	-12	0.0	410	22.0	-	-	-13	0.9	423	23.9	-	-	-14	0.1
HA-15mg/L	47	2.0	42	1.1	-20	2.1	44	0.3	49	3.8	-20	3.1	49	0.9	49	13.4	-17	0.5
HA-10mg/L	56	2.7	53	5.2	-24	4.8	44	0.7	46	1.3	-21	2.4	51	0.7	78	15.7	-18	0.7
HA-2mg/L	53	2.8	49	4.4	-24	1.8	41	0.4	49	0.5	-18	0.7	45	0.9	47	2.8	-28	2.2
FA-15mg/L	51	1.7	41	1.4	-23	1.8	42	0.3	49	3.8	-23	4.3	52	1.0	55	8.9	-22	1.4
FA-10mg/L	54	1.3	44	3.1	-21	2.8	43	0.2	43	1.2	-23	1.8	50	1.1	52	3.4	-24	2.1
FA-2mg/L	54	1.6	43	2.5	-26	1.8	45	0.5	43	1.2	-24	2.1	45	0.7	49	6.4	-23	4.2
SDS-15mg/L	46	0.9	45	1.0	-34	1.7	43	0.5	44	2.3	-32	2.8	48	0.7	52	3.3	-32	2.4
SDS-10mg/L	49	0.1	49	2.5	-27	2.4	46	0.8	46	2.5	-26	1.9	52	0.3	47	2.6	-35	1.8
SDS-2mg/L	45	1.8	42	4.8	-25	2.0	43	0.4	44	2.3	-35	1.8	51	1.2	48	2.4	-29	3.9
AEO-15mg/L	45	0.4	45	3.2	-25	0.7	42	0.4	46	1.7	-25	2.8	49	1.2	45	1.4	-29	2.6
AEO-10mg/L	46	1.8	49	4.3	-28	3.9	45	1.1	42	0.9	-23	0.8	50	0.3	48	3.6	-34	3.2
AEO-2mg/L	46	0.4	48	5.3	-31	0.8	49	0.7	43	2.7	-34	1.0	56	1.3	44	0.9	-38	3.1

*Std Dev of Z-Average Size and Zeta Potential are calculated with at least 12 sub-measurements of two replicates. Std Dev of Average size is calculated with 3 video images of one sample. “-“ means no data avaliable

Table A.6: Z-Average Size (DLS), Average Size (NTA) and Zeta Potential of AgNP-PVP in different mixture of synthetic media

Media	1 Hour						1 Day						1 Week					
	Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)	
	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
Ionic	51	0.9	63	3.2	-9	0.1	53	0.8	62	1.5	-11	0.5	63	1.0	63	1.3	-10	0.3
Ionic+HA	67	1.0	110	2.2	-12	1.6	70	0.4	75	1.5	-11	0.5	77	0.6	75	2.3	-13	1.0
Ionic+FA	53	0.4	56	16.6	-9	0.0	56	0.2	61	2.6	-9	0.1	70	1.7	72	0.8	-11	0.5
Ionic+SDS	60	0.8	71	4.2	-11	1.7	66	0.4	75	2.6	-12	0.7	71	0.4	72	1.2	-9	1.4
Ionic+AEO	51	0.6	57	7.0	-9	0.5	49	0.8	62	7.7	-11	0.6	53	0.5	55	1.2	-11	0.3
Ionic+HA+FA	72	1.7	68	5.1	-13	0.4	84	5.2	82	17.7	-14	0.4	93	3.5	91	6.7	-14	0.7
Ionic+HA+SDS	71	1.6	68	1.6	-14	0.5	79	1.6	70	5.4	-14	0.1	84	1.2	81	1.4	-14	0.3
Ionic+HA+SDS+AEO	64	0.2	65	3.0	-13	0.3	66	1.4	59	2.7	-13	2.0	78	3.9	78	3.0	-14	0.6
Ionic+HA+AEO	69	0.5	68	1.9	-12	1.4	76	3.8	66	0.7	-13	0.2	82	0.5	76	1.0	-14	0.2
Ionic+HA+FA+SDS	79	2.8	74	3.6	-13	0.2	64	1.0	68	0.0	-11	0.3	105	3.8	95	15.3	-13	-
Ionic+HA+FA+SDS+AEO	59	0.9	63	0.6	-13	0.5	49	0.1	51	0.2	-12	1.3	70	1.0	69	2.3	-11	1.2

*Std Dev of Z-Average Size and Zeta Potential are calculated with at least 12 sub-measurements of two replicates. Std Dev of Average size is calculated with 3 video images of one sample. “-“ means no data avaliable

Table A.7: Z-Average Size (DLS), Average Size (NTA) and Zeta Potential of TiO_2 in different single synthetic media

Media	1 Hour						1 Day						1 Week					
	Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)	
	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
NaCl	236	23.1	210	18.8	-30	7.6	324	1.4	-	-	-28	1.2	499	28.5	-	-	-20	1.7
KCl	344	55.4	243	31.7	-17	5.6	454	39.3	-	-	-20	1.6	522	50.3	-	-	-17	1.2
CaCl2	454	22.0	259	35.0	-6	2.9	812	28.8	-	-	-4	0.5	1825	-	-	-	-	-
MgCl2	440	13.2	274	66.7	-7	0.1	802	65.2	-	-	-4	1.4	-	-	-	-	-	-
NaNO3	408	15.7	226	40.4	-20	3.9	258	19.0	-	-	-23	1.0	965	137.1	-	-	-7	1.3
Na2CO3	416	15.7	221	6.7	-26	8.4	815	101.4	-	-	-24	2.5	303	1.9	-	-	-42	0.8
Na2SO4	416	0.2	261	39.8	-17	1.6	843	47.6	-	-	-10	0.6	1064	34.6	-	-	-2	1.5
NaCl+KCl	339	2.3	-	-	-29	6.6	331	8.6	-	-	-29	2.3	1327	4.9	-	-	-4	1.9
NaCl+NaNO3	374	7.8	-	-	-24	5.2	490	30.6	-	-	-28	2.1	2581	202.2	-	-	-1	0.4
NaCl+CaCl2	403	35.9	-	-	-9	0.9	601	58.3	-	-	-7	0.9	1296	115.3	-	-	-2	0.8
NaCl+MgCl2	429	6.7	-	-	-13	3.6	606	41.8	-	-	-7	3.8	1706	467.2	-	-	-1	0.6
CaCl2+MgCl2	412	67.1	-	-	-8	0.1	541	27.8	-	-	-7	1.8	1280	108.3	-	-	-6	1.1
HA-15mg/L	325	1.9	221	41.2	-31	-	466	27.3	244	41.7	-28	2.3	427	21.5	167	7.5	-19	3.9
HA-10mg/L	469	14.1	228	15.6	-29	-	607	29.0	172	12.3	-32	2.6	573	40.0	174	7.6	-24	2.3
HA-2mg/L	353	25.2	208	11.1	-34	-	546	4.3	243	8.6	-37	0.0	454	15.2	192	18.2	-11	34.5
FA-15mg/L	305	13.5	185	14.3	-33	-	445	39.8	260	35.8	-32	4.2	383	18.1	149	16.1	-34	0.7
FA-10mg/L	474	12.5	225	13.1	-33	-	491	21.8	204	5.0	-36	0.7	479	8.2	165	6.2	-34	2.3
FA-2mg/L	348	15.0	209	14.1	-33	-	467	19.0	-	-	-37	-	467	7.3	157	44.0	-30	1.7
SDS-15mg/L	370	41.9	222	22.7	-26	1.6	540	34.9	218	6.5	-	-	503	23.7	327	4.5	-15	2.8
SDS-10mg/L	345	18.5	196	42.3	-9	3.0	493	9.0	216	30.9	-19	1.8	484	12.0	210	12.2	-9	3.0
SDS-2mg/L	394	39.4	215	45.9	-6	2.1	476	17.9	212	12.8	-16	1.7	513	59.5	221	7.0	-6	2.1
AEO-15mg/L	408	14.3	240	20.2	-21	1.8	438	14.1	237	13.3	-	-	382	10.4	215	10.5	-19	0.9
AEO-10mg/L	460	41.1	189	10.2	-21	3.5	379	8.0	239	10.3	-22	0.9	556	13.1	207	34.9	-19	2.5
AEO-2mg/L	456	8.6	249	15.7	-19	6.0	418	20.1	248	8.2	-19	0.1	375	13.1	175	4.5	-20	0.4

*Std Dev of Z-Average Size and Zeta Potential are calculated with at least 12 sub-measurements of two replicates. Std Dev of Average size is calculated with 3 video images of one sample. “-“ means no data avaliable

Table A.8: Z-Average Size (DLS), Average Size (NTA) and Zeta Potential of TiO_2 in different mixture of synthetic media

Media	1 Hour						1 Day						1 Week					
	Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)		Z-Average Size (nm)		Average Size (nm)		Zeta Potential (mV)	
	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
Ionic	507	56.9	188	16.1	-11	0.1	1087	85.0	0	-	-11	1.1	1038	109.3	-	-	-17	0.8
Ionic+HA	297	33.6	213	-	-14	4.2	648	9.9	241	75.5	-15	0.2	1060	59.4	-	-	-14	1.4
Ionic+FA	334	2.9	185	31.7	-13	2.0	501	39.3	192	68.6	-13	0.7	988	69.4	-	-	-18	0.8
Ionic+SDS	349	7.5	197	21.1	-14	-	706	61.0	261	36.1	-7	1.2	1218	196.8	-	-	-1	0.5
Ionic+AEO	381	40.4	212	-	-9	2.6	658	34.7	-	-	-10	0.4	1176	106.0	-	-	-8	3.1
Ionic+HA+FA	313	22.8	200	14.0	-13	0.8	525	8.4	139	18.8	-15	0.2	608	45.5	-	-	-10	1.3
Ionic+HA+SDS	323	50.1	228	12.5	-13	1.3	574	0.5	193	46.0	-13	1.4	2247	281.4	-	-	-11	1.5
Ionic+HA+SDS+AEO	292	8.0	232	17.7	-13	1.1	539	25.1	-	-	-13	0.5	872	27.2	-	-	-13	0.7
Ionic+HA+AEO	324	59.1	241	2.2	-13	0.3	667	26.0	-	-	-13	1.4	1565	9.0	-	-	-10	0.7
Ionic+HA+FA+SDS	360	26.4	200	14.9	-14	0.4	-	-	190	8.0	-	-	653	26.0	-	-	-12	1.1
Ionic+HA+FA+SDS+AEO	406	138.0	238	26.1	-12	0.9	613	32.4	160	19.6	-13	1.1	751	155.1	-	-	-17	1.0

*Std Dev of Z-Average Size and Zeta Potential are calculated with at least 12 sub-measurements of two replicates. Std Dev of Average size is calculated with 3 video images of one sample.

CHAPTER 4
AGGLOMERATION OF AG AND TIO2 NANOPARTICLES IN SURFACE AND WASTE WATER: ROLE OF CALCIUM IONS AND OF ORGANIC CARBON FRACTIONS

ABSTRACT

This study aims to investigate factors leading to agglomeration of citrate coated silver (AgNP-Cit), polyvinylpyrrolidone coated AgNPPVP and titanium dioxide (TiO_2) nanoparticles in surface waters and wastewater. ENPs (1 mg/L) were spiked to unfiltered, filtered, ultrafiltered (<10 kDa and <1 kDa) samples. Z-average particle sizes were measured after 1 hour, 1 day and 1 week. AgNP-PVP was stable in all fractions of the samples and kept their original size around 60 nm over 1 week. Agglomeration of AgNP-Cit and TiO_2 was positively correlated with Ca^{2+} concentration, but dissolved organic carbon concentrations > 2mg/L contributed to stabilizing these NP. Moreover, agglomeration of AgNP-Cit in the various organic matter fractions showed that high molecular weight organic compounds such as biopolymers provide stabilization in natural water. A generalized scheme for the agglomeration behavior of AgNP-Cit, AgNP-PVP and TiO_2 in natural waters was proposed based on their relation with Ca^{2+}, Mg^{2+} and DOC concentration.

Emel Topuz, Jacqueline Traber, Laura Sigg, Ilhan Talinli, Agglomeration of Ag and TiO_2 nanoparticles in surface and waste water: role of divalent ions and of organic carbon fractions, 2015, Environmental Pollution, 204, 313-323.

4.1 Introduction

Nowadays, an increasing number of companies are attracting the customer`s interest with the consumer products that are consisting of engineered nanoparticles (ENPs) for a better functionality or quality. Silver and TiO_2 are among the most commonly encountered nanoparticles applied in consumer products in recent years (www.nanotechproject.org). However, their prevalent usage is of concern because of their possible release to the environment (Al-Kattan et al. 2014; Gondikas et al., 2014; Gottschalk and Nowack, 2011; Hedberg et al., 2014; Kaegi et al., 2013; Lombi et al., 2014), which may result in exposure of the ecosystem components and lead to adverse effects (Ali et al., 2014). Agglomeration and dissolution studies are providing valuable contributions for the speciation of the nanoparticles in the environment which are essential to develop an understanding of the mobility and transformation of ENPs. It is known that divalent cations can screen the surface charge of the ENPs and lead to agglomeration because of the decreased energy barrier for stability based on Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Domingos et al., 2010). Presence of natural organic matter (NOM) may lead to steric stabilization due to their higher affinity to bind on the surfaces (Akaighe et al., 2011), but they may also cause agglomeration because of bridging effects between ENPs (Gao et al., 2012).

According to our previous study in synthetic media (Topuz et al. 2014), the agglomeration behaviour of citrate coated Ag nanoparticles (AgNP-Cit) and TiO_2 depends on the concentration of Ca^{2+} and the presence of NOM, which can provide steric stabilization of particle size over 1 week. In contrast, polyvinylpyrrolidone AgNP (AgNP-PVP) were stable over 1 week in the presence of various ions except Mg^{2+} at high concentrations. Complexation of Ca^{2+} with citrate (El-Badawy et al., 2010) and Mg^{2+} with PVP coating (Topuz et al., 2014) can lead to agglomeration of AgNP-Cit and AgNP-PVP, respectively. Therefore, a surface water simulated medium was proposed for the agglomeration studies. However, the behavior of these ENPs could differ in natural waters which are more complex with various components, so that our motivation for this study was to conduct agglomeration studies in natural water media to establish more realistic relations between agglomeration and water chemistry (Behra et al. 2013; Chambers et al., 2014; Yu et al., 2014). Although there are some studies conducted with natural water samples, they are lacking in different aspects to make an overall evaluation for the agglomeration behavior of Ag and TiO_2 nanoparticles. Stabilization effect of dissolved organic carbon (DOC) in natural water samples was shown in some studies for AgNPs (Chinnapongse et al., 2011; Delay et al., 2011; Gao et al., 2009; Thio et al., 2012).

Keller et al. (2010) concluded that the electrophoretic mobility of TiO_2, ZnO and CeO_2 depends on ionic strength and DOC by comparing their behavior in seawater, lagoon, river and groundwater. Ottofuelling et al. (2011) proposed a test scheme to predict the behavior of TiO_2 in natural water (verified with natural water samples) and suggested to apply similar approaches to different kinds of ENPs and to study the effect of different types of NOM for further studies. Therefore, a detailed study for the prediction of agglomeration behavior of AgNPs is lacking in the literature, as well as time resolution data for the stability of ENPs in natural water media (Reidy et al. 2013). Hammes et al. (2013) also classified European surface waters according to the colloid stability behavior, which was based on theoretical calculations of Debye length, however without direct comparison to experimental data.

Furthermore, it is of interest to evaluate the ENP agglomeration behavior in different fractions of natural water such as unfiltered, filtered, ultrafiltered (< 10 kDa and < 1 kDa cut-off) to understand which NOM fraction may have a dominant effect for stabilization of ENPs. Analyzing both unfiltered and filtered water is essential to critically evaluate the experiments with synthetic water media in terms of their relevance for the real environment. Louie et al. (2013) studied the agglomeration of gold nanoparticles in 2 different Suwannee River NOM media with an average molecular weight of 691 and 12.8 kDa, respectively. They showed that low amounts of the media with higher molecular weight were more effective for nanoparticle stabilization than high amounts of the media with lower molecular weight due to the steric effects.

This study has two major aims, namely first to examine the possible correlations of the size levels of AgNP-Cit, AgNP-PVP and TiO_2 after 1 hour, 1 day and 1 week with the concentration of Ca^{2+} and DOC in natural waters of different composition. The second aim is to evaluate which fractions of the NOM are playing a major role for ENP stabilization or agglomeration by fractionating natural water samples by filtration and ultrafiltration. Therefore, this study provides novelty to the literature with its results for the long term agglomeration (1 week) in different fractions of natural water samples based on filtration and fractionation of organic matter by molecular weight. The obtained results can then be applied for ENP risk assessment to predict their size and fate in natural water, and so their hazard and exposure, and they can be used as a guide for the preparation of exposure media for toxicity experiments.

4.2 Materials and Methods

4.2.1 Chemicals

TiO_2 P25 were obtained from Evonik Industries in powder form and and AgNPs from NanoSys GmBH (Switzerland) as suspensions at 1g/L concentration. The manufacturers reported the nominal size of AgNPs (1 g/L) and TiO_2 (0.1 mg/L) as 25 nm. TiO_2 in powder form was suspended in nanopure water (NW) to prepare a stock solution (100 mg/L), and sonication in a bath sonicator was applied to both stock and working suspensions of TiO_2 for 2 hours. Agglomeration experiments were conducted with daily prepared working suspensions of AgNP-Cit, AgNP-PVP and TiO_2 (5 mg/L) in NW. The Z-average sizes of AgNP-Cit, AgNP-PVP and TiO_2 (1 mg/L) in NW were 61 ± 0.6 nm, 42 ± 0.4 nm and 317 ± 85 nm, respectively.

4.2.2 Sample Site Selection

Samples were taken from six surface water sources and three wastewater treatment plants. The critical parameters for the agglomeration of ENPs (Ca^{2+} , Mg^{2+} , DOC and alkalinity), at their respective concentrations (Topuz et al., 2014) were employed to make a preliminary classification for the sample site selection, based on the rivers in the NADUF (National long-term surveillance of Swiss rivers) project database (http://www.bafu.admin.ch/). Concentration ranges were assigned for each parameter (Table B1 in Appendix B) and 8 classes were developed not to miss out a critical concentration level. The sample numbers were optimized to have one representative sample for each class. Finally, five river sampling sites (Glatt-Rheinsfelden, Thur-Andelfingen, Aare Brugg, Rhein- Rheinsfelden, Rhein-Diepoldsau) were selected. The peat bog lake Etang de la Gruère with a very high (13.1 mg/L) concentration of DOC compared to river water samples was also included in the samples to observe a marginal class. Wastewater treatment plants (WWTPs) were selected considering the population they serve and their configuration which likely cause differences in terms of wastewater characteristics. All of the units in one of the selected WWTPs were sampled to investigate the possible differences in agglomeration behavior of ENPs at different stages of WWTP. Duebendorf WWTP (Duebendorf, Switzerland) was selected for sampling unit by unit since it has advanced biological wastewater treatment technology for the removal of nutrients (nitrogen and phosphorus). In addition, Duebendorf WWTP serves more than 50,000 Population Equivalency (PE) which means that high organic matter load is expected in the effluent sample. Effluent and influent samples were taken from two other WWTPs to examine

the possible effect of different treatment configurations for biological wastewater treatment. Kuesnacht and Zumikon were selected to represent relatively lower PE and different treatment technologies (Table B2). Samples were taken in glass bottles, which were muffled at 550 ºC for 24 hours in order to prevent organic matter contamination. All samples were filtered as soon as they were brought to laboratory and kept at 4 °C for the experiments and ultrafiltration.

4.2.3 Characterization of Nanoparticle Suspensions and Water Quality

Major ions were analyzed by ion chromatography according to standard methods at the analytical laboratory of Eawag. Each molecular weight fraction of the water samples was analyzed with Liquid Chromatography-Organic Carbon Detection-Organic Nitrogen Detection (LC-OCD-OND) which is based on the theory of Size Exclusion Chromatography-OCD technique (Huber et al., 2011) to identify the concentrations of biopolymers, humic substances, building blocks, low molecular weight humics and acids, neutrals. Zetasizer (Nano ZS, Malvern Instruments) equipped with a red laser (633 nm) was used for DLS measurements. Z-average size (based on intensity mean) was measured in triplicate with at least 12 sub-measurements and zeta potential (ZP) was calculated from electrophoretic mobility using the Henry equation. ENPs were imaged in selected samples with NTA using a NanoSight LM10 equipped with a LM14 temperature controller (NanoSight Ltd.). NanoSight NTA 2.2 Software was used to get mode and mean size of the ENPs in suspension with analyzing at least 3 different videos of ENPs. UVIKON 930 (Kontron Instruments) was used to measure UV-vis absorbance of AgNP suspensions in selected samples between 200 and 800 nm.

4.2.4 Experimental set up

Samples were fractionated as shown in Figure B1. This fractionation scheme is based on preliminary experiments performed with water from the small stream Chriesbach (Duebendorf, Switzerland). These preliminary experiments showed that AgNP-Cit were better stabilized in unfiltered samples than in filtered samples and that samples filtered over 0.2 µm and 0.45 µm resulted in very similar Z-Average sizes. The second trials showed that Minimate Tangential Flow Filtration (TFF) Capsule (Pall Coop.) capsules were effective for molecular weight fractionation of organic matters under 10 kDa and 1 kDa cut off and that agglomeration levels were different for these fractions (Figure B2). Therefore, unfiltered, 0.2 µm filtered, < 10 kDa and < 1 kDa cut off fractions were included in the experiments. After an aliquot was put aside

as unfiltered water, remaining samples were filtered over 0.2 µm filters (Cellulose acetate filters, Millipore) under vacuum. For the < 10 kDa and < 1 kDa cut off, filtered samples were passed through TFF of 10 kDa and 1 kDa, respectively. A water sample was fed from a stirring cell inlet to the TFF capsule with a vacuum pump, and the filtrate was collected in muffled glass bottles. Before filtering each sample, capsules were backwashed and flushed with NW until DOC was under limit of detection. Water quality parameters were measured only in the 0.2-µm filtered fraction, whereas organic matter characterization was performed for the < 0.2 µm and the < 10 kDa and < 1 kDa ultrafiltered fractions. Agglomeration experiments for unfiltered and filtered samples were conducted within 5 hours as soon as they were brought to laboratory. Molecular weight fractionations of filtered samples were completed within 24 hours.

Three replicates of the samples were spiked with 1 mg/L of ENPs, shaken at 100 rpm for 1 hour and time series measurements were done with DLS after 1 hour, 1 day and 1 week. The concentration of ENPs, spiking procedure and agglomeration experiments were as defined previously in Topuz et al. (2014) which are also quoted in Appendices E Information of this study.

4.3 Results

4.3.1 Characterization of Sample Sites

NADUF project database provided a precise selection of the most representative surface waters for the corresponding classes in Table B1, since the water quality characterization of the samples (Table 4.1 and 4.2) were similar to the average concentrations in the NADUF database. All filtered samples were analyzed with DLS and the Z-average sizes and zeta

Table 4. 1: Characterization of water quality for surface water samples.

Parameter (mM)	Glatt	Thur-Andelfingen	Aare Brugg	Rhein-Rheinsfelden	Rhein-Diepoldsau	Etang de la Gruere
pH	8.26	8.20	8.16	8.27	7.98	6.56
Alkalinity	4.83	4.05	3.08	2.86	1.89	0.69
Hardness	2.67	2.20	1.82	1.74	1.18	0.40
Na^{+}	1	0.6	0.3	0.28	<MQL	0.49
K^{+}	0.2	0.2	0.1	0.04	<MQL	<MQL
Ca^{++}	2	1.7	1.6	1.3	0.9	0.3
Mg^{++}	0.7	0.5	0.3	0.38	0.2	<MQL
Cl^{-}	1	0.5	0.2	0.26	0.14	0.54
NO_3^{-}	0.1	0.04	0.03	0.02	0.01	<MQL
SO_4^{2-}	0.2	0.12	2.3	0.32	0.27	<MQL
Ionic Strength	6.9	5.3	4.6	4.3	2.8	1.1
DOC (mg/L) filtered (< 0.2 µm)	1.55	1.99	3.82	1.36	0.929	13.1
DOC (mg/L) ultrafiltered(< 10 kDa)	1.50	1.76	2.14	1.29	0.645	4.67
DOC (mg/L) ultrafiltered(< 1 kDa)	1.25	1.49	1.58	1.16	0.624	3.26

Table 4.2: Characterization of water quality for wastewater samples.

Parameter (mM)	Duebendorf Influent	Aerated	Anaerobic	Biological Effluent	Clarification Effluent	Filtration Effluent	Kuesnacht Influent	Kuesnacht Effluent	Zumikon Influent	Zumikon Effluent
pH	8.0	7.91	7.81	7.99	7.81	7.96	8.22	7.67	8.00	6.70
Alkalinity	9.09	6.91	8.46	6.29	6.91	6.93	5.84	3.29	5.2	1.35
Hardness	3.47	3.47	3.40	3.30	3.31	3.32	2.42	2.46	2.42	2.43
Na^{+}	5.25	5.29	5.17	3.96	5.35	5.41	3.74	2.49	2.58	2.93
K^{+}	0.70	1.04	0.89	0.62	0.83	0.84	0.57	0.59	0.67	0.73
Ca^{++}	2.52	2.59	2.52	2.43	2.46	2.49	1.79	1.74	1.57	1.72
Mg^{++}	0.85	0.79	0.83	0.77	0.76	0.77	0.69	0.64	0.57	0.59
Cl^{-}	3.87	4.3	4.04	3.13	3.96	3.83	2.95	2.18	2.08	2.85
NO_3^{-}	<0.05	0.18	<0.05	0.13	0.18	0.18	<0.05	0.23	0.1	0.47
SO_4^{2-}	0.53	0.56	0.55	0.54	0.58	0.59	0.44	0.59	0.69	1.10
Ionic Strength	25.4	26.6	25.7	22.8	25.5	25.7	9.44	8.70	8.38	10.33
DOC (mg/L) filtered (< 0.2 µm)	60.7	6.8	9	5	7.3	6.5	24.3	3.5	16.1	3.1
DOC (mg/L) ultrafiltered(< 10 kDa)	53.8	5.7	6.8	4	5.7	5.5	17.8	3.0	11	2.9
DOC (mg/L) ultrafiltered(< 1 kDa)	49.2	4.9	6.3	1.6	5.1	4.8	11.7	2.5	9.7	2.7

Table 4.3: Concentration of organic matter compounds in the fractions of surface water and wastewater effluent samples (µg/L).

Samples	Dissolved Organic Carbon	Bio polymers	Humic Substances	Building Blocks	Low MolecularWeight Humics	Low MolecularWeight Acids	Neutrals
Rhein-Rheinsfelden-< 0.2 µm	1357	41	836	203	53	0	184
Rhein-Rheinsfelden -<10 kDa	1289	6	817	166	56	0	159
Rhein-Rheinsfelden -<1 kDa	1155	1	637	166	64	0	183
Etang de la Gruere - < 0.2 µm	13058	82	9370	1182	333	0	1595
Etang de la Gruere -<10 kDa	4670	14	2791	770	219	0	461
Etang de la Gruere - <1 kDa	3257	6	1582	566	179	59	514
Thur Andelfingen - < 0.2 µm	1994	68	943	319	89	0	394
Thur Andelfingen - <10 kDa	1756	5	957	239	112	0	277
Thur Andelfingen - <1 kDa	1488	1	663	275	99	14	300
Glatt – Rheinsfelden- < 0.2 µm	1552	63	770	202	79	0	235
Glatt – Rheinsfelden -<10 kDa	1504	4	874	273	67	0	183
Glatt - Rheinsfelden -<1 kDa	1248	1	630	228	66	17	219
Aare Brugg - < 0.2 µm	3821	63	848	471	140	944	1184
Aare Brugg - <10 kDa	2139	21	888	417	140	35	445
Aare Brugg - <1 kDa	1584	3	926	252	74	0	219
Rhein-Diepoldsau - < 0.2 µm	929	44	543	106	51	0	148
Rhein-Diepoldsau - <10 kDa	645	8	318	74	46	13	134
Rhein-Diepoldsau - <1 kDa	624	0	311	70	48	12	148
Zumikon Effluent - < 0.2 µm	3088	161	900	691	105	56	700
Zumikon Effluent- <10 kDa	2884	24	813	643	101	98	671
Zumikon Effluent- <1 kDa	2674	16	716	622	89	92	633
Kuesnacht Effluent- < 0.2 µm	3515	195	1059	687	123	38	722
Kuesnacht Effluent- <10 kDa	2974	9	929	617	106	45	659
Kuesnacht Effluent- <1 kDa	2475	4	720	538	93	59	593

potentials were between 100-200 nm and -10: -20 mV, respectively (Table B3). LC-OCD-OND results for organic matter characterization are presented in Table 3.3 and chromatograms are provided in Figure B3-4. Average molecular weights of biopolymers and humic substances were much higher than 10 kDa and around 1 kDa, respectively. Organic matter compounds with average molecular weights lower than 1 kDa include building blocks, low molecular weight humics, acids and neutrals. In all natural waters, the most abundant fraction consisted of humic substances, whereas biopolymers and lower molecular weight acids were present at lower concentrations.

4.3.2 Agglomeration behavior of ENPs in surface water and wastewater

Agglomerations of ENPs were characterized with the change in size measured with DLS. The data quality was checked with polydispersity indexes which were lower than 0.5 if not otherwise specified. The sizes of AgNPs for 1 hour experiments were also checked with NTA measurements (Table B4) and they were comparable for most of the samples in which AgNPs had mean size up to 200 nm. Rhein-Rheinsfelden sample (having an average DOC and Ca^{2+} concentration) were tested with UV-Vis Spectrometer (Figure B5). AgNP-Cit in Rhein-Rheinsfelden has a broader peak and smaller absorbance than in NW showing that it was agglomerated after 1 hour. AgNP-PVP resulted in almost the same peak in NW and in Rhein-Rheinsfelden showing its stability in natural water. The results were comparable with the results of DLS and NTA. Therefore, evaluations for all samples were made with DLS measurements. Zeta potentials were in the range -10 to -20 mV for all samples (Figures B6-16), and no clear trend was observed.

Z-average sizes of AgNPs and TiO_2 in Rhein- Diepoldsau and Etang de la Gruère samples followed a different trend from other samples. Higher sizes were measured in the unfiltered fractions than in the filtered ones, but their polydispersity indexes were around 0.7 which is not acceptable for good quality measurements. Therefore, unfiltered fractions of these samples were not reported in the results. After 1 hour, AgNP-Cit (65±5 nm) was agglomerated (in the range of 150-300 nm) in all fractions of all surface water samples (Figure 4.1 and Table B4) except for Etang de la Gruere where no agglomeration occurred. Agglomeration of AgNP-Cit in unfiltered fractions gradually increased after 1 day and 1 week (200-700 nm) except for Aare-Brugg. However, unfiltered samples resulted in lower Z-average sizes of AgNP-Cit than other fractions after 1 day and 1 week.

AgNP-PVP was stable with average sizes around 50 – 70 nm in all fractions of all surface water samples (Figure 4.2 and Table B4). Agglomeration of AgNP-PVP did not occur over 1 week under various water quality conditions such as the concentration of Ca^{2+} and DOC in the range of 0.3 - 2 mM and 0.9 - 13 mg/L, respectively. TiO_2 agglomerated (around 500 nm) in all samples except Etang de la Gruère after 1 hour (Figure 4.3). While the Z- average size did not change in 1 day for unfiltered and filteed samples,it increased in ultrafiltered samples. TiO_2 agglomerated up to micro meter scale in all samples over one week.

Since the WWTP influent and unit samples contain high suspended matters relative to effluent and surface water samples, particle sizes were only evaluated with filtered samples. AgNP-Cit agglomerated to average sizes in the range of 200 – 300 nm in unfiltered and filtered fractions of the WWTP samples and to around 400 nm in ultrafiltered samples after 1 hour (Figure 4.4).

AgNP-PVP were stable (<100 nm) in all of the WWTP samples after 1 day except in the Duebendorf influent sample (Figure B17). TiO_2 nanoparticles agglomerated in all of the WWTP samples after 1 hour (Figure 4.4); however, agglomeration levels were higher in Duebendorf WWTP samples (more than 450 nm). For 1 day, agglomeration increased for all samples, again especially in Duebendorf WWTP unit samples and increase of Z-averages sizes after 1 day were more pronounced for ultrafiltered fractions of all samples.

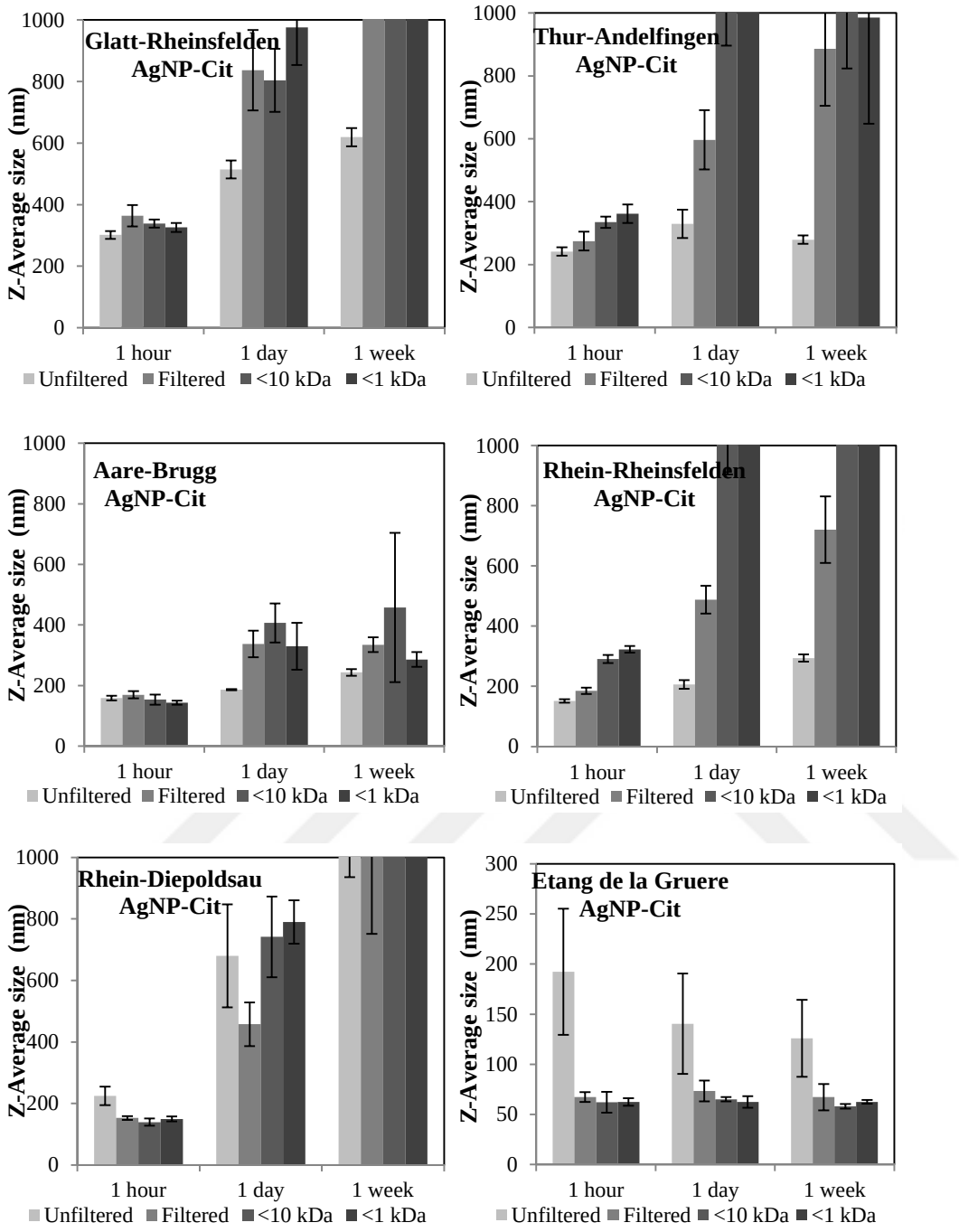


Figure 4.1: Z-Average size levels of AgNP-Cit in different fractions of surface water samples (Error bars represents the standard deviation of triplicates).

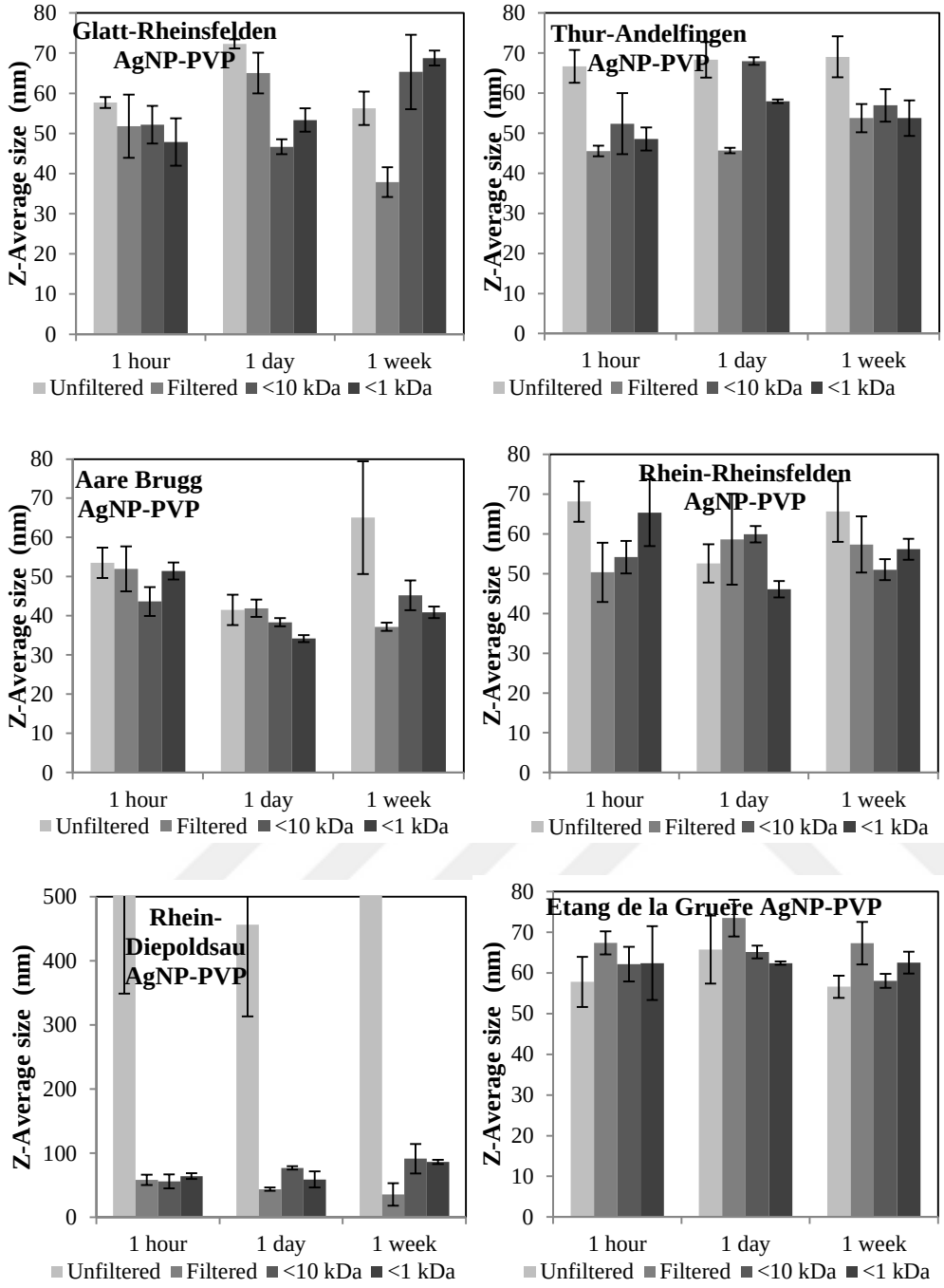


Figure 4.2: Z-Average size levels of AgNP-PVP in different fractions of surface water samples (Error bars represents the standard deviation of triplicates).

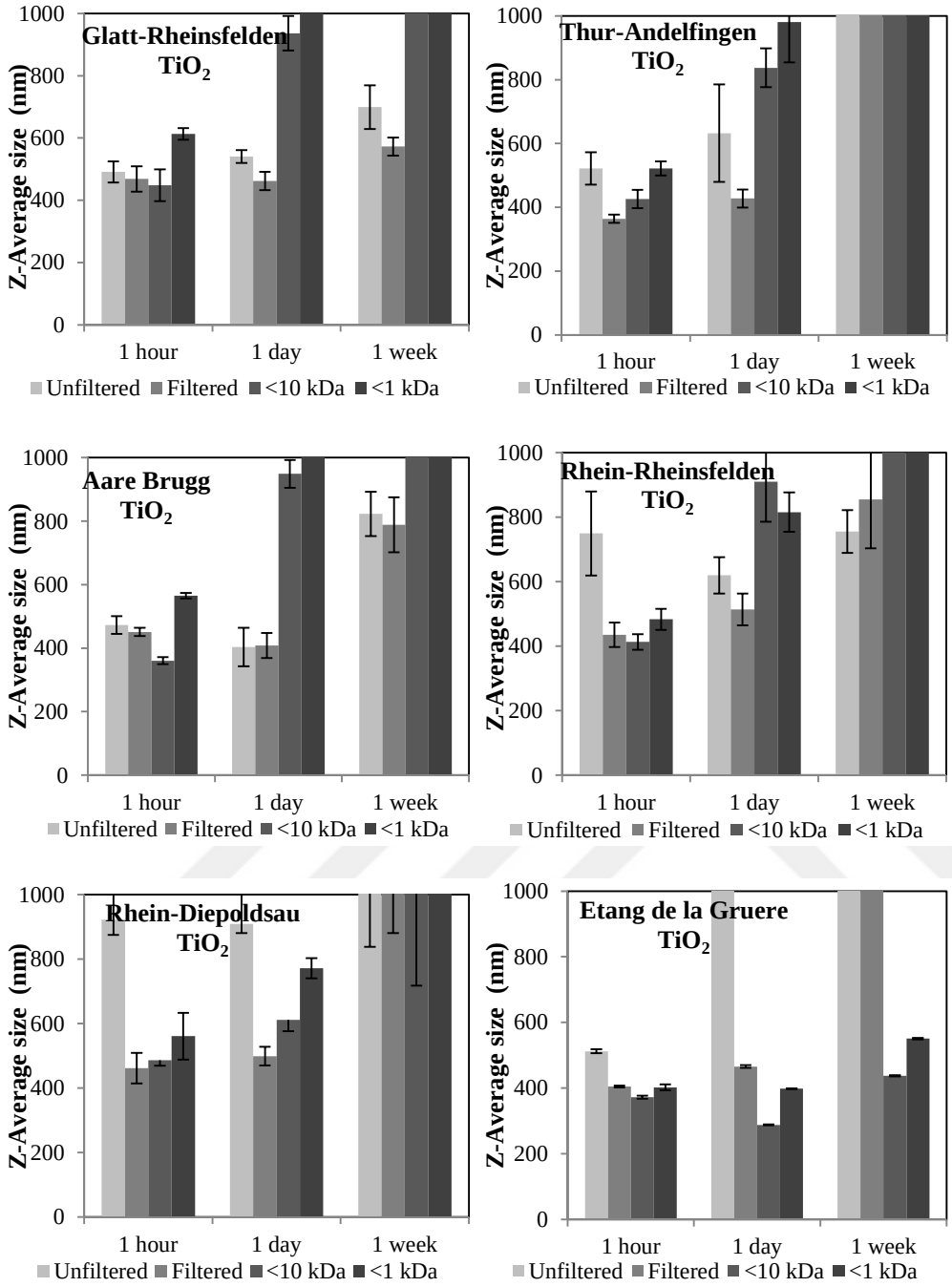


Figure 4.3: Z-Average size levels of TiO_2 in different fractions of surface water samples (Error bars represents the standard deviation of triplicates).

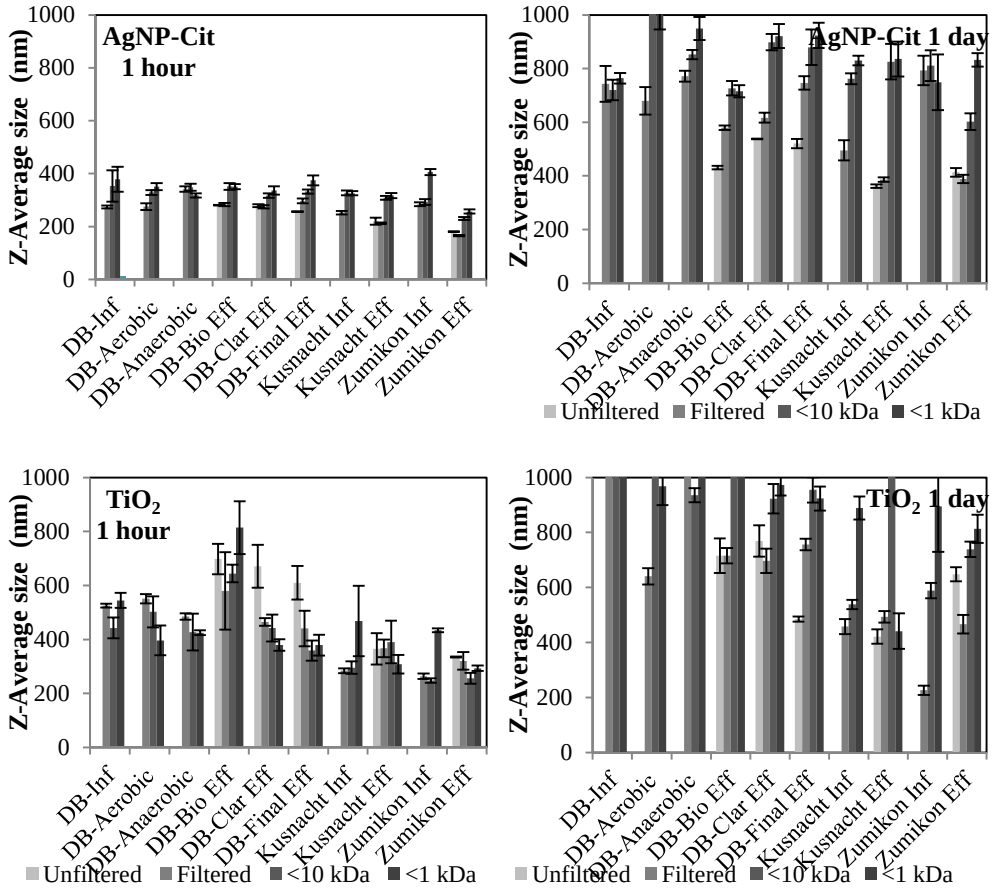


Figure 4.4: Z-Average size levels in different fractions of wastewater samples (Error bars represents the standard deviation of triplicates).

4.4 Discussion

4.4.1 Role of Ca^{2+} and dissolved organic matter concentration in surface and wastewater for the agglomeration of ENPs

Unfiltered fractions of Rhein- Diepoldsau and Etang de la Gruère samples reveal that surface waters with high natural suspended matters may result in unexpected agglomeration behavior for ENPs due to the possibility of heteroaggregation or misleading results of DLS due to overestimation. Z-Average sizes of ENPs were significantly higher in unfiltered fractions than in filtered ones for only these 2 samples (10 samples in total). Although it is known that DLS is not sensitive for the samples with high colloidal matter and polydispersity index (>0.7) were the highest for these samples, heteroaggregation also seems to be critical for the regulation of the agglomeration behavior in natural water samples with higher suspended matter (Quik et al., 2014). In such cases, the heteroaggregation process may be predominant and lead to higher Z-Average size levels of ENPs independently from the concentration of

Ca^{2+} and DOC in natural water. Therefore, heteroaggregation of ENPs in natural water, which was not in the scope of this study, should be further studied in the future to make its role clear for the agglomeration of ENPs in natural water.

For the agglomeration of AgNP-Cit in 1 hour, correlations between Z-average size of ENPs and Ca^{2+} concentrations were examined in filtered fractions to be able to include Rhein Diepoldsau and Etang de la Gruere samples, since there were no significant differences between average sizes in unfiltered and 0.2 μm filtered fractions of the other samples. Z-average sizes of AgNP-Cit in filtered surface water and wastewater samples (n=16) after 1 hour were highly correlated with the concentration of Ca^{2+} (R^2:0.741), as shown in Figure 4.5a. It was clear that the effect of divalent cations in electronic double layer suppression was the predominant factor resulting in higher Z-average size levels and the effect of Ca^{2+} was more pronounced than of Mg^{2+} due to its affinity for complexation with the citrate coating (El-Badawy et al., 2010). Moreover, most of the data points, which slightly deviate from the regression line, were attributed to the DOC concentration in the corresponding samples, which was thus the secondary parameter affecting the agglomeration of AgNP-Cit. It is known that NOM can improve the stability of AgNP-Cit which may be due to the replacement of citrate coatings with the natural organic matter and may lead to both steric and charge stabilization (Cumberland and Lead, 2009). The samples with high DOC concentration (>3.8 mg/L) (except for Kuesnacht, Zumikon effluent and Duebendorf anaerobic tank sample) resulted in lower Z-average sizes of AgNP-Cit than expected from the correlation in Figure 4.5a, whereas lower DOC concentration (< 2 mg/L) led to higher agglomeration than expected from correlation especially when the Ca^{2+} concentration was also higher than 1.5 mM (Glatt-Rheinsfelden and Thur-Andelfingen).

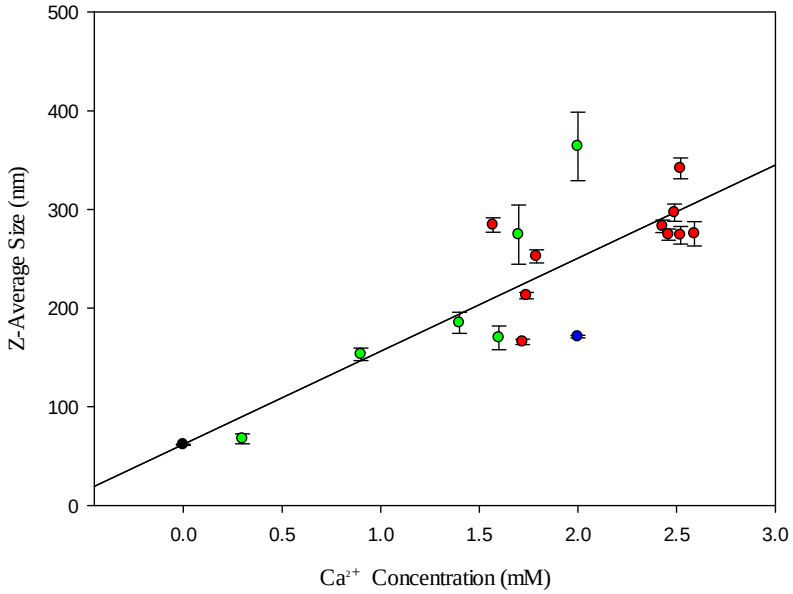


Figure 4.5(a): Correlation of Z-Average size of AgNP-Cit and Ca^{2+} concentration in filtered samples after 1 hour.

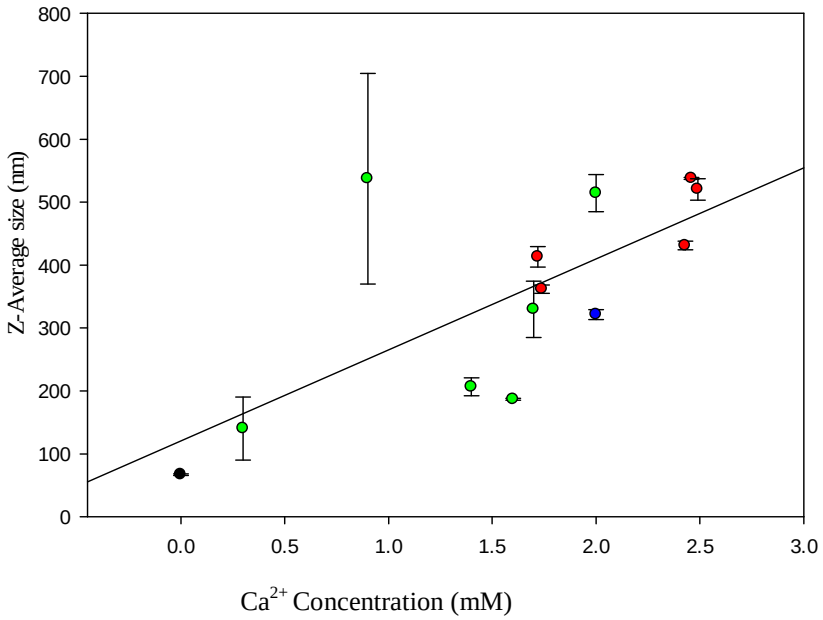


Figure 4.5(b): Correlation of Z-Average size of AgNP-Cit and Ca^{2+} concentration in unfiltered samples after 1 day (Error bars represents the standard deviation of triplicates. Green data points represent surface water samples and red data points represent the wastewater samples. Blue data points show Z-average size of AgNP-Cit in freshwater simulated medium which was reported in Topuz et al.2014).

King and Jarvie et al. (2012) hypothesized that high DOC can suppress the cation effect. Based on the data in Figure 4.5a, DOC higher than 2 mg/L appears to be effective. However, AgNP-Cit agglomeration in WWTP influent samples was slightly stronger than expected in spite of their high DOC concentration which could be due to their more complex composition.

Z-average sizes of AgNP-Cit in surface water simulated media (Ca: 2 mM, DOC: 5 mg/L) in our previous study (Topuz et al., 2014) were also lower than expected from correlations as shown in Figure 4.5 (blue data point) because of the high DOC concentration (> 2 mg/L). Gao et al. (2009) and Thio et al. (2012) also demonstrated that Z-average sizes of AgNP-Cit in filtered freshwater samples were lower than 100 nm (Ca<0.5 mM) and around 150 nm (Ca:1 mM), respectively, which were fitting with our correlation.

For the agglomeration of AgNP-Cit in 1 day, the correlation between Z-Average size levels and Ca^{2+} concentration were made for unfiltered water to be realistic since filtered fractions lead to much higher Z-average size levels. The correlation (Figure 4.5b; R^2:0.54) was lower than for 1 hour showing the less pronounced effect of Ca^{2+} in 1 day than in 1 hour. After 1 week, the agglomeration trend and the role of parameters were similar to the results of after 1 day. Stabilization of AgNP-Cit after 1 day up to 1 week could be attributed to replacement of citrate coating of AgNP with DOC after 1 day. In addition to the similarity of Z-average sizes of AgNP-Cit after1 hour, the time resolution results were also consistent with our previous study in which surface water simulated media were used (Topuz et al., 2014). With synthetic media including only ions, agglomeration of AgNP-Cit were very high (up to 1000 nm after 1 day) in the cases of Ca^{2+} concentration between 1.7 and 3.3 mM. On the other hand, the agglomeration (350 nm after 1 week) of AgNP-Cit in the surface water simulated media including ions, NOM and surfactants (Ca: 2mM and DOC 5 mg/L) was very slight due to the stabilization effect of DOC.

Owing to the steric hindrance of PVP with its bulky neutral structure (Thio et al., 2012) and strong bonds with N atoms (Kvitek et al., 2008), AgNP-PVP were stable in all of the unfiltered water samples and kept their original size over 1 week in the presence of Ca^{2+} concentration up to 2.5 mM and Mg^{2+} concentration up to 0.8 mM (Figure 4.2). El Badawy et al. (2010) also stated that AgNP-PVP was stable despite of high ionic strength. However, agglomeration of AgNP-PVP only occurred in the filtered fraction of Duebendorf influent sample after 1 day which had the highest Mg^{2+} concentration (0.85 mM). In our previous study, presence of Mg^{2+} (>2.5 mM) also led to agglomeration of AgNP-PVP which could be

due to the specific interactions between PVP and Mg^{2+}, however, surface water simulated media (Mg^{2+}: 0.5 mM) provided stability over 1 week (Topuz et al. 2014). Apparently, AgNP-PVP has potential to be affected by the presence of high Mg^{2+} concentrations which are not commonly detected in surface or wastewater media.

Although Z-average size levels of TiO_2 in unfiltered samples after 1 hour were slightly increasing with increasing concentration of Ca^{2+}, there was no clear correlation with Ca^{2+} concentrations (R^2 = 0.274) (Figure 4.6). However, TiO_2 in Kuesnacht and Zumikon WWTP samples and Thur-Andelfingen were stabilized around their original size which might be due to the high DOC concentration in the samples. In a similar way as for AgNP-Cit, agglomeration effect of Ca^{2+} was suppressed by high DOC concentration (DOC >2 mg/L), whereas it did not happen in the case of high Ca^{2+} concentration (>2.5 mM). When the correlation was applied again by omitting the samples with DOC >2 mg/L, it was improved significantly (R^2 : 0.569). Therefore, TiO_2 agglomerates up to the same levels (350-550 nm) for a wide range of Ca^{2+} concentration (0.3-2.5 mM) and is only stabilized in the case of low Ca^{2+} (<1.8 mM) and high DOC concentration. Moreover, TiO_2 agglomerated to the same levels in surface water simulated media including 2 mM of Ca^{2+} and 5 mg/L of NOM (Topuz et al., 2014), which was similar to the correlation as shown in Figure 4.6 (blue data point).

Shih et al. (2012) and Mukherjee et al. (2010) also showed that Z-average size levels of TiO_2 in synthetic media became around 600 nm (Ca: 1-10 mM) and 450 nm (Ca: 3-5 mM), respectively. Although there was a slight increase in Z-average levels of TiO_2 after 1 day, they were not significantly different from the levels after 1 hour which shows that agglomeration of TiO_2 nanoparticles mostly occur within 1 hour. However, agglomeration of TiO_2 in surface water was nearly up to micrometer scales over 1week. In conclusion, TiO_2 was only slightly affected by Ca^{2+} and DOC concentration after 1 day or 1 week.

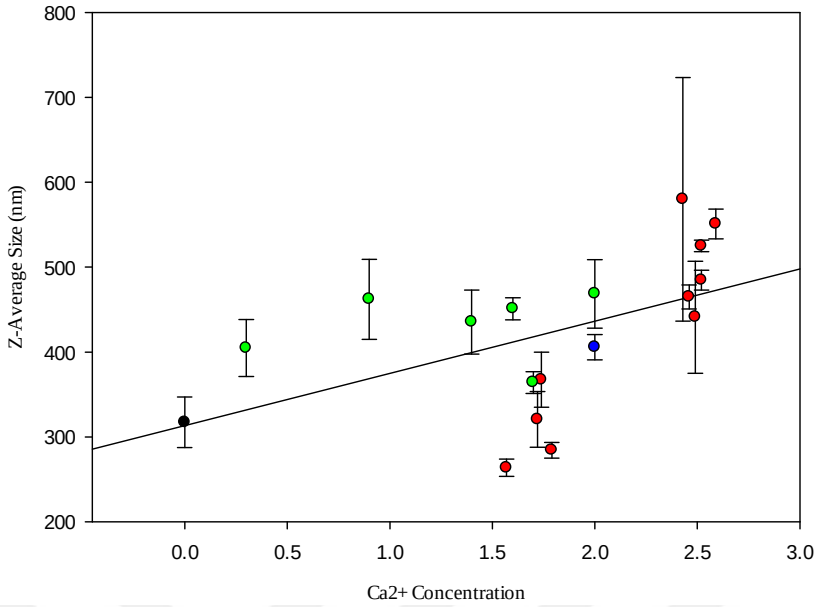


Figure 4. 6: Correlation of Z-Average size of TiO_2 and Ca^{2+} concentration in the filtered samples after 1 hour (Error bars represents the standard deviation of triplicates. Green data points represent surface water samples and red data points represent the wastewater samples. Blue data point shows Z-average size of TiO_2 in a freshwater simulated medium (Topuz et al. 2014).

4.4.2 Role of dissolved organic matter fractions for agglomeration of ENPs

The biopolymer fraction which comprises mostly polysaccharides and proteins (Stewart et al. 2013) decreased dramatically for 10 kDa cut off. The humic acid fractions were lower in 10 kDa cut off than in filtered sample only in the case of Etang de la Gruère, where humic acids of terrestrial origin may be predominant. Z-average sizes of ENPs in unfiltered water, 0.2 µm filtered, < 10 kDa and < 1 kDa cut off fractions were compared over 1 week.

As it is expected due to the strong steric stabilization of PVP for AgNP particles, AgNP-PVP were stable in all fractions over 1 week (Figure 4.2). After 1 day, Z-average sizes of AgNP-Cit in unfiltered, filtered and 10 kDa cut off fractions became different for all samples (Figure 4.1) except Gruere with low Ca^{2+} concentration and very high content of humic substances. Unfiltered water samples, except Rhein-Diepoldsau, resulted in the lowest Z-average sizes of AgNP-Cit and Z-average sizes in filtered samples were significantly higher than in unfiltered water (Figure 4.1). Moreover, 10 kDa cut off led to high agglomeration of AgNP-Cit in Thur-Andelfingen, Rhein-Rheinsfelden and Rhein-Diepoldsau (Figure 4.1) samples like the

synthetic media including only ions (Topuz et al., 2014). It was likely due to the fact that high molecular weight organic compounds (>10 kDa) provide stabilization after 1 day up to 1 week and that those compounds were removed by ultrafiltration. Aare Brugg sample has the highest concentration of biopolymers in < 10 kDa cut off, which might be the reason for similar Z-average sizes of AgNP-Cit in all fractions. Although the concentration of biopolymers were relatively low in filtered samples (>10 kDa), these biopolymers may be more effective in stabilizing NPs, as also mentioned by Louie et al. (2013) for the stabilization of Au nanoparticles by high molecular weight compounds at very low concentration levels. Joshi et al. (2012) and Dimpka et al. (2011) figured out that extracellular polymeric substances, which are a kind of biopolymers, caused induction of aggregation of AgNPs. Humic acids were also proved to provide more electrostatic stabilization for AgNP-PVP under high ionic strength conditions due to their high molecular weight (Yang et al., 2014).

TiO_2 had the same Z-average size levels in all fractions of the samples after 1 hour (Figure 4.3) which might prove the previous argument in Section 4.2 that TiO_2 was not significantly affected by Ca^{2+} or DOC concentration in short term. However, TiO_2 was usually more stable in filtered samples than in unfiltered fractions after 1 day for most of the samples (Figure 4.3). This could be due to the fact that its higher original size and low zeta potential at pH 7 – 8 make it more vulnerable to heteroaggregation than AgNP-Cit. Within 1 day, TiO_2 was more stable in filtered samples (> 10 kDa) (Figure 3.4), which also seems to be due to the presence of high molecular weight organic compounds. TiO_2 was more stabilized in Etang de la Gruère filtered and ultrafiltered samples with high amounts of humic substances. Thio et al. (2011) also showed that deposition of TiO_2 nanoparticles were decreased in the presence of humic acid with high molecular weight organic fractions. Gondikas et al. (2012) mentioned the importance of molecular weight and aromaticity of organic carbon for the aggregation process of TiO_2 and accelerating effect of low molecular weight compounds on aggregation. Nevertheless, TiO_2 agglomerated up to micrometer scale after 1 week in all fractions of most samples (Figure 4.3). According to our study with natural water samples, significantly higher Z-average sizes of AgNP-Cit and TiO_2 in filtered fractions than in unfiltered ones also indicate the role of higher molecular weight organic matter fractions, such as biopolymers for stabilization over time.

4.5 Conclusion

A generalized scheme for the agglomeration behavior of AgNP-Cit, AgNP-PVP and TiO_2 in natural waters might be proposed based on their relation with Ca^{2+}, Mg^{2+} and DOC concentration (Figure 4.7). Since AgNP-Cit was more sensitive to various natural water conditions, a more detailed classification approach in terms of agglomeration is proposed in Figure B18. If the Ca^{2+} concentration is lower than 0.5 mM, the agglomeration potential is not considered, whereas, for higher concentrations, 7 classes with different size levels were developed for different Ca^{2+} concentrations. However, heteroaggregation potential and the pH of surface water which are not tested in this study (out of 6.26-8.27) should be considered for the possible deviations from Figure 4.7 and Figure B18.

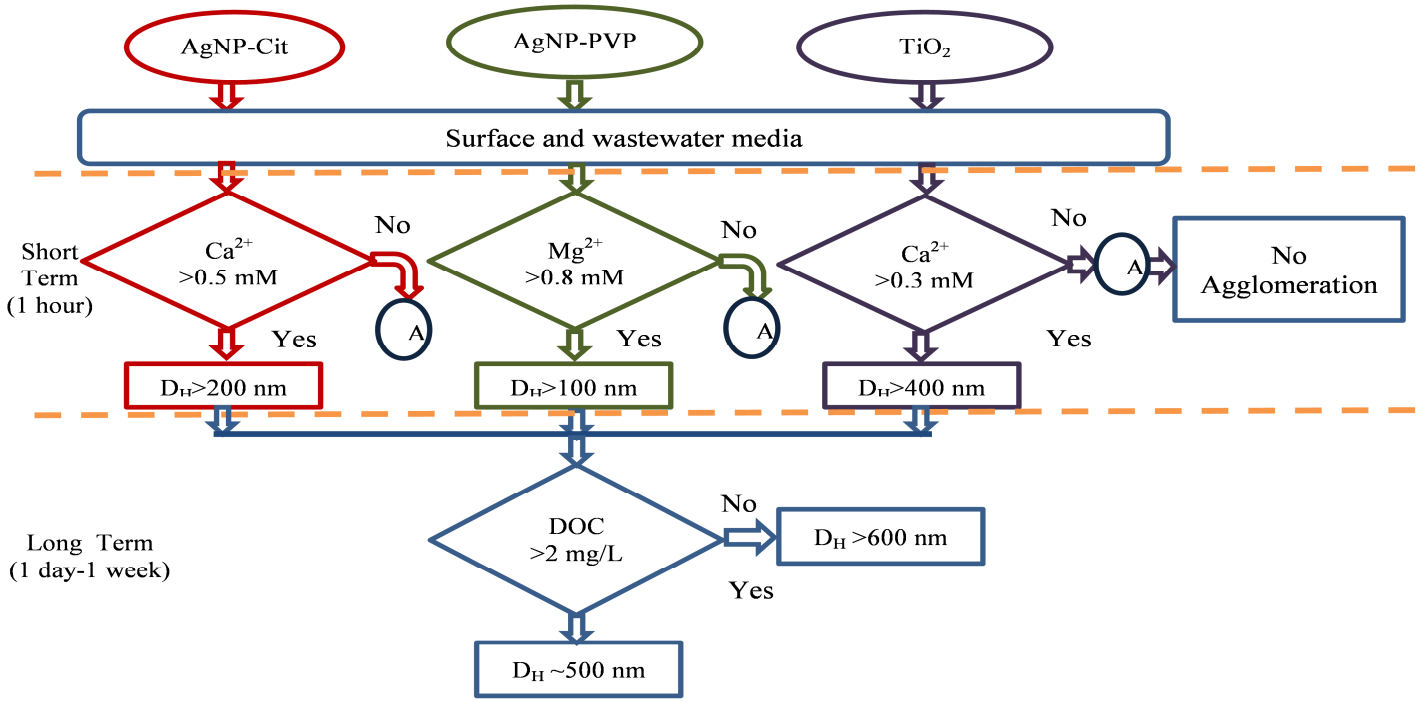


Figure 7. A generalized scheme for the agglomeration behavior of AgNP-Cit, AgNP-PVP and TiO_2 in surface and wastewater

Figure 4. 7: A generalized scheme for the agglomeration behavior of AgNP-Cit, AgNP-PVP and TiO_2 in surface and wastewater.

Furthermore, it is suggested to decrease the number of samples considering the results of this study and develop a scheme (if it would be different) for lower concentrations of these ENPs (more environmentally relevant) with advanced size characterization techniques. Correlations suggested in this study can be applicable for the environmental risk assessment studies. Firstly, they could give an idea in which compartment they could partition in surface water or wastewater treatment plants such as water column vs sediment or effluent water vs sewage sludge, respectively. Especially for surface water bodies, they can easily be used in fate models or water quality models since they provide estimations over time which makes spatial distribution analysis possible. In addition, the predictions for the partition provide foresights about their bioavailability to the organisms which live respective compartments of the medium.

Agglomeration of ENPs in natural water is also affected by the molecular weight based fractions of organic matter. The high molecular weight organic carbon fraction (> 10 kDa), mostly comprising biopolymers, provides stabilization of ENPs in even at very low concentrations and this effect becomes more pronounced in 1 day. Therefore, the studies conducted with filtered water or the studies which omit the presence of high molecular weight organic carbon could lead to overestimated size results of ENPs especially for the long term (1 day-week). Our results showing the higher stability in unfiltered samples compared to filtered ones highlight the importance of studying with mesocosms for both fate and toxicity evaluations for more realistic approaches. However, more studies should be conducted with applying molecular weight fractionation to the natural water samples to better understand the mechanism of this effect. Further studies are suggested to investigate the kinetics of the stabilization depending on the concentration levels and different structures of organic matter present in surface and wastewater media.

APPENDIX B: Chapter 4

Table B.1: Classification of surface water based on concentration levels

Class	Ca (mM)	Mg (mM)	Alkalinity (mM)	DOC (mg/L)
Low (L)	<0.5	<0.2	<1.5	<0.5
Low-Medium (LM)	0.5-1	0.2-0.3	1.5-2	0.5-1
Medium-Low (ML)	1-1.2	0.3-0.5	2-2.5	1-1.5
Medium (M)	1.2-1.5	0.5-0.6	2.5-3	1.5-2
Medium-High (MH)	1.5-1.7	0.6-0.7	3-3.5	2-2.5
High-Medium (HM)	1.7-2	0.7-0.9	3.5-4	2.5-4.0
High (H)	2-2.5	0.9-1	4-5	4.0-5.0
Very High (VH)	>2.5	> 1	>5	>5.0

Table B.2: Wastewater sampling points

Sampling plant	Process	Population Eq.
Duebendorf	Bio. Nut.+Flocculation	>50000
Kuesnacht	No nitrification/denitrification	10000-50000
Zumikon	Only nitrification	2000-10000

Table B.3: Z-average size and Zeta potentials of blank samples (0.2 µm filtered) (± gives standard deviations for triplicates)

Sample	Z-Average Size (nm)	Zeta Potential (mV)	Sample	Z-Average Size (nm)	Zeta Potential (mV)
Glatt – Rheinsfelden	128±3.85	-17.1±2.91	Zumikon Influent	156±14.7	-10.3±2.76
Thur – Andelfingen	149±14.6	-7.52±2.60	Zumikon Effluent	142±5.1	-12.2±3.69
Aare-Brugg	177±10.6	-13.4±3.69	Duebendorf Influent	161±9.5	-13.5±5.45
Rhein-Rheinsfelden	129±2.79	-16.9±1.89	Duebendorf Aerobic	135±9.7	-12.7±0.33
Rhein-Diepoldsau	188±4.51	-15.6±2.87	Duebendorf Anaerobic	173±4.8	-11.7±1.36
Etang de la Gruere	192±11.1	-11.4±5.54	Duebendorf Clarification	160±6.3	-15.9±2.95
Kuesnacht Influent	133±9.8	-11.9±1.22	Duebendorf Filtration	137±4.4	-16.6±1.75
Kuesnacht Effluent	128±5.3	-13.8±3.51	Duebendorf Effluent	189±10.6	-12.4±3.95

Table B.4: Z-average size and Mean size of ENPs in surface water samples measured with DLS and NTA, respectively, after 1 hour

Samples	AgNP-Cit Z-Average Size (nm)	Mean (nm)	AgNP-PVP Z-Average Size (nm)	Mean (nm)
Glatt –Rheinsfelden <0.2 µm	364±34.6	190±28.6	51.8±7.86	60.4±0.97
Glatt –Rheinsfelden <10 kDa	339±13.3	180±24.8	52.2±4.66	60.9±3.42
Glatt –Rheinsfelden < 1 kDa	326±14.4	161.4±4.80	47.9±5.91	60.3±5.24
Thur –Andelfingen <0.2 µm	274±30	191±31.5	45.6±1.31	52±2.31
Thur –Andelfingen <10 kDa	335±18.1	168±13.6	52.4±7.63	50.2±0.45
Thur –Andelfingen < 1 kDa	361±29.5	162±10.6	48.6±2.87	54±0.29
Aare-Brugg <0.2 µm	170±12.1	114±4.29	52±5.74	53±1.51
Aare-Brugg <10 kDa	154±16.7	124±4.56	43.6±3.67	50.1±1.06
Aare-Brugg < 1 kDa	144±6.3	111±5.64	51.4±8.64	54.02±6.04
Rhein-Rheinsfelden <0.2 µm	185±10.7	151±4.73	50.4±7.44	52.3±3.86
Rhein-Rheinsfelden <10 kDa	291±13.4	166±5.22	54.2±4.09	52.3±3.86
Rhein-Rheinsfelden < 1 kDa	323±11.4	157±2.47	65.4±8.39	54.3±1.29
Rhein-Diepoldsau <0.2 µm	153±6.22	111.1±0.91	58.55±8.01	54.2±0.40
Rhein-Diepoldsau <10 kDa	140±11.9	116±1.0	56.3±10.8	52.5±0.77
Rhein-Diepoldsau < 1 kDa	150±8.06	116±3.04	64.4±4.61	52±0.77
Lake de la Gruere <0.2 µm	67.4±4.94	64.5±4.32	58.6±2.86	44.7±1.22
Lake de la Gruere <10 kDa	62.2±10.4	69.8±4.0	65.6±4.24	50.4±3.85
Lake de la Gruere < 1 kDa	62.4±3.85	54.7±1.54	49.8±9.11	52.8±4.27

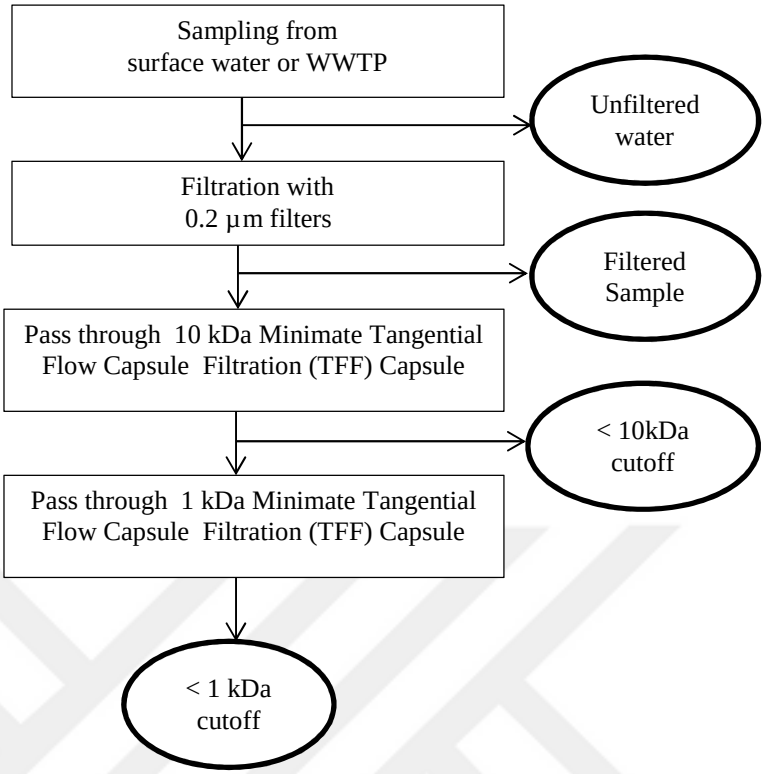


Figure B.1: Fractionation of surface water and WWTP samples

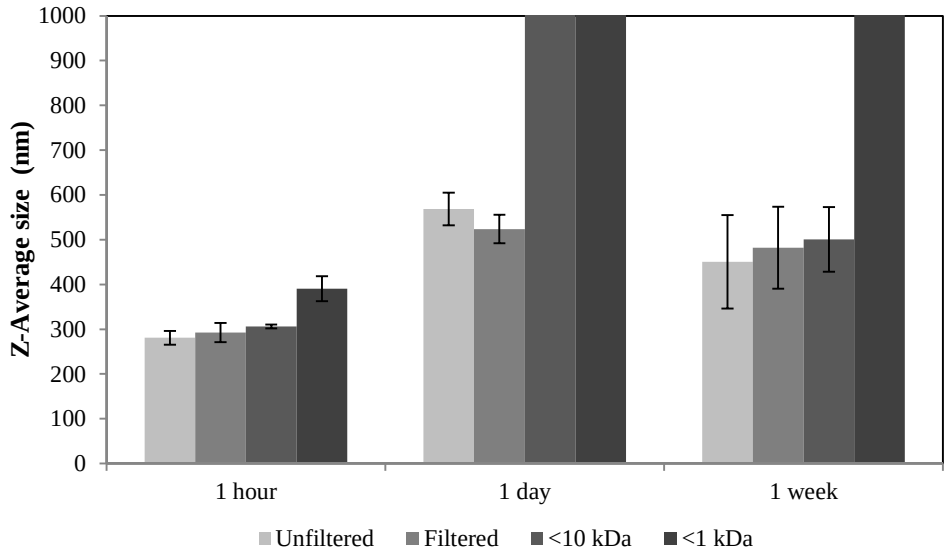


Figure B.2: Z-Average size of AgNP-Cit in the fractions of Chriesbach stream (Duebendorf, Switzerland)

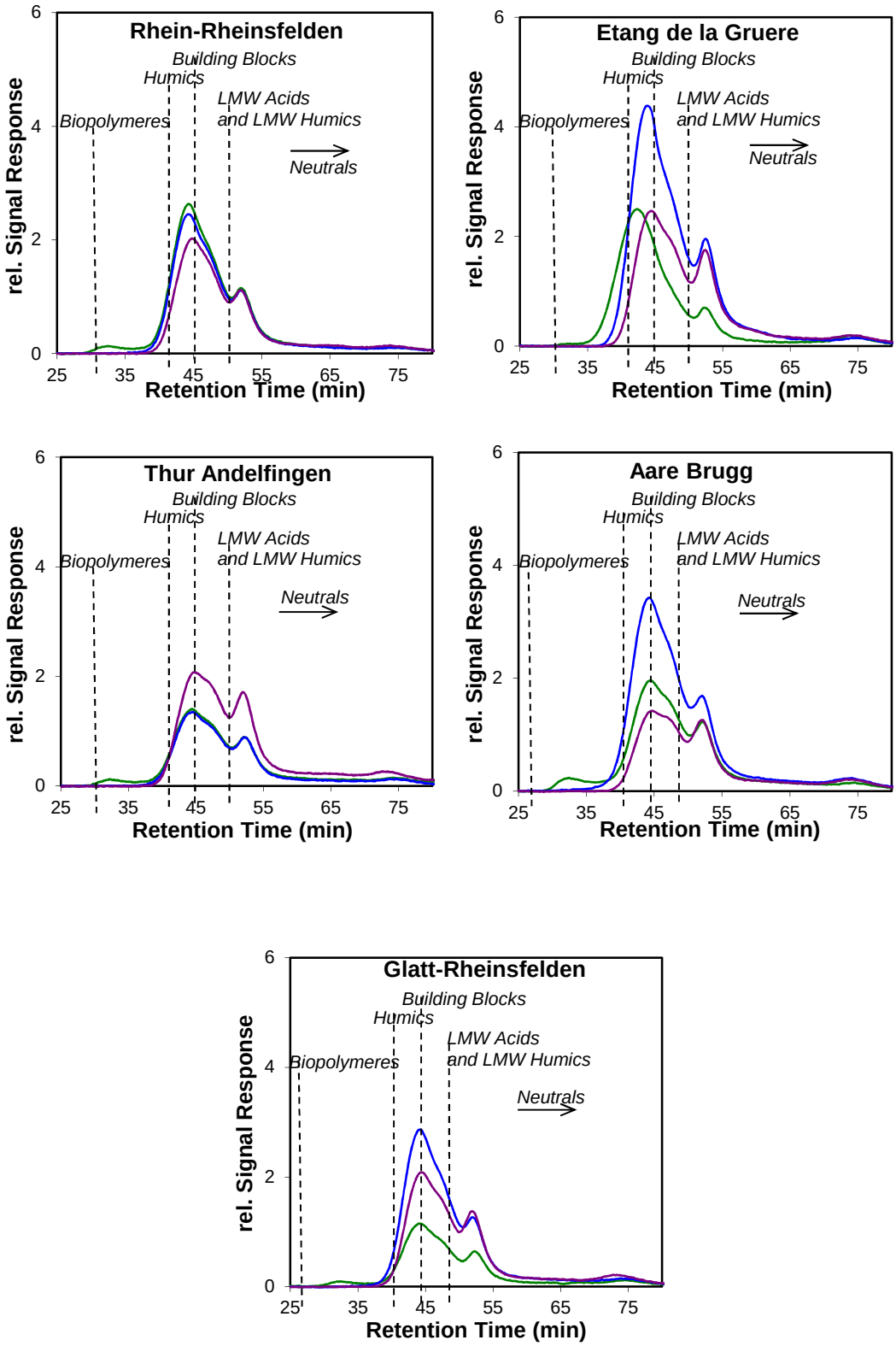


Figure B.3: LC-OCD-OND chromatograms for the filtered (0.2 µm) surface water samples

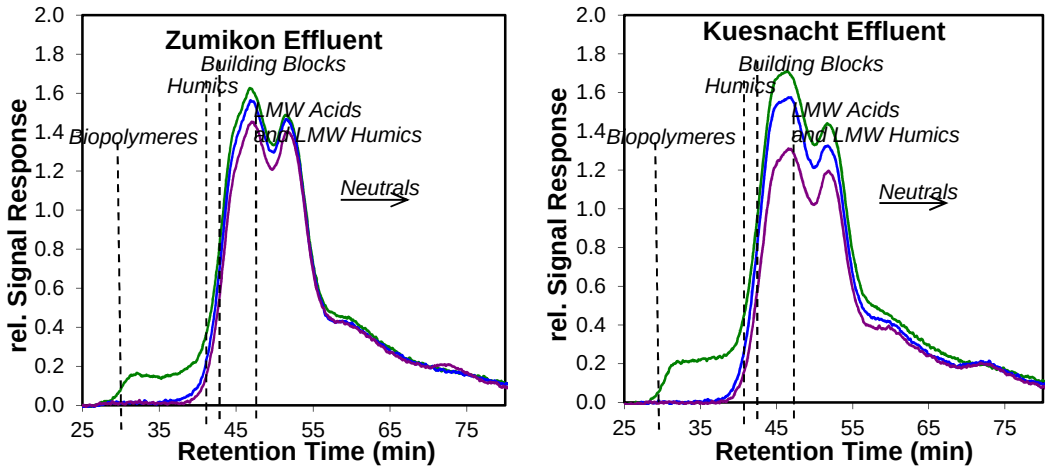


Figure B.4: LC-OCD-OND chromatograms for the filtered (0.2 µm) wastewater effluent samples

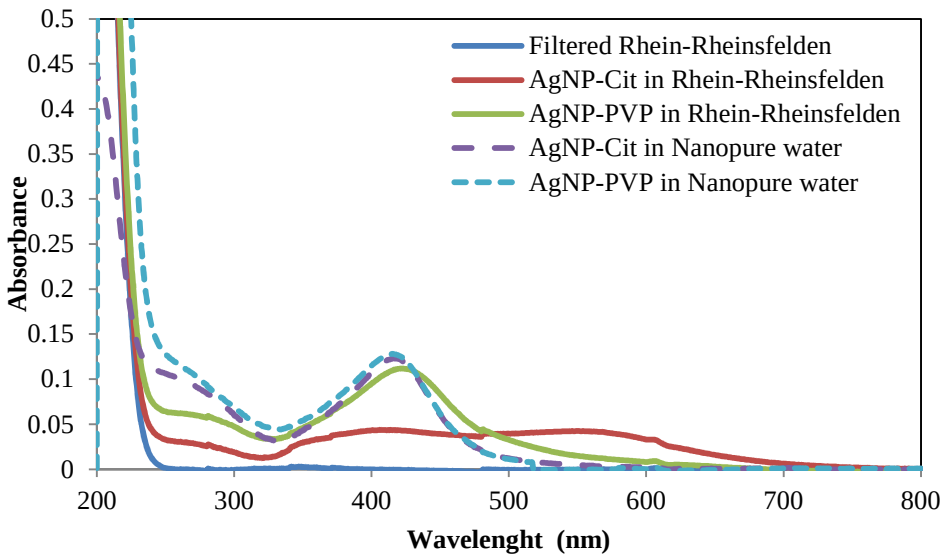


Figure B.5: UV absorbance for filtered Rhein-Rheinsfelden, AgNP-Cit and AgNP-PVP in filtered Rhein-Rheinsfelden and nanopure water

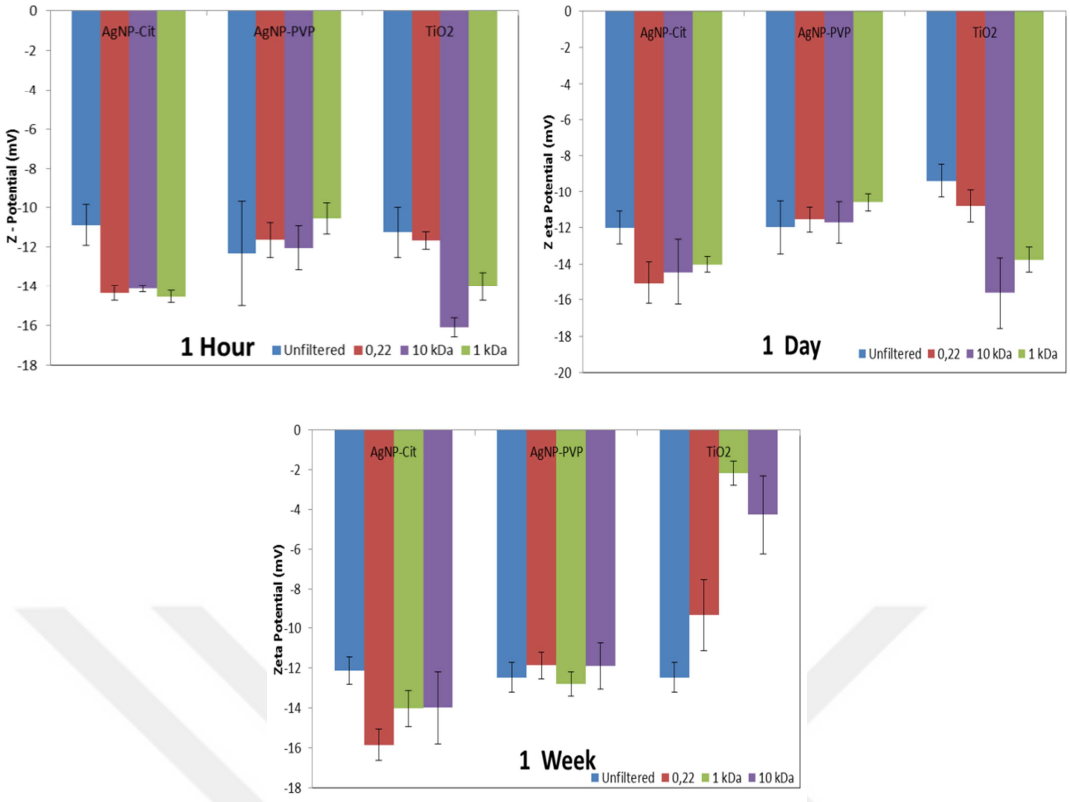


Figure B.6: Zeta Potential of ENPs in the fractions of Rhein Rheinsfelden sample over time (Error bars represent the standard deviation of duplicate samples)

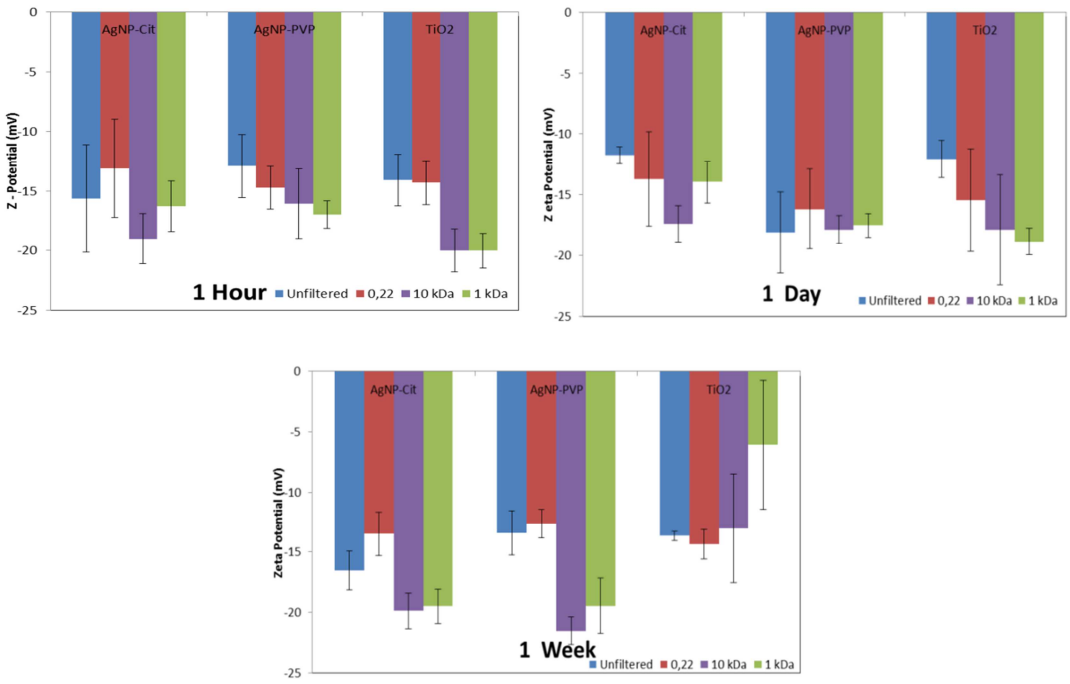


Figure B.7: Zeta Potential of ENPs in the fractions of Etang de la Gruere sample over time (Error bars represent the standard deviation of duplicate samples)

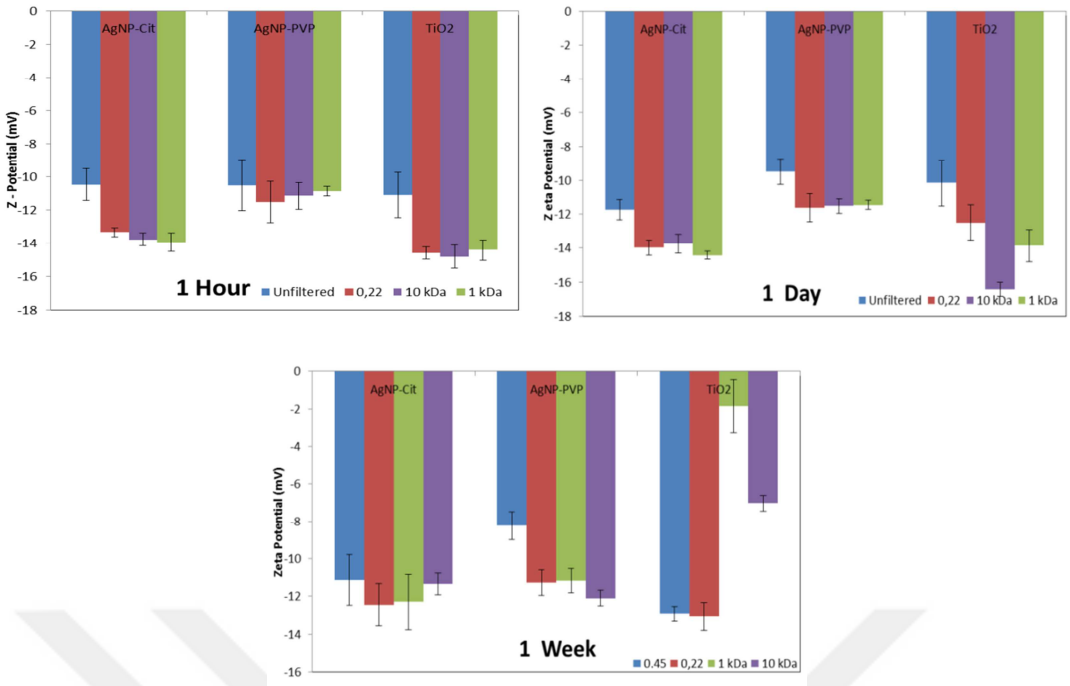


Figure S8. Zeta Potential of ENPs in the fractions of Thur Andelfingen sample over time (Error bars represent the standard deviation of duplicate samples)

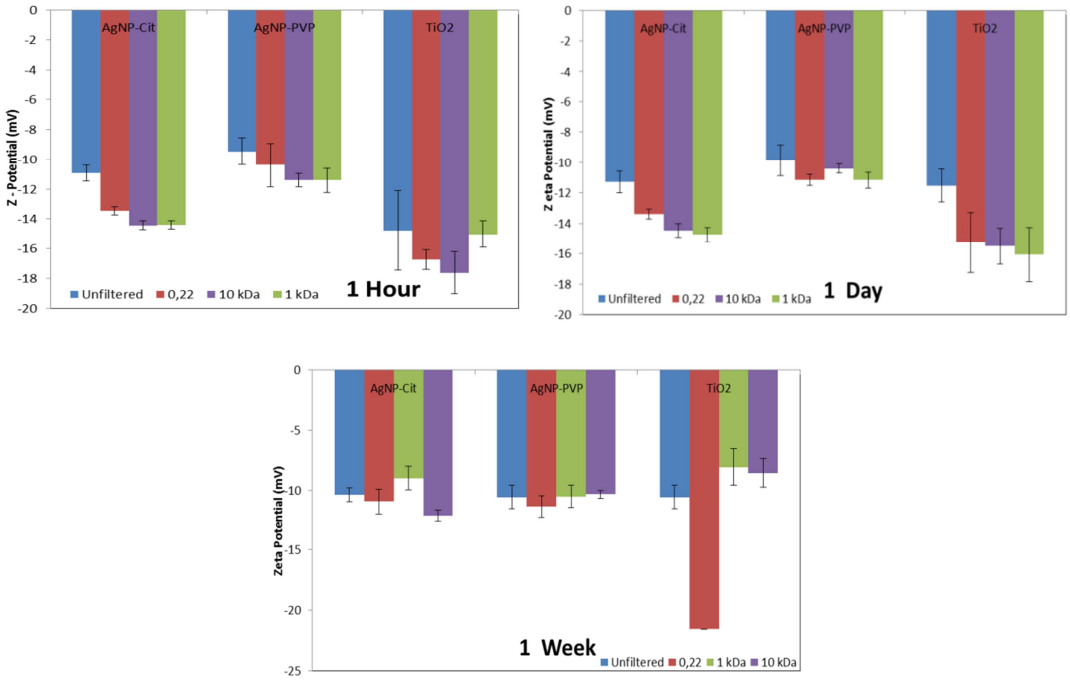


Figure B.9: Zeta Potential of ENPs in the fractions of Glatt Rheinsfelden sample over time (Error bars represent the standard deviation of duplicate samples)

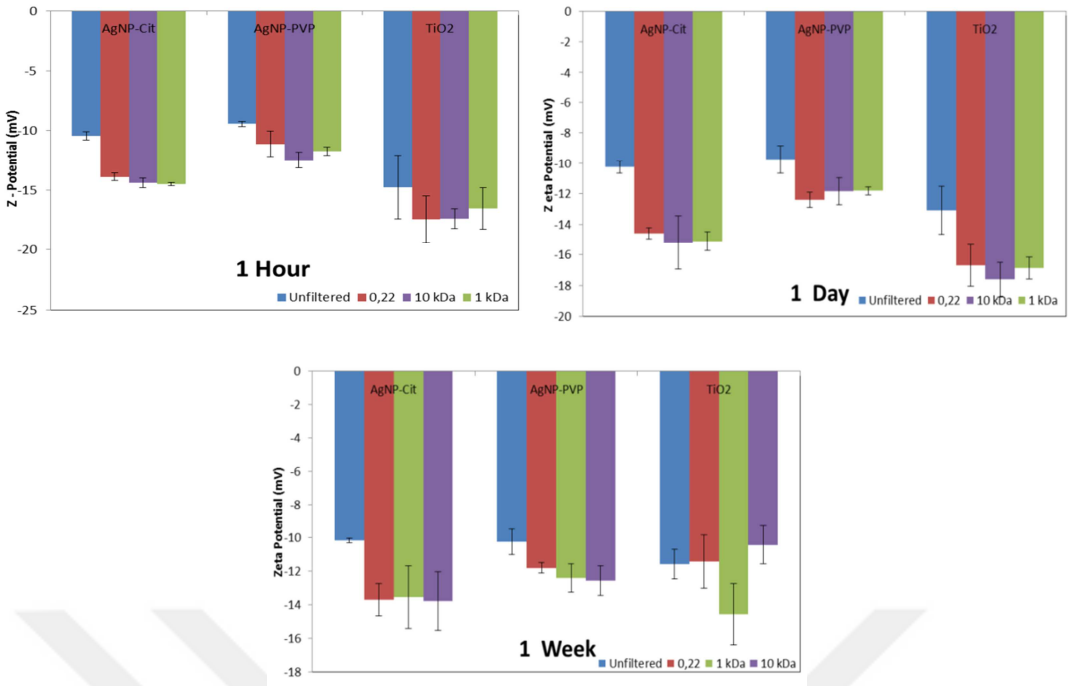


Figure B.10: Zeta Potential of ENPs in the fractions of Etang de la Gruere sample over time (Error bars represent the standard deviation of duplicate samples)

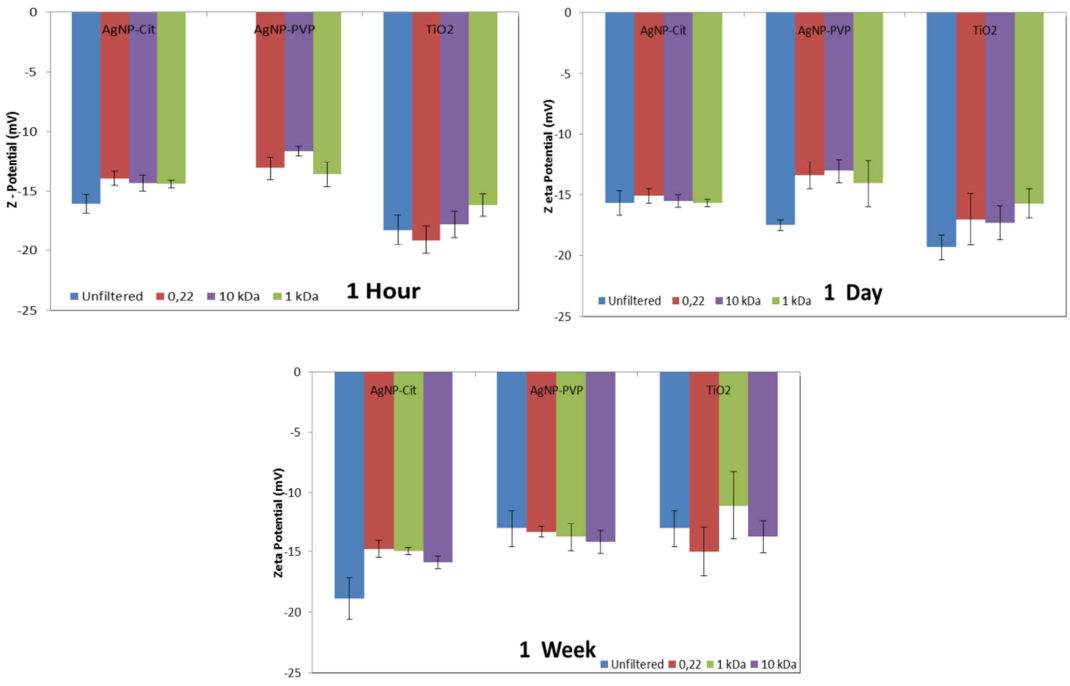


Figure B.11: Zeta Potential of ENPs in the fractions of Rhein Diepoldsau sample over time (Error bars represent the standard deviation of duplicate samples

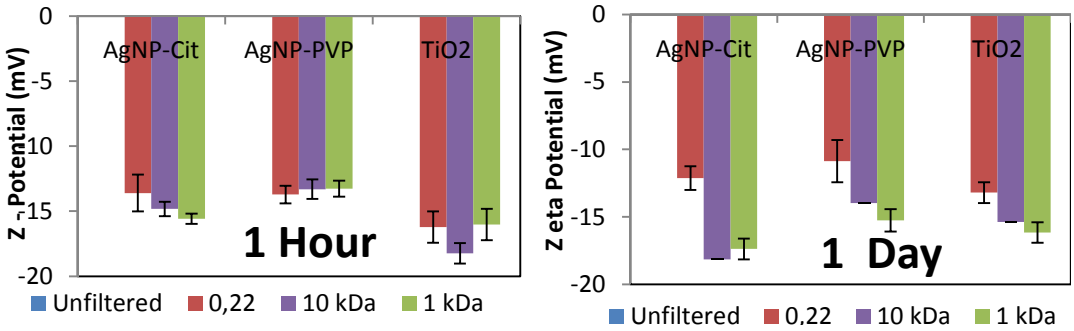


Figure B.12: Zeta Potential of ENPs in the fractions of Duebendorf WWTP Influent sample over time (Error bars represent the standard deviation of duplicate samples)

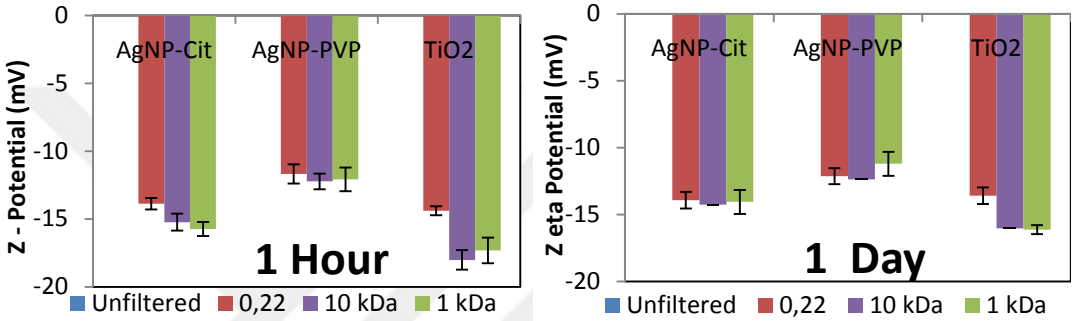


Figure B.13: Zeta Potential of ENPs in the fractions of Duebendorf WWTP Aerobic Tank supernutant sample over time (Error bars represent the standard deviation of duplicate samples)

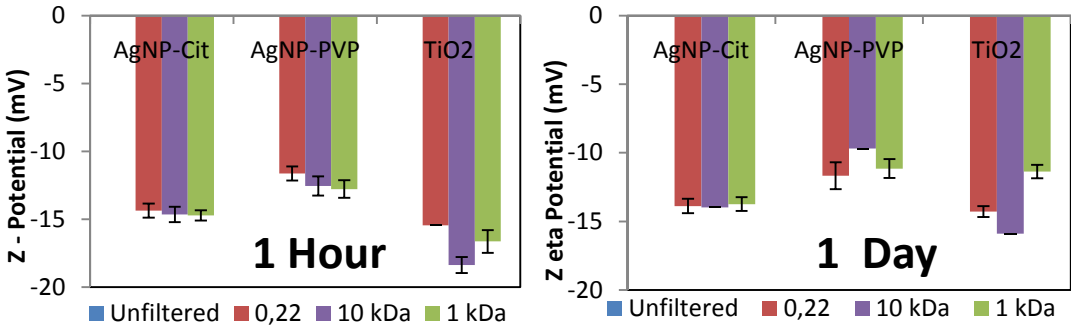


Figure B.14: Zeta Potential of ENPs in the fractions of Duebendorf WWTP Anaerobic Tank supernutant sample over time (Error bars represent the standard deviation of duplicate samples)

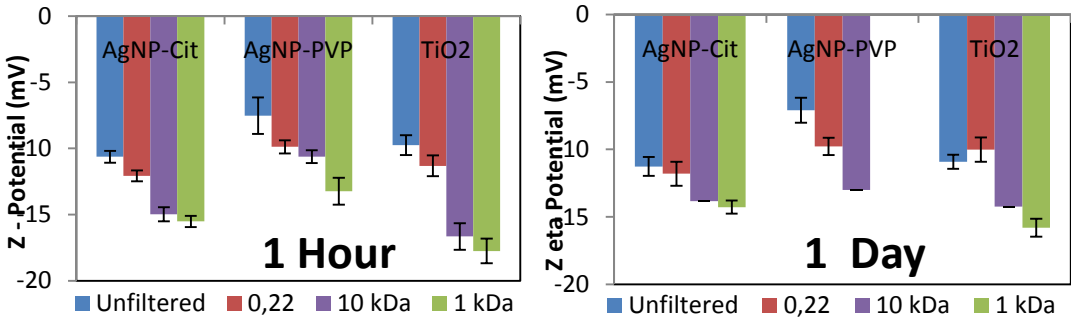


Figure B.15: Zeta Potential of ENPs in the fractions of Duebendorf WWTP Clarification Tank effluent sample over time (Error bars represent the standard deviation of duplicate samples)

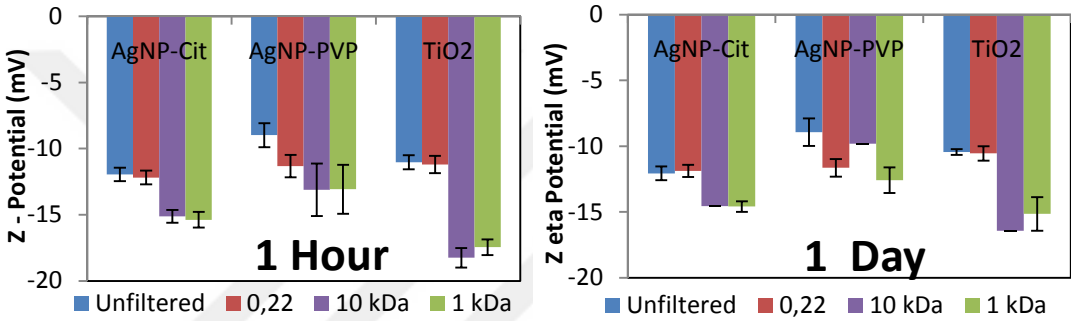
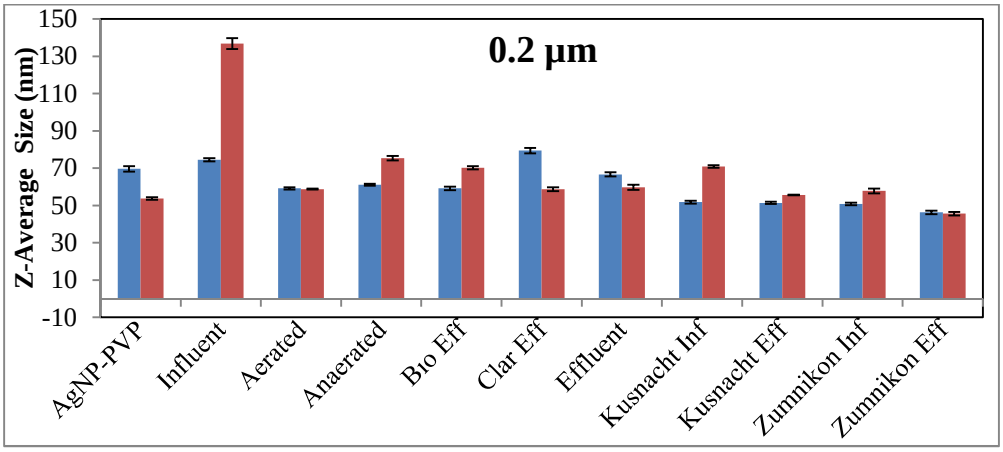
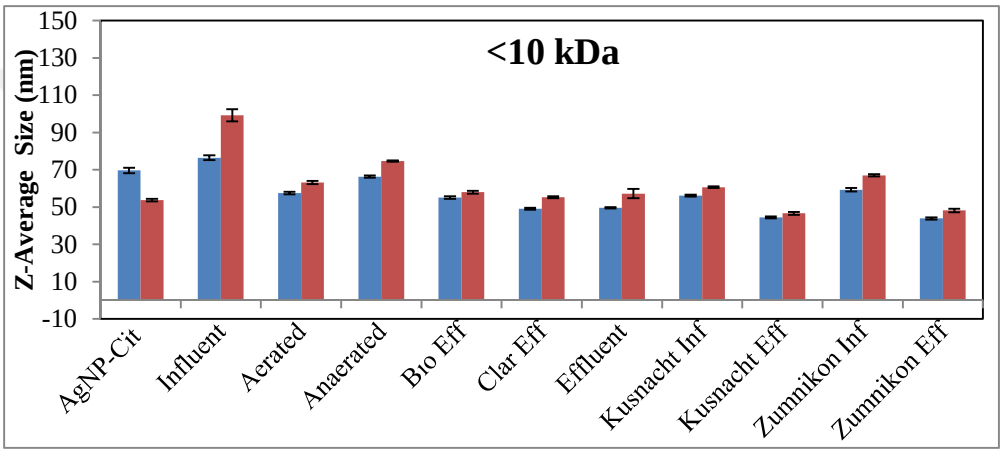


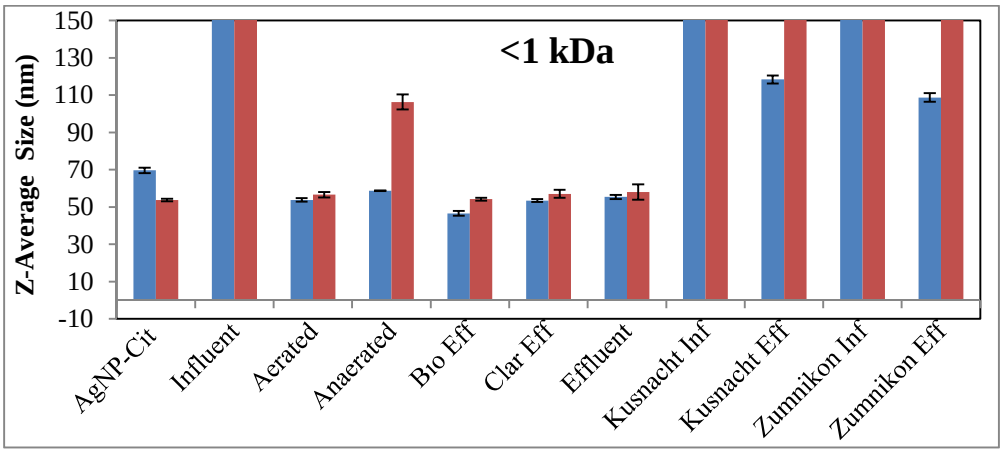
Figure B.16: Zeta Potential of ENPs in the fractions of Duebendorf WWTP FiltrationTank effluent sample over time (Error bars represent the standard deviation of duplicate samples)



(a)



(b)



(c)

Figure B.17: Z-Average size levels of AgNP-PVP in the fractions of wastewater samples a) Unfiltered b) Filtered c) <10 kDa d) <1 kDa

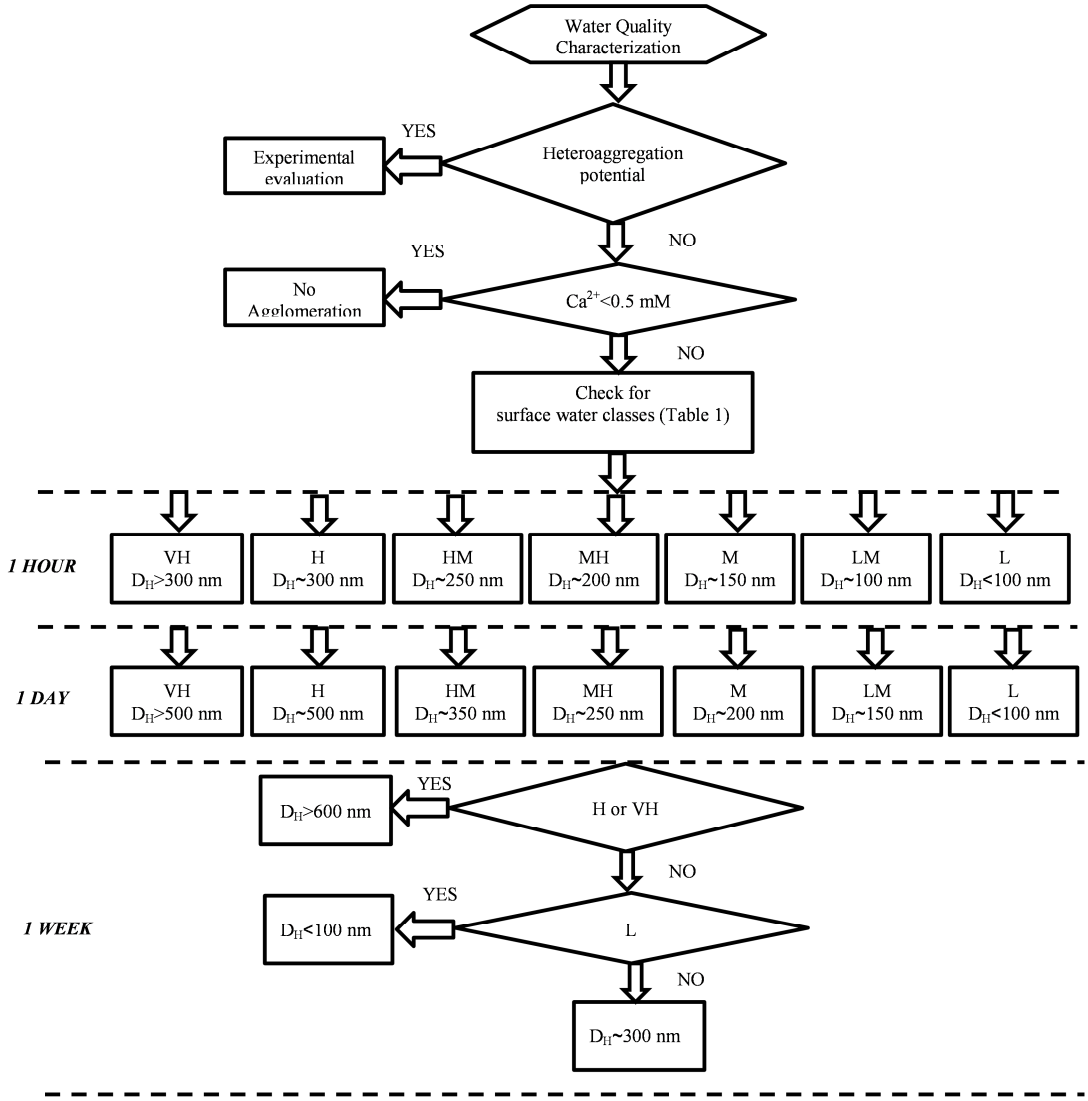


Figure B.18: A proposal for surface water classification for the agglomeration of AgNP-Cit

CHAPTER 5
TOXICOKINETICS AND TOXICODYNAMICS OF DIFFERENTLY COATED SILVER NANOPARTICLES AND SILVER NITRATE IN ENCHYTRAEUS CRYPTICUS UPON AQUEOUS EXPOSURE IN AN INERT SAND MEDIUM

Abstract

The aim of this study is to evaluate the effect of AgNPs on *Enchytraeus crypticus*, applying a combined toxicokinetics and toxicodynamics approach to understand the relationship between survival and the development of internal Ag concentrations in the animals over time. Toxicity tests were conducted in medium composed of well-defined aqueous solutions added to inert quartz sand to avoid the complexity of soil conditions. Citrate (AgNP-Cit) and polyvinylpyrrolidone coated AgNPs (AgNP-PVP) were tested and compared with $AgNO_3$ which was used as positive control for Ag ion effects. LC50 values based on Ag concentrations in the solution phase of the test medium decreased over time and reached steady state after 7 days, with $AgNO_3$ and AgNP-PVP being more toxic than AgNP-Cit. Slow dissolution may explain the low uptake kinetics and lower toxicity of AgNP-Cit compared to the other two Ag forms. LC50s based on internal Ag concentrations in the animals were almost stable over time, highlighting the importance of integrating toxicokinetics and toxicodynamics and relating survival with internal Ag concentrations. Both survival based elimination rates and internal LC50s in the organisms did not show any significant evidence of nano-specific effects for both AgNPs although it suggested some uptake of particulate Ag for AgNP-Cit. It is concluded that toxicity of both AgNPs probably is mainly due to the release of Ag ions.

Emel Topuz and Cornelis A.M. van Gestel, Toxicokinetics and toxicodynamics of differently coated silver nanoparticles and silver nitrate in Enchytraeus crypticus upon aqueous exposure in an inert sand medium, 2015, Environmental Toxicology and Chemistry, doi: 10.1002/etc.3123

5.1 Introduction

Silver nanoparticles (AgNPs) have been used in an increasing number of consumer products (Nanotech Project, 2013), and ecosystems might be exposed to AgNPs due to their possible release from these products into the environment (Cleveland et al., 2012). The release of the AgNPs from textiles has already been proved by a few recent studies (Hedberg et al., 2014; Lombi et al., 2014; von Goetz et al., 2012). It is likely that they will end up in waste water treatment systems and ultimately in receiving environmental media like surface waters and soils (Nowack, et al., 2012). Several studies are dealing with the possible consequences of the presence of AgNPs in the aquatic environment. These studies conclude that AgNPs may cause substantial harm at very low concentration levels (µg/L) to various species of aquatic organisms such as the algae *Pseudokirchneriella subcapitata* or *Chlamydomonas reinhardtii* (Ribeiro et al., 2014; Navarro et al., 2008), *Daphnia magna* (Ribeiro et al., 2014; Zhao and Wang, 2011), and the fish *Danio rerio* (Ribeiro et al., 2014; Zhao and Wang, 2011; Powers et al., 2010).

Soil organisms are also likely exposed to AgNPs. AgNPs may partition to sediments or soil media due to the agglomeration over time depending on the water chemistry (Topuz et al., 2014). In addition, AgNPs that enter wastewater treatment plants mostly partition to the sewage sludge fraction during treatment (Kaegi t al., 2013) and soil organisms might be exposed to AgNPs through the land application of sewage sludge (Navarro et al., 2014). Therefore, it also is essential to investigate the possible interactions of AgNPs for a diverse range of soil organisms. However, the fate of AgNPs in soil is even more complicated than in water due to their interaction with both the soil solution and the soil stationary phase (Klitzke et al., 2014). Complexity of the behavior of AgNPs in aquatic media is usually due to their agglomeration/aggregation and/or dissolution rates, which may vary depending on the presence of various dissolved inorganic/organic compounds and/or colloids (Behra et al., 2013). Similar processes also play an important role in soil (Tourinho et al., 2012). So, it would be better to first figure out the exposure of soil organisms to AgNPs at the soil-solution interface and its consequent effects. This relation may be more accurately investigated by integrating toxicokinetics (uptake and elimination of the compound over time) and toxicodynamics (effects on the organism over time) approaches (He and van Gestel, 2013).

In addition to the environmental factors, the properties of AgNPs, which are mainly dependent on their capping agents, are also determining variations in their environmental fate and effects

(Hyunh and Chen, 2011). Therefore, such studies may also provide substantial knowledge on the difference in toxicity of AgNPs with different capping agents.

Hence, the aim of this study is to investigate the toxicokinetics and toxicodynamics of AgNPs with different coatings in a model soil organism and understand the relationship between survival and the development of internal Ag concentrations in the animals over time. AgNPs with citrate (AgNP-Cit) and polyvinylpyrrolidone (AgNP-PVP) coating, which are among the most widely used in products, were tested and compared, while also ionic Ag ($AgNO_3$) exposures were included for comparison. *Enchytraeus crypticus* was chosen since they are ecologically relevant soil organisms playing a crucial role in decomposition and bioturbation in soils (Castro-Ferreira et al., 2012). Enchytraeids are commonly used for toxicity testing due to their sensitivity to the wide range of stressors (Didden and Rombke, 2001). To avoid disturbance by complex environmental conditions usually affecting the fate of NPs and released metal ions in soil, the uptake, elimination and toxicity of ionic Ag and AgNPs were determined in a medium composed of well-defined aqueous solutions added to inert quartz sand. This medium was shown to provide a convenient living medium for the test organisms (He and van Gestel, 2013).

5.2 Materials and Methods

5.2.1 Test organisms

Enchytraeus crypticus (Enchytraeidae; Oligochaeta; Annelida) has been cultured for over 10 years in the laboratory of the Department of Ecological Science, VU University, Amsterdam, The Netherlands. Details about culturing the test organisms have been explained in Castro-Ferreira et al. (2012). Briefly, *E. crypticus* were cultured in agar media prepared with soil extract and kept in the dark in a climate room at constant temperature (16 °C) and relative humidity (75%). The culture was fed twice a week with a mixture of oatmeal, dried yeast, egg yolk powder, and fish oil. For the experiments, adult *E. crypticus* with white spots in the clitellum region were selected.

5.2.2 Test compounds and test medium

AgNPs, AgNP-PVP and AgNP-Cit, and ionic silver in the form of $AgNO_3$ were tested in this study. $AgNO_3$ also served as a positive control to distinguish possible effects originating from nanoparticles from those of the released Ag^+ ions. AgNP-Cit and AgNP-PVP were purchased from NanoSys GmBH (Switzerland) and obtained as suspensions in water at concentrations of

1 g/L and 10 g/L AgNPs, respectively. $AgNO_3$ (Sigma-Aldrich, >99%) was dissolved in deionized water. Nominal size of AgNP-Cit and AgNP-PVP provided by the manufacturer was around 25 nm; detailed characterization of both AgNPs has been reported in Topuz et al. (2014).

The experiments were conducted in quartz sand which was pretreated to obtain an inert matrix free of residuals such as organic carbon etc. Pretreatment of quartz sand and its characteristics has been explained in detail by (He and van Gestel, 2013). In brief, after combustion at 600 °C for 2 hours, the quartz sand was rinsed with 0.7 M HNO_3 (Sigma-Aldrich, 65%), tap water and deionized water, and finally air dried.

The test medium was prepared by dissolving $Ca(NO_3)_2$, $MgSO_4$ $7H_2O$, $NaNO_3$, and KNO_3 (Sigma-Aldrich, >99%) in deionized water. The test medium was diluted by spiking with $AgNO_3$ or AgNP stock solution to prepare the test solutions with the desired Ag test concentrations, and having ionic concentrations of 0.2 mM Ca^{2+}, 0.05 mM Mg^{2+}, 2.0 mM Na^{+}, and 0.078 mM K^{+}. Because of its known influence on the speciation of silver, the use of chloride was avoided in the test medium. The pH of the test solutions was adjusted to approx. 6.0 using 0.75 g/L MOPS (3-[N morpholino] propane sulfonic acid) (AppliChem, >99%), 0.75 mg/L MES (2-[N-morpholino] ethane sulfonic acid) (Sigma-Aldrich, >99%) or 1 mM NaOH (Sigma-Aldrich, >99%), if needed.

5.2.3 Toxicity tests

Toxicity tests were designed according to the results of preliminary experiments in which *E. crypticus* were exposed to 10 mg/L of Ag and AgNPs for 7 days in triplicates. All the animals died in the presence of $AgNO_3$, while the survival was 90±0% and 43±40% for AgNP-Cit and AgNP-PVP, respectively. Based on these results, nominal external test concentrations selected were 1.5625, 3.125, 6.25, 12.5, 25 and 50 mg Ag/L for AgNP-Cit and AgNP-PVP to be able to obtain a full dose-response curve and to compare the toxicity of the AgNPs. For $AgNO_3$, concentrations tested were 0.5, 1, 2, 4, 8, and 16 mg Ag/L. Controls without Ag were also included. Five different time points (2, 3, 5, 7, and 10 days) were selected to determine the effect of exposure time on Ag bioaccumulation and on survival as the toxicity endpoint. Three replicates were prepared for each test concentration and time point.

After placing 20 g of pretreated quartz sand in glass jars (100 mL), 6 mL of test solution was added and the sand-solution mixture was conditioned overnight. The experiments were started by the introduction of 10 adult *E. crypticus* into each jar. The jars were covered with

perforated aluminum foil to limit water loss and kept in a climate room at 20±1 °C and 12h:12h light:dark. Water evaporation was checked twice a week and deionized water was added if necessary. At each time point, *E. crypticus* was collected from three jars per treatment after the addition of 5 mL deionized water, counted to determine survival and frozen at -18 ºC for Ag analysis. Test solutions were filtrated through 0.45 µm membrane filters (Pall Inc.) which had been conditioned with 0.1 M $Cu(NO_3)_2$ (Alfa Aesar, purity >98%) to prevent Ag loss by sorption to the filters (Cornelis et al., 2010). Filtered samples were stored at +4 ºC for Ag analysis after adding 0.625 mL concentrated HCl (Sigma Aldrich, 37%) and 0.275 mL H_2O_2 (Sigma Aldrich, 35%). The glass jars with sand matrix were opened and stored under the fume hood to evaporate until dryness before extraction for Ag analysis.

5.2.4 Ag analysis

E. crypticus, filtered test solutions and sand matrix were analyzed for total Ag at the end of the toxicity tests. *E. crypticus* were freeze dried overnight, weighted using an analytical balance and transferred into thoroughly cleaned Pyrex glass tubes. After placing the tubes on a hotplate, 300 µL of a mixture of concentrated HNO_3 (Mallbaker Ultrex Ultra Pure, 65%) and $HClO_4$ (Mallbaker Ultrex Ultra Pure, 70%) (7:1) was added. The animals were digested in different heating steps, at 85, 120 and 165 °C for 30, 30 and 45 minutes, respectively, after which the digestion mixture was completely evaporated at 180 °C. Residues were taken up in 1 M HCl for Ag measurements.

Test solutions and sand were digested following the method described in Riberio et al. (2014) for total Ag measurements. Filtered test solutions were shaken at 100 rpm for 24 hours and evaporated to around 1 mL in a water bath at 50 °C. Then, 3 mL of concentrated HCl (Sigma Aldrich, 35%) and 1 mL of concentrated HNO_3 (Sigma Aldrich, 65%) were added to digest the samples for 1 hour in the same water bath. Digested samples were made up to 50 mL with 1 M HCl in polyprolydine falcon tubes for the measurements. Completely dried sand samples were extracted by adding 10 mL of deionized water, 0.625 mL concentrated HCl and 0.275 mL H_2O_2 and shaking the mixture at 100 rpm for 24 hours. Supernatants were filtered over 0.45 µm membrane filters conditioned with 0.1 M $Cu(NO_3)_2$. Analyses were conducted by graphite furnace atomic absorption spectrophotometry (AAS; Perkin Elmer 5100ZL and Analytic-Jena contrAA 700).

5.2.5 Kinetic calculations

Internal Ag concentrations in *E. crypticus* (C_o, mg/kg dry body weight) as a function of time (t) were calculated with a 1-compartment model, assuming that exposure concentration (C_w, mg Ag/L) was constant over time (Equation 5.1).

$$C_0(t) = \frac{K_w \times C_w}{K_{e1}} \times (1 - e^{-K_{e1} \times t}) \quad \textbf{(5.1)}$$

where K_w is uptake rate constant (L/kg dry body weight/day) and K_{e1} is elimination rate constant (1/day).

To link Ag concentrations in *E. crypticus* (C_o in mg Ag/kg dry body weight) after 7 days with exposure concentrations in the water fraction of the test medium (C_w in mg Ag/L), a Langmuir equation was applied:

$$C_0 = \frac{K_L * Cmax * C_w}{1 + K_L * C_w} \quad \textbf{(5.2)}$$

where C_{max} (mg/kg dry body weight) is the maximum internal Ag concentration that can be accumulated by the enchytraeids and K_L is the adsorption coefficient (L/kg dry body weight) which may indicate affinity of the different Ag forms for uptake by the test animals.

Lethal concentrations killing 50% of the test organisms (LC50) at the different points in time were calculated with the trimmed Spearman–Karber method (Hamilton et al., 1997).

Median lethal concentration (LC50, mg Ag/L) can also be expressed as a function of time as:

$$LC50(t) = \frac{LC50\infty}{1 - e^{-K_{e2} \times t}} \quad \textbf{(5.3)}$$

Where LC50∞ is the ultimate LC50 (mg Ag/L) and K_{e2} is the survival-based elimination rate constant (1/day). Lethal Body Concentration (LBC, mg/kg dry body weight) was related to LC50∞ (mg Ag/L) using K_w (L/kg dry body weight/day) and K_{e1} (1/day).

$$LBC = LC50\infty \times \frac{K_w}{K_{e1}} \quad \textbf{(5.4)}$$

The relationship between survival (S, %) and time (t, day) was determined with a logistic survival model.

$$S(t) = \frac{e^{-\mu \times t}}{1 + (\frac{C_w}{LC50(t)})^b} \quad \textbf{(5.5)}$$

where C_w (mg Ag/L) is the exposure concentration, μ is the natural mortality rate (1/day), LC50(t) is LC50 determined at time t, and b is the slope factor.

Logistic dose-response equations were also used for analyzing the relationship between survival (S, %) and internal Ag concentrations in the organisms (C_o, mg/kg dry body weight)

$$S = \frac{S_0}{(1+\frac{C_0}{LC50inter})^b} \quad \textbf{(5.6)}$$

Where S_0 is the control survival (%), LC50inter is the median lethal concentration based on internal Ag concentrations (mg/kg dry body weight) and b is the slope parameter.

5.2.6 Statistical Analysis

IBM SPSS Statistics 21 was used for all kinetic calculations and statistical analysis. Non-linear regression analyses were used to fit corresponding equations to the data to obtain overall Ag uptake (K_w) and elimination (K_{e1}) rate constants, natural mortality rate (μ) and survival-based elimination (K_{e2}) rate constants, ultimate LC50 (LC50∞) and LC50inter. Estimated parameters for $AgNO_3$, AgNP-Cit and AgNP-PVP were compared using likelihood-ratio tests.

5.3 Results

Measured Ag concentrations in the spike solutions generally were in agreement with nominal ones with recoveries higher than 80% (Table C1 in the Appendix C). Ag concentrations in the sand (Table C2) and solution fractions (Table C3) of the test medium, measured after 2 and 7 days, did not differ much, which demonstrates constant exposure conditions during the time period used in this study. Most of the Ag was partitioned to the sand (see Tables C2 and C3). Recoveries of the spiked Ag, calculated from a mass balance of sand and solution phase concentrations, mostly were acceptable at 80-120% (Table C4). Toxicity, toxicokinetics and toxicodynamics of the different Ag forms are related to the average Ag concentrations measured in the solution phase of the test medium reported in Table C3.

Survival decreased with increasing time and exposure concentration (Figure 5.1). At the highest three concentrations of $AgNO_3$ (0.215-0.595 mg Ag/L) and AgNP-PVP (0.348-1.08 mg Ag/L) survival reached steady state within 7 days of exposure, while for the lower concentrations steady state was reached within 10 days of exposure. For AgNP-Cit, steady state was reached after 7 days at all test concentrations.

Total Ag concentrations taken up by *E. crypticus* are shown in Tables C5-C7 and plotted against time in Figure 5.2. The uptake of the silver from $AgNO_3$ and both AgNPs was affected by time and exposure concentration.

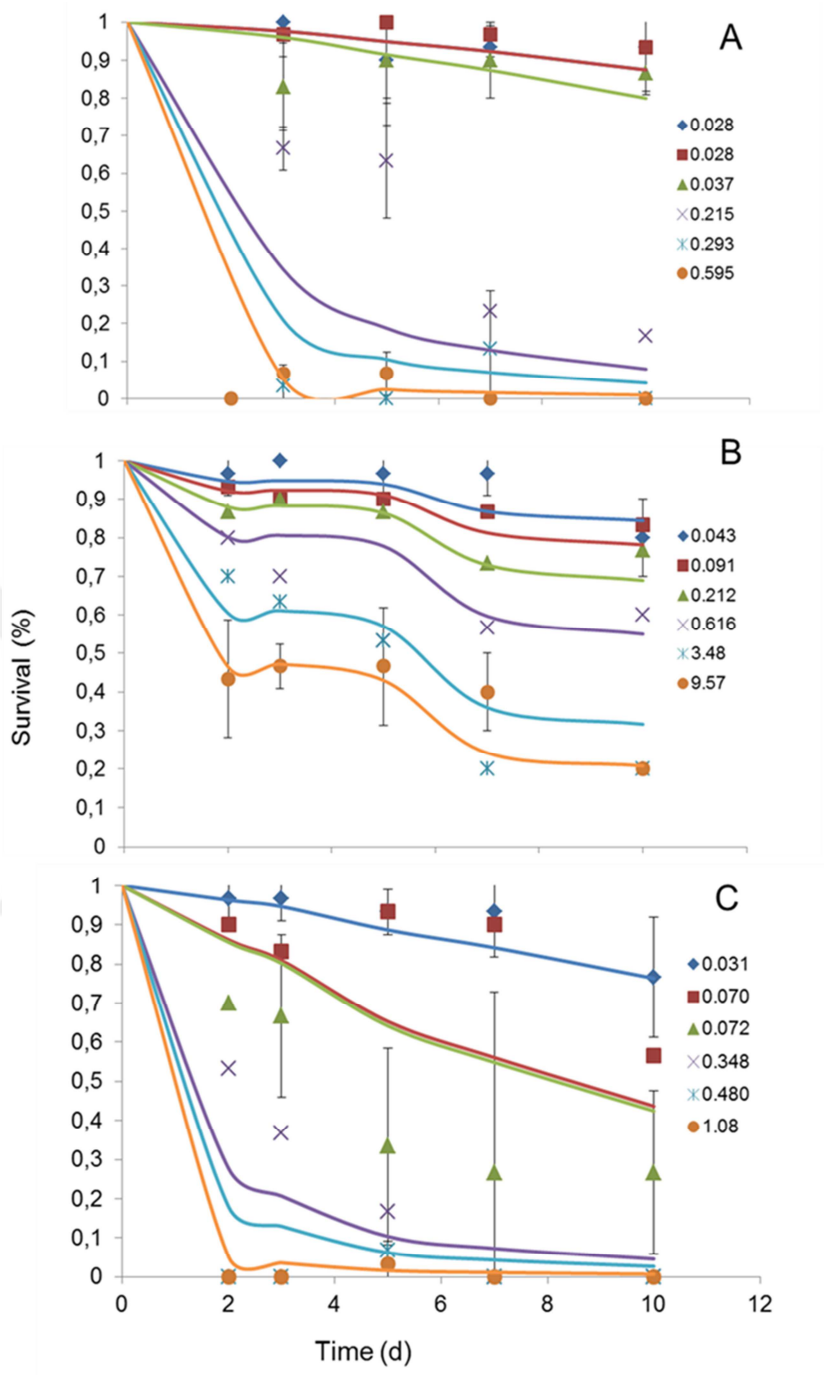


Figure 5. 1: Development with time of the survival of *Enchytraeus crypticus* exposed to a) $AgNO_3$, b) AgNP-Cit, and c) AgNP-PVP in sand-solution media. Exposure concentrations are measured concentrations in the solution phase of the sand-solution medium, in mg Ag/L, obtained after 0.45 µm filtration. Data points show survival at different sampling times, the solid lines represent the fit of a logistic dose-response model (Equation 3) to the data. Error bars represent the standard deviations of the survival in triplicate experiments.

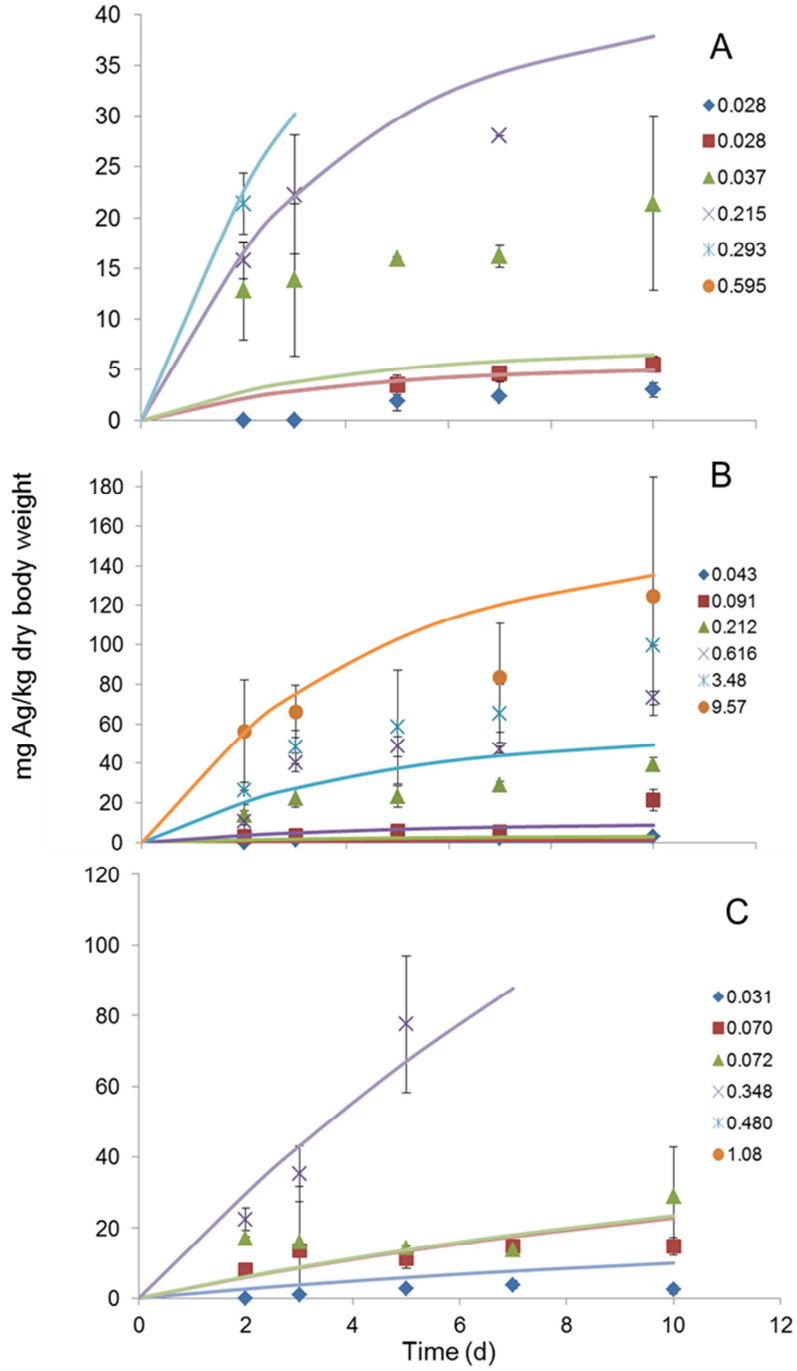


Figure 5. 2: Development with time of internal total Ag concentrations in *Enchytraeus crypticus* exposed to a) $AgNO_3$, b) AgNP-Cit, and c) AgNP-PVP in a sand-solution medium. Data points show the measured total Ag concentrations in *E. crypticus* at each sampling time, the solid lines represent the fit of the one-compartment model (Equation 1) to the data for the different measured exposure concentrations in the solution phase of the sand-solution medium (in mg Ag/L; obtained after 0.45 µm filtration). Error bars represent the standard deviations of the concentrations measured in triplicate samples.

Body concentrations approached steady state after 7 days of exposure. No data were obtained for the highest two concentrations of $AgNO_3$ (0.293 and 0.595 mg Ag/L) and AgNP-PVP (0.48 and 1.08 mg Ag/L), where all animals died. The highest total Ag concentrations in *E. crypticus* measured were 28.1, 125 and 77.5 mg Ag/kg for $AgNO_3$ (at 0.215 mg Ag/L), AgNP-Cit (at 9.57 mg Ag/L) and AgNP-PVP (at 0.348 mg Ag/L) after 7, 10 and 5 days of exposure, respectively.

When all data points for all concentrations and sampling times were fitted to Equation 5.1, the overall K_w (uptake) and K_{e1} (elimination) rate constants for $AgNO_3$, AgNP-Cit and AgNP-PVP were 49.5, 3.62, 45.8 L/kg dry body weight/day and 0.259, 0.231 and 0.071 1/day, respectively (Table 5.1). R^2 values for the model fit (Figure 5.2) ranged from 0.318-0.415 for $AgNO_3$ and AgNP-Cit to 0.876 for AgNP-PVP. K_W and K_{e1} values for AgNP-Cit and AgNP-PVP were significantly different (χ^2 = 10.8 and 17.8, respectively; $p<0.05$). K_w increased with increasing exposure concentration up to 0.037 mg Ag/L, 0.212 mg Ag/L and 0.072 mg Ag/L for $AgNO_3$, AgNP-Cit and AgNP-PVP, respectively, and then decreased again with increasing concentration (see Tables C5-C7). BCFs calculated from the overall K_w and K_{e1} values were 190, 16 and 646, respectively (Table 5.1), but especially the BCFs for the AgNPs are flattered by deviating values at the highest exposure levels where uptake probably was hampered by high toxicity (see Figure 5.1 and Tables C5-C7). Omitting the higher concentrations led to average BCFs of approx. 150 and 205 L/kg for AgNP-Cit and AgNP-PVP, respectively.

When all Ag uptake data for all concentrations after 7 days exposure were fitted to Equation 4.2, Langmuir adsorption coefficients (K_L) and maximum internal Ag concentrations (C_{max}) were estimated at 1.53, 1.80 and 7.47 L/kg dry body weight and 58.7, 44.4 and 84.4 mg Ag/kg dry body weight for $AgNO_3$, AgNP-Cit and AgNP-PVP, respectively (Table 5.1; Figure C1). Neither the K_L and nor the C_{max} values of $AgNO_3$ and the AgNPs were significantly different ($\chi^2<3.84$; n.s.)

After 10 days of exposure, LC_{50} values for the effect of $AgNO_3$, AgNP-Cit and AgNP-PVP on enchytraeid survival based on Ag concentrations in the spike solutions were 1.53, 15.9 and 3.37 mg Ag/L, respectively. LC50s based on measured concentrations in the solution phase of the test medium for AgNP-Cit and AgNP-PVP decreased from 7.43 to 0.88 mg Ag/L and from 0.2 to 0.06 mg Ag/L, respectively, in 10 days. For $AgNO_3$, LC50 decreased from 0.15 to 0.07 mg Ag/L (Figure 5.3). R^2 values for the fit of LC50 versus time using Equation 3 ranged from 0.709 for AgNP-Cit to 0.982 for AgNP-PVP (Table 5.1).

Table 5. 1: Estimated toxicokinetics and toxicodynamics parameters (±standard error) for the uptake and toxicity of Ag in *Enchytraeus crypticus* upon exposure to $AgNO_3$, AgNP-Cit and AgNP-PVP in quartz sand-solution media. All parameters are estimated by fitting different equations to the data, using measured Ag concentrations in the solution phase of the test medium (see Table S3). See Figures 1-4 for the corresponding curves showing toxicity-time relationships, uptake curves and relating toxicity to accumulated Ag concentrations in the test organisms. See the text for the Equations used.

Parameter	Symbol	Unit	$AgNO_3$		AgNP-Cit		AgNP-PVP		Equation
			Value	R^2	Value	R^2	Value	R^2	
Uptake rate	K_w	L/kg dry body weight/d	49.5±18.5	0.318	3.62±1.46	0.415	45.8±8.01	0.876	(1)
Elimination rate	K_{e1}	1/d	0.259±0.190		0.231±0.151	0	0.071±0.069		(1)
Bioconcentration factor	BCF	L/kg	190		16		645		K_w/K_{e1}
Mortality rate	u	1/d	0.000±0.006	0.939	0.000±0.005	0.895	0.000±0.011	0.800	(5)
Slope	b	-	2.07±0.302	0.971	0.552±0.064		1.73±0.000		(5)
10-day LC50		mg/L	0.07		0.88		0.06		-
Ultimate LC50	LC50∞	mg Ag/L	0.081±0.019	0.736	0.322±3.54	0.709	0.047±0.011	0.982	(3)
Survival based elimination rate	K_{e2}	1/d	0.324±0.137		0.018±0.209		0.129±0.038		(3)
LCinternal	LCinter	mg Ag/kg dry body weight	21.8±1.84	0.567	57.6±4.04	0.851	21.8±3.85	0.611	(6)
Slope	b	-	4.21±1.64		1.51±0.215	0.851	1.45±0.497		(6)
Lethal Body Concentration	LBC	mg Ag/kg dry body weight	15.5		5.06		30.4		(4)
Adsorption coefficient	K_L	L/kg dry body weight	1.53±1.17	0.943	1.80±0.692	0.863	7.47±5.01	0.735	(2)
Maximum Ag concentration in *E. crypticius*	Cmax	mg Ag/kg dry body weight	58.7±28.1		84.4±7.88	0.863	50.8±15.8		(2)

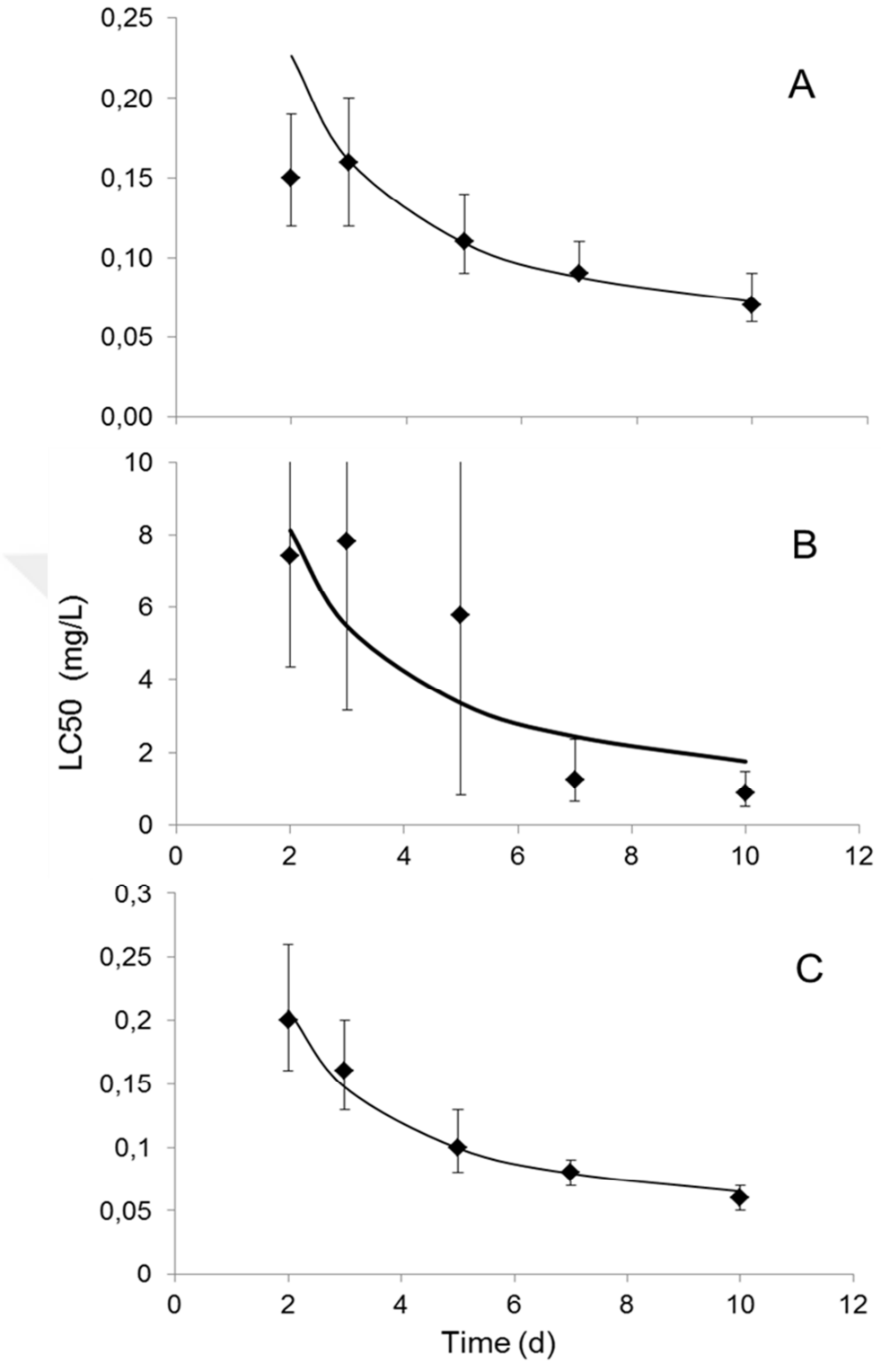


Figure 5. 3: Median lethal concentration (LC50) for the effect of a) $AgNO_3$, b) AgNP-Cit, and c) AgNP-PVP on the survival of *Enchytraeus crypticus* upon exposure in sand-solution media for different periods of time. Data points show the LC50 values calculated with the trimmed Spearman-Karber model (Hamilton et al., 1977) expressed on the basis of average measured concentrations in the solution phase of the test media (see Table S3); the solid lines represent the fit of Equation 4 to the data.

AgNP-PVP and $AgNO_3$ had the lowest ultimate LC50∞ (0.047 and 0.081 mg Ag/L, respectively) while LC50∞ for AgNP-Cit (0.322 mg Ag/L) was higher (Table 5.1). Especially due to the large error for the LC50∞ for AgNP-Cit, there was no significant difference between the LC50∞ values. Survival-based elimination rates (K_{e2}) determined with Equation 5.3 were 0.324, 0.018 and 0.129 1/day for $AgNO_3$, AgNP-Cit and AgNP-PVP, respectively (Table 5.1). K_{e2} was significantly different for $AgNO_3$ and AgNP-Cit (χ^2=18.0; $p<0.05$). Lethal body concentration (LBC), which was related to ultimate LC_{50} with Equation 5.4, was 15.5, 48.3 and 9.6 mg Ag/kg dry body weight, respectively when using the overall BCF estimated from uptake kinetics for $AgNO_3$ and the average BCFs over the lower exposure concentrations for the AgNPs (Table 5.1).

Natural mortality rate μ (calculated with Equation 5.5) was 0 day^{-1} for all three Ag forms (Table 5.1). Although the survival-time data fitted very well to the logistic survival model with $R^2>0.8$ for all three Ag forms (Figure 5.1), natural mortality rate constants showed quite high variability (Table 5.1).

LC50inter concentrations calculated with Equation 4.6 were almost equal for $AgNO_3$ and AgNP-PVP at 21.8 mg Ag/kg dry body weight, and much higher for AgNP-Cit (57.7 mg Ag/kg dry body weight) (Table 5.1). The latter value was significantly different from those for $AgNO_3$ and AgNP-PVP according to the likelihood ratio test (χ^2=12.7 and χ^2=12.6, respectively; $p<0.05$). R^2 values for the fit of all data to Equation 5.6 (Figure 5.4) ranged from 0.57 for $AgNO_3$ to 0.85 for AgNP-Cit. When the data for each time point was fitted to Equation 5.6 individually, LC50inter for each time point did not differ significantly for all Ag compounds.

5.4 Discussion

This study showed that, when related to the dissolved Ag fraction in the 0.45 μm filtered solution phase of the test medium, AgNP-PVP and $AgNO_3$ were about 10 times more toxic to *E. crypticus* than AgNP-Cit. Uptake and toxicity of all three Ag forms reached steady state after 7-10 days. Internal LC50s did not change much with time and were about twice as high for AgNP-Cit than the values for the other two Ag forms.

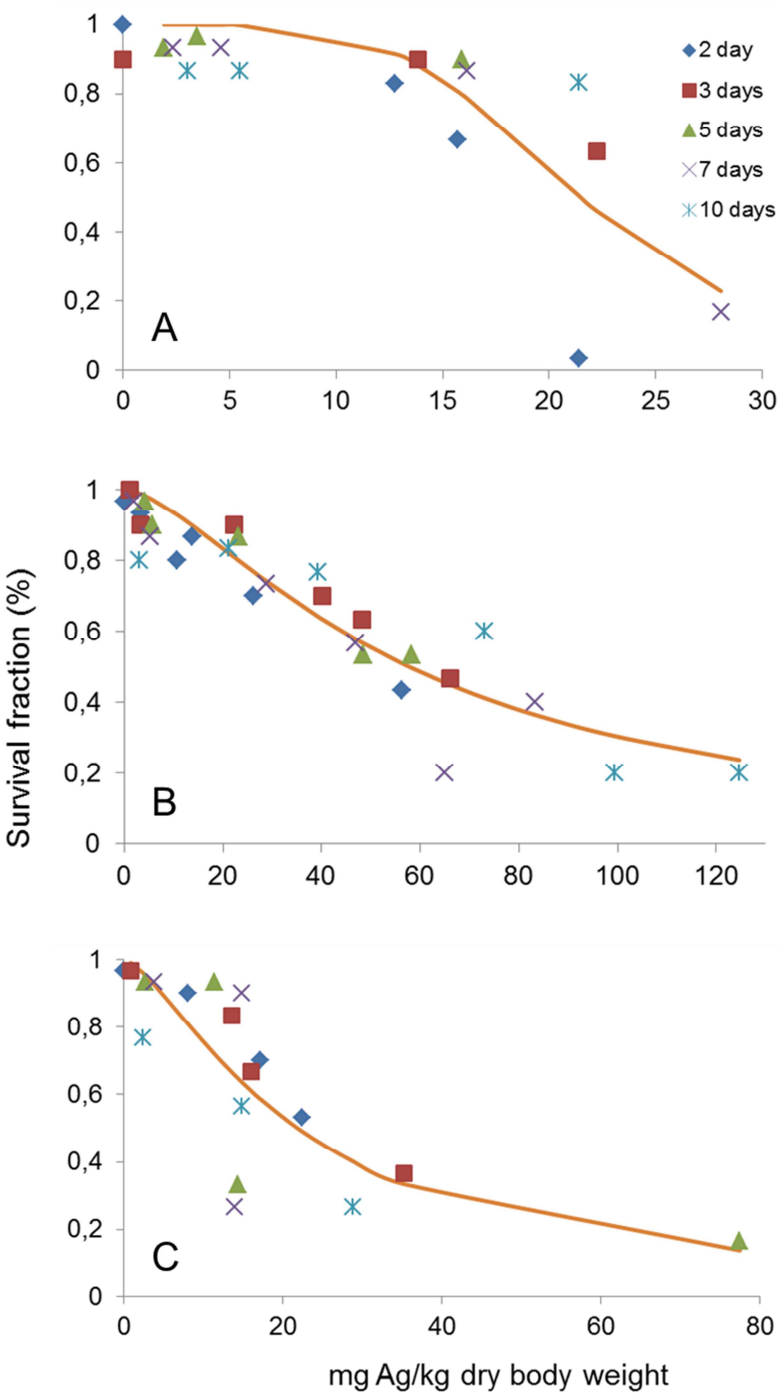


Figure 5. 4: The relation between the survival of *Enchytraeus crypticus* and total body Ag concentrations in animals after different times of exposure to a) $AgNO_3$, b) AgNP-Cit, and c) AgNP-PVP in sand-solution media. Data points stand for the measured total Ag concentrations in surviving *E. crypticus* at different sampling times, the solid lines represent the fit of a logistic dose-response equation (Equation 6) to the data.

5.4.1 Ag concentrations in the test medium

Our results showed that also in case of $AgNO_3$, only a fraction of the total dose was present in the solution phase of the test medium (Table C3). This indicates that, although we expected the sand medium to be inert, it still did bind a considerable amount of ionic Ag. We found the similar concentrations of Ag in the solution phase of our test medium for $AgNO_3$ and AgNP-PVP. Considering the expected higher sorption of AgNPs, the Ag concentrations for AgNP-PVP are in agreement with its dissolution in aqueous media reported by (Odzak et al., 2014) in which the same AgNP-PVP were used. Therefore, it seems that the concentration of ionic Ag in the solution phase of the test medium was similar for $AgNO_3$ and AgNP-PVP. This seems, however, not the case for AgNP-Cit, where at the same total concentrations as for AgNP-PVP much higher Ag concentrations were measured in the solution phase of the test medium. This could on one hand be due to the higher affinity of the more lipophilic AgNP-PVP for binding to the sand, on the other hand it might also suggest a higher release of Ag^+ ions or that part of the AgNP-Cit did pass the 0.45 µm filter.

5.4.2 Toxicity of different Ag forms to *E. crypticus*

Most studies in the literature reported higher toxicity of $AgNO_3$ to soil organisms compared to AgNPs. The EC50 for the effects of AgNPs on the reproduction of *Enchytraeus albidus*, *Eisenia andrei* and *Folsomia candida* was significantly higher than that for $AgNO_3$ (Gomes et al., 2013; Schlich et al., 2013; Waalewijn-Kool et al., 2014). Likewise, the survival of *Eisenia fetida* (Heckmann et al., 2010) and *E. andrei* (Kwak et al., 2014) was higher when exposed to AgNPs than to $AgNO_3$ while the growth and cocoon production of *Lumbricus rubellus* were more reduced by $AgNO_3$ than by AgNPs (van der Ploeg et al., 2012). It should be noted that toxicity in these studies was related to total soil concentrations, not to available concentrations in the soil solution.

The higher toxicity of $AgNO_3$ may indicate that ionic Ag is the major cause of the toxicity of AgNPs (Kittler et al., 2010). This suggests that in our study the dissolved fraction of AgNP-PVP was mainly present as Ag^+ ions, as its toxicity was similar to that of $AgNO_3$ when expressed on the basis of the concentration in the solution phase of our test medium. The LC50 for AgNP-Cit was a factor of 10 higher than that of $AgNO_3$, suggesting that only a fraction of the Ag measured in the solution phase of the test medium was present as Ag^+ ions. As much more Ag from AgNP-Cit was found in solution compared to $AgNO_3$ or AgNP-PVP, this also suggests that part of the AgNP-Cit did pass the 0.45 µm filter and stayed in solution.

The dissolution of AgNP-PVP was reported to be faster than that of AgNP-Cit (Hsiao et al., 2015), which again supports the idea that the fraction of Ag^+ ions was lower for AgNP-Cit than for $AgNO_3$ and AgNP-PVP and that the toxicity of AgNP-PVP and AgNP-NO_3 was mainly due to released Ag^+ ions.

When expressed on the basis of concentrations in the test animals, AgNP-Cit again was least toxic with highest LCinter and highest LBC. The similarity of LCinter for $AgNO_3$ and AgNP-PVP suggests that in both cases uptake of the same Ag form, probably ionic Ag, occurred. AgNP-Cit is more prone to agglomeration because of the electrostatic stabilization of citrate and might agglomerate especially in the presence of Ca^{2+} within hours (Topuz et al., 2014). So, once taken up AgNP-Cit could be aggregated in biological fluids with various ingredients inside the body of the organism and its (internal) bioavailability might be limited in spite of being taken up by the organism. Kwak et al. (2013) also mentioned decreased bioavailability of AgNP-Cit to *E. andrei* due to its agglomeration in biological fluids. The higher LC50 and higher LCinter for AgNP-Cit suggest on one hand that uptake of nanoparticulate Ag occurred but on the other hand that the low toxicity of internalized AgNP-Cit might due to internal agglomeration. A recent study (Hsiao et al., 2015) confirmed this by imaging Ag speciation inside cells. In the cells the ratio of AgNPs to total Ag was much lower than in the extracellular media due to reactions with H_20_2 (intracellular reactive oxygen species) to form Ag ions. In cells, most of the Ag ions bind with thiol groups of proteins to form complexes which are known to be less toxic (Lock and Janssen, 2001).

Both types of AgNPs produced an increasing toxicity pattern over time which was similar to that of $AgNO_3$, with their LC50s decreasing and reaching equilibrium after approximately 10 days. These results emphasize that exposure duration of a (standard) toxicity tests could be critical since the toxicity is changing over time until equilibrium is reached.

5.4.3 Toxicokinetics and toxicodynamics of different Ag forms to *E. crypticus*

The overall Ag uptake rate constant (K_w) was lower for AgNP-Cit (3.62 L/kg dry body weight/day) than for the other two Ag forms, while elimination rate constant (K_{e1}) was lowest for AgNP-PVP (Table 5.1). The similarity in K_w values for $AgNO_3$ and AgNP-PVP supports the conclusion that exposure was mainly to ionic Ag in both cases. The lower overall K_w for AgNP-Cit suggests that its bioavailable fraction was much lower. However, it should be noted that on one hand K_w calculated for the lower AgNP-Cit exposure concentrations were somewhat higher (Table C6), but not as high as the values for $AgNO_3$ and AgNP-PVP. On the

other hand, Ag concentrations measured in *E. crypticus* were quite similar for all three Ag forms at similar exposure concentrations (Tables C5-C7), but at the higher exposure concentrations of AgNP-Cit they did exceed the toxic level (LCinter) determined for the other two Ag forms. This seems to support the suggestion that at least some of the AgNP-Cit was taken up in the nano form.

Since fit of the toxicokinetics model was poor for $AgNO_3$ and Ag-Cit, K_w and K_{e1} values were also calculated for each concentration level separately. Individual K_w values increased with increasing concentration up to a certain concentration level and then they decreased with increasing concentration (Tables C5-C7). The same trend was also observed in studies with Ni 3.42-30.6 L/kg dry body weight/day (He and van Gestel, 2013) and Cd (0.104-0.214 kg/kg dry body weight/day) (Lock and Jansen, 2001). He et al. (2013) explained this trend from the limited number of metal ion transporters in the membrane of the organism which could be occupied at higher concentrations. In addition, toxic effects at high exposure concentrations may reduce metal uptake rate. This might also be the case for Ag. Figure C1 supports these observations: internal total Ag concentrations increased with total Ag concentrations in the water fraction and approached equilibrium at a certain exposure concentration. Fitting the Langmuir equation enabled quantification of the affinity of the different Ag forms for uptake, which may resemble K_L, while the maximum amount accumulated in the enchytraeid (C_{max}) may provide some idea on the type of Ag accumulated (ionic or nano form) in the body. Unfortunately K_L and C_{max} values of $AgNO_3$ and the AgNPs were not significantly different, so no firm conclusions can be drawn from these data.

The overall K_{e1} value for $AgNO_3$ and AgNP-Cit was higher than that for AgNP-PVP (Table 5.1), however, when looking at the individual values for the different test concentrations they all range between 0.1 and 0.5 day^{-1} (Tables C5-C7). Since elimination rates basically depend on organism characteristics, this suggests that the form in which Ag was present in the test organisms may have been the same for all Ag compounds. Although the survival-based elimination rates (K_{e2}) were significantly different for $AgNO_3$ and AgNP-Cit, they all had the same order of magnitude. From this, we may not conclude that the type of Ag accumulated was different.

LC50inter was calculated with Equation 5.6, which relates survival to internal body concentrations. Where LC50s changed dramatically over time, LC50inter values for the different Ag compounds were quite similar across all time points. A similar result was obtained for Ni by He and van Gestel (2013). LC50inter might be a better parameter than

LC50 to represent the toxicity of the compounds since the effects of time on toxicity are taken into account. However, internal concentrations may not always be a good representative parameter since the effective concentration (toxicodynamic process) that leads to toxicity is not taken into account. Lethal Body Concentrations (LBC) of all Ag forms, calculated using BCF values for all ($AgNO_3$) or the lower exposure concentrations (AgNPs) were quite similar to the LC50inter value, which indicates that internal concentration and effective concentration are almost the same.

5.5 Conclusion

This study is the first to determine the bioaccumulation and effects of AgNPs in *E. crypticus.* The toxicity of AgNP-PVP, expressed on the basis of Ag concentrations in the solution phase of our test media was similar to that of $AgNO_3$, while AgNP-Cit was less toxic. This shows that toxicity is strongly coating material dependent, which may be related to different adsorption to the sand matrix of the test medium. While LC50 based on external concentrations in the test medium decreased with time, LC50inter based on internal body concentrations of Ag did not change significantly over time. LC50inter therefore can be more representative for toxicity. This emphasizes the necessity for the integration of toxicodynamics and toxicokinetics studies for AgNPs. Moreover, standard toxicity tests with duration of shorter than 10 days may cause overestimation of LC50s because of non-equilibrium conditions. Future studies should focus on the long-term exposure of organisms to AgNPs to assess their potential toxicity under realistic environmental conditions and effect of dissolution rate. Internal Ag concentrations will also provide insight into the bioaccumulation of AgNPs upon exposure to soil pore water, which represents the most likely route of exposure for many organisms in soil. The results of this study may be helpful for the planning of the future toxicity studies with environmentally relevant soil media.

APPENDIX C: Chapter 5

Table C.1: Ag concentrations measured in the stock solutions of $AgNO_3$, AgNP-Cit and AgNP-PVP used to spike test systems used for exposing *Enchytraeus crypticus*. All concentrations are in mg/L. Standard deviations are calculated with triplicates.

$AgNO_3$		AgNP-Cit		AgNP-PVP	
Nominal	Measured	Nominal	Measured	Nominal	Measured
0.5	0.400±0.029	1.56	1.56±0.045	1.56	1.62±0.034
1	0.507±0.415	3.13	2.70±0.074	3.13	2.70±0.053
2	1.01±0.107	6.25	5.53±2.269	6.25	6.74±0.207
4	3.06±0.151	12.5	9.86±0.678	12.5	19.7±0.300
8	6.63±1.274	25	22.2±0.200	25	23.2±2.05
16	14.8±0.893	50	43.4±	50	50.5±2.22

Table C.2: Mean total Ag concentrations (± standard deviation; n=3) measured after 2 and 7 days in the sand fraction of a quartz sand - solution medium used for exposing *Enchytraeus crypticus* to $AgNO_3$, AgNP-Cit and AgNP-PVP. Measured concentrations are in mg Ag/kg dry sand and nominal spiked concentrations are in mg/L.

Nominal	Measured		Nominal	Measured		Nominal	Measured	
$AgNO_3$	Day 2	Day 7	AgNP-Cit	Day 2	Day 7	AgNP-PVP	Day 2	Day 7
0.50	0.141±0.011	0.133±0.008	1.56	0.270±0.016	0.393±0.006	1.56	0.337±0.024	0.279±0.002
1.0	0.315±0.013	0.202±0.006	3.13	1.31±0.12	0.971±0.159	3.13	1.24±0.204	0.849±0.003
2.0	0.585±0.03	0.573±0.03	6.25	1.87±0.147	1.73±0.624	6.25	2.09±0.198	2.14±0.01
4.0	1.23±0.023	1.22±0.041	12.5	2.80±0.273	3.91±0.186	12.5	4.16± 0.35	4.62±0.015
8.0	2.42±0.141	2.36±0.35	25.0	6.99±0.16	6.13±0.500	25.0	8.17±0.33	9.65±0.216
16.0	4.73±0.283	4.90±0.246	50.0	11.9±0.49	15.9±0.422	50.0	15.3±1.15	13.6±0.111

Table C.3: Mean Ag concentrations (± standard deviation; n=3) measured after 2 and 7 days in the solution fraction of a quartz sand solution medium used for exposing *Enchytraeus crypticus* to $AgNO_3$, AgNP-Cit and AgNP-PVP. All test solutions were 0.45 µm filtered. All concentrations are in mg/L.

$AgNO_3$				AgNP-Cit				AgNP-PVP			
Nominal	Measured			Nominal	Measured			Nominal	Measured		
	Day 2	Day 7	average		Day 2	Day 7	average		Day 2	Day 7	average
0.500	0.016±0.003	0.040±0.005	0.028	1.56	0.047±0.005	0.039±0.003	0.043	1.56	0.033±0.004	0.028±0.008	0.031
1.00	0.044±0.002	0.012±0.001	0.028	3.13	0.099±0.055	0.082±0.045	0.091	3.13	0.081±0.016	0.058±0.013	0.070
2.00	0.038±0.003	0.035±0.006	0.037	6.25	0.194±0.018	0.230±0.089	0.212	6.25	0.078±0.008	0.065±0.028	0.072
4.00	0.237±0.050	0.192±0.012	0.215	12.5	0.654±0.051	0.577±0.182	0.616	12.5	0.370±0.025	0.325±0.034	0.348
8.00	0.317±0.080	0.269±0.068	0.293	25.0	3.36±0.456	3.60±1.09	3.48	25.0	0.403±0.086	0.557±0.168	0.480
16.0	0.620±0.083	0.569±0.108	0.595	50.0	9.74±1.17	9.40±1.39	9.57	50.0	1.07±0.059	1.08±0.269	1.08

Table C.4: Mean recoveries of Ag from the test medium used for exposing *Enchytraeus crypticus* to $AgNO_3$, AgNP-Cit and AgNP-PVP in an inert sand medium. Recoveries are based on the mass balance of Ag concentrations measured in the sand fraction (Table S2) and the solution phase (Table S3) of the test medium after 2 and 7 days exposure of the enchytraeids.

Nominal	Recovery (%)		Nominal	Recovery (%)		Nominal	Recovery (%)	
$AgNO_3$	Day 2	Day 7	AgNP-Cit	Day 2	Day 7	AgNP-PVP	Day 2	Day 7
0.50	96	103	1.56	86	60	1.56	61	74
1.0	68	106	3.13	106	143	3.13	92	135
2.0	98	99	6.25	96	103	6.25	115	113
4.0	106	109	12.5	109	80	12.5	126	126
8.0	102	105	25.0	96	106	25.0	131	111
16.0	98	102	50.0	125	99	50.0	93	104

Table C.5: Development with time of total Ag concentrations in *Enchytraeus crypticus* upon exposure to $AgNO_3$ at different concentrations in the solution phase of a sand-solution medium. Shown are the average measured concentrations in the solution phase (see Table S3) and the mean concentrations in the animals with standard deviation (n=3). Also added are the uptake (K_w) and elimination (K_{e1}) rate constants and the BCF ($=K_w/K_{e1}$) estimated for each individual exposure concentration using equation 1.

Average Ag concentration in solution (mg/L)	Ag Concentration (mg/kg dry body weight) Time (days)					K_w (L/kg body weight/ day)	K_{e1} (1/day)	BCF (L/kg)
	2	3	5	7	10			
0.028	<LOD[a]	<LOD	1.92±0.250	2.33±1.48	3.04±0.670	17.0	0.099	171
0.028	-	-	3.49±0.970	4.62±0.080	5.50±0.780	33.9	0.12	283
0.037	12.8±4.84	13.9±7.55	15.9±0.230	16.2±1.12	21.4±8.60	233	0.44	526
0.215	15.7±1.82	22.3±5.91	-	28.1[b]	-	57.9	0.41	140
0.293	21.4±2.99	-	-	-	-	39.8	0.088	451
0.595	-	-	-	-	-			

[a]LOD:0.05 µg/L;
[b]Only one sample available

Table C.6: Development with time of total Ag concentrations in *Enchytraeus crypticus* upon exposure to AgNP-Cit at different concentrations in the solution phase of a sand-solution exposure medium. Shown are the average measured concentrations in the solution phase (see Table S3) and the mean concentrations in the animals with standard deviation (n=3). Also added are the uptake (K_w) and elimination (K_{e1}) rate constants and the BCF ($=K_w/K_{e1}$) estimated for each individual exposure concentration using equation 1.

Average Ag concentration in solution (mg/L)	Ag Concentration (mg/kg dry body weight)					K_w (L/kg body weight/ day)	K_{e1} (1/day)	BCF (L/kg)
	Time (days)							
	2				10			
0.043	<LOD[a]	1.12±0.140	4.17±0.72	2.02±1.06	3.06±0.550	24.1	0.33	74.0
0.091	3.22±0.250	3.33±1.28	5.62±1.62	5.32±1.53	21.3±5.49	-[c]	-[c]	-[c]
0.212	13.9±5.27	22.4±4.75	23.1±5.27	28.7±1.85	39.3±3.36	37.1	0.18	210
0.616	10.7±6.35	40.2±11.9	48.5±18.4	46.8±6.22	73.1±44.35	18.7	0.11	170
3.48	26.1[b]	48.2±8.46	58.2±28.7	65.0±14.8	99.5[b]	4.31	0.10	42.7
9.57	56.2±25.0	66.2±13.2	-	83.4±27.5	125±60.24	3.17	0.25	12.9

[a]LOD:0.05 µg/L;
[b]Only one sample available
[c]no proper fit possible

Table C.7: Development with time of total Ag concentrations in *Enchytraeus crypticus* upon exposure to AgNP-PVP at different concentration in the solution phase of a sand-solution exposure medium. Shown are the average measured concentrations in the solution phase (see Table S3) and the mean concentrations in the animals with standard deviation (n=3). Also added are the uptake (K_w) and elimination (K_{e1}) rate constants and the BCF ($=K_w/K_{e1}$) estimated for each individual exposure concentration using equation1.

Average Ag concentration in solution (mg/L)	Ag Concentration (mg/kg) Time (days)					K_w (L/kg body weight/ day)	K_{e1} (1/day)	BCF (L/kg)
	2				10			
0.031	<LOD[a]	0.81±0.09	2.67±0.500	3.75±1.15	2.43±1.23	24.9	0.22	115
0.070	8.06±0.990	13.4±0.930	11.3±2.74	14.8±1.61	14.8±2.61	104	0.49	211
0.072	17.1±0.540	16.0±1.57	14.3±0.660	13.9[b]	28.8±14.1	155	0.54	286
0.348	22.4±3.19	35.2±7.80	77.5±19.5	-	-	-[c]	-[c]	-[c]
0.480	-	-	-	-	-			
1.08	-	-	-	-	-			

[a]LOD:0.05 µg/L;
[b]Only one sample available
[c]no proper fit possible

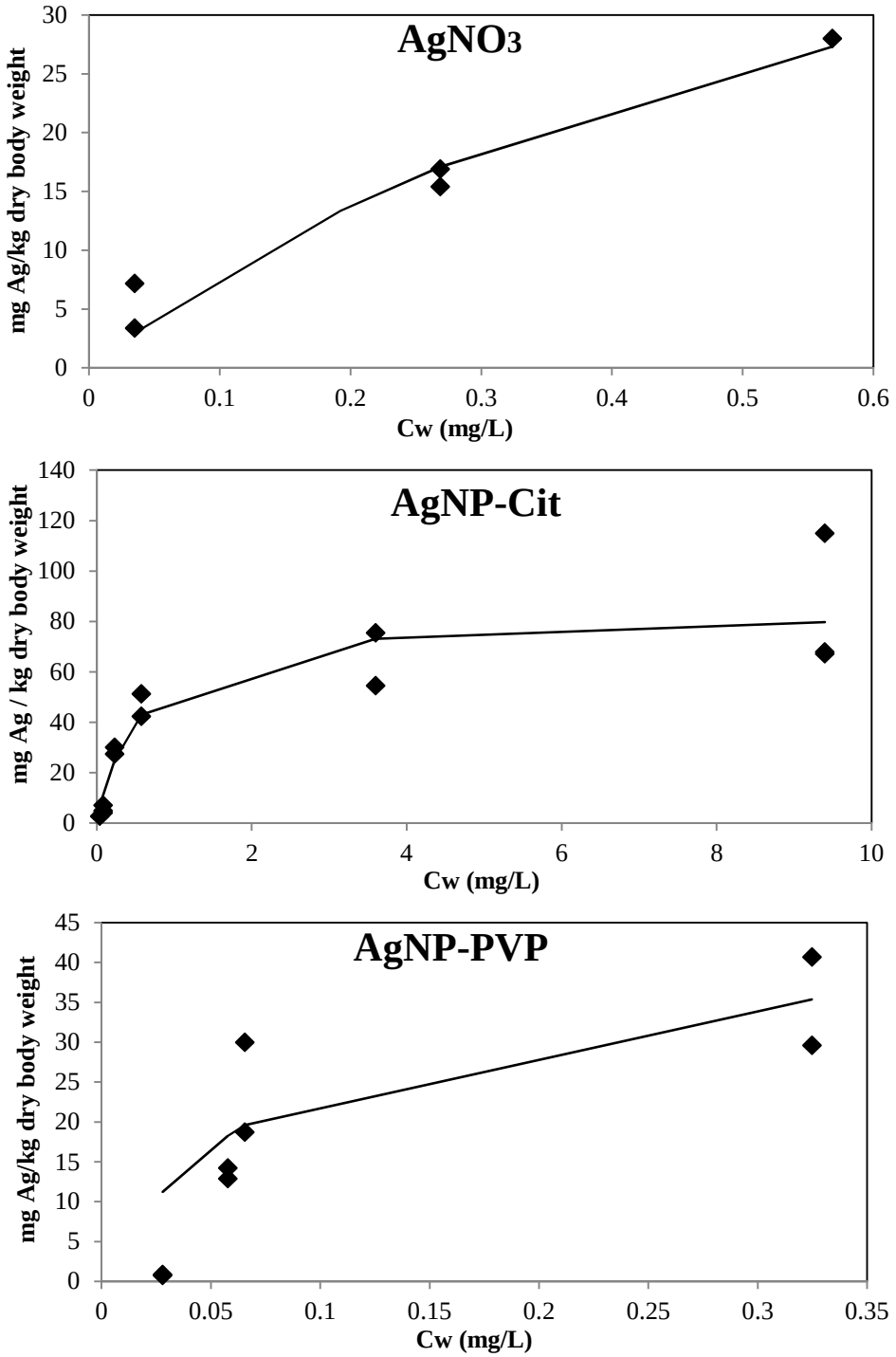


Figure C.1: Internal total Ag concentrations in *Enchytraeus crypticus* as a function of measured Ag concentrations in the solution phase of a sand-solution medium after 7 days exposure to a) $AgNO_3$, b) AgNP-Cit, and c) AgNP-PVP. Data points show the measured total Ag concentrations in *E. crypticus* at each concentration level at 7 days. Solid lines represent the fit of Langmuir equation (Equation 2) to the data.

CHAPTER 6
EFFECT OF SOIL PROPERTIES ON THE TOXICITY OF SILVER NANOPARTICLES AND SILVER NITRATE TO ENCHYTRAEUS CRYPTICUS

Abstract

Recent studies showing the possible accumulation of silver nanoparticles (AgNPs) in soil increased concerns about their possible toxicity to soil organisms. Therefore, this study determined the toxicity of AgNPs to *Enchytraeus crypticus*, an ecologically relevant soil organism. Standard natural soils with different organic matter contents and pH (Lufa 2.2, Lufa 2.3 and Lufa 5M) were spiked with ionic Ag ($AgNO_3$) and AgNPs with different coatings (polyvinyl pyrrolidone (PVP) and Citrate) to determine the effects of soil properties and Ag source. *Enchytraeus crypticus* were exposed for 21 days to assess effects on survival and reproduction. Soils, pore water and surviving animals were analyzed for Ag. LC50s were higher for $AgNO_3$ than for AgNP-PVP (111 and 335 mg Ag/kg dry soil, respectively), however, a minor contribution of nano-specific properties to toxicity could not be excluded. No effect of soil properties on enchytraeid survival was observed. EC50s for effects of both $AgNO_3$ and AgNP-PVP on reproduction were similar and negatively correlated with soil organic matter content, while soil pH seemed not to affect toxicity of both Ag forms. AgNP-Cit had similar toxicity as AgNP-PVP, but caused a dramatic dose-related increase of soil pH, hampering proper assessment of EC50s. Ag uptake in the enchytraeids was higher at higher organic matter content. NOEC (<30 mg Ag/kg dry soil in general) was close to the Ag concentrations predicted in the sewage sludge regardless of Ag source and soil types.

6.1 Introduction

The numbers of the consumer products that contain nanoparticles (NPs) have been increasing dramatically recently. Silver nanoparticles (AgNPs) are among the most widely used NPs in various products including cosmetics, paints, textiles etc. (The Project on Emerging Technologies). There is increasing concern about their release from products which may lead to exposure of the receiving environmental bodies and posing harm to ecosystems. While most previous work focused on effects in aquatic environments, there is increasing concern about the effects of AgNPs on soil organisms since recent studies showed that the agricultural application of sewage sludge (Blaster et al., 2008), washing of paints with rainwater and sedimentation (depending on the water chemistry; Topuz et al., 2015 *submitted*) may lead to spread and accumulation of AgNPs also in soils.

So far, earthworms are the most commonly used organisms for assessing AgNP toxicity in soil. Bioaccumulation, reproductive toxicity (Shoults-Wilson et al., 2011a) and avoidance response to polyvinyl pyrrolidone coated AgNPs (AgNP-PVP) (Shoults-Wilson et al., 2011b) were reported. Heckmann et al. (2011) observed no effect of AgNPs on the survival and reproduction of the earthworm *Eisenia fetida* at concentrations as high as 1000 mg Ag/kg dry soil. Hu et al. (2012) evaluated responses of the antioxidant system, acid phosphatase and ATPase of *E. fetida* following exposure to AgNPs. The reproductive toxicity and cytotoxicity of AgNP on *Eisenia andrei* were determined by Schlich et al. (2013) and Kwak et al. (2014). AgNPs did affect the growth and reproduction of the earthworm *Lumbricus rubellus* (Van der Ploeg et al., 2014). Waalewijn-Kool et al. (2014) observed no effects on the survival and reproduction of the springtail *Folsomia candida* at AgNP concentrations up to 800 mg Ag/kg dry soil.

However, knowledge on the toxicity of AgNPs to enchytraeids is lacking in the literature. Enchytraeids are ecologically relevant soil organisms owing to their crucial role in decomposition and bioturbation in soils (Castro-Ferreira et al., 2011) and are commonly preferred for toxicity testing due to their sensitivity to a wide range of stressors (Didden and Römbke, 2001). Santorufo et al. (2013) showed that metal bioaccumulation in *Enchytraeus crypticus* was higher than in *E. andrei* and *F. candida*. Although Gomes et al. (2013) found no effect of AgNP-PVP on the survival of *Enchytraeus albidus* and EC50 for effects on

reproduction was 225 mg/kg, AgNPs should also be tested with *E. crypticus* which is a convenient model for soil ecotoxicology (Castro-Ferreira et al., 2012).

The stability of AgNPs is affected by the environmental conditions in the soil solution (Klitzke et al. 2015). Therefore evaluation of the toxicity of $AgNP_S$ in soil should also consider their speciation in pore water. Moreover, data on the effect of soil properties on the toxicity of Ag nanoparticles to soil organisms are lacking. Since Ag may complex with organic matter, altering its speciation and bioavailability (Cornelis et al., 2012), soil organic matter content may affect the toxicity of AgNPs. Soil pH may also affect the speciation of silver by its influence on the processes of dissolution, agglomeration and adsorption (Liu and Hurt, 2010). In addition to the effect of soil properties, there are still doubts about the form of Ag causing toxicity. While some studies report an effect of nano-specific properties (Colman et al., 2013; Eom et al., 2013), others are pointing at a dominant role of ionic silver in causing toxicity of AgNPs (Heckmann et al., 2011; Gomes et al., 2013; van der Ploeg et al., 2014).

This study has the following major aims: a) to determine Ag bioaccumulation and the lethal and reproductive toxicity of two differently coated AgNPs to *E. crypticus* in three different soils, b) to assess the effect of Ag source on Ag uptake and toxicity by including $AgNO_3$ as a ionic Ag form, c) to assess the effect of organic matter content and pH on Ag bioaccumulation and toxicity, d) to relate toxicity and Ag uptake by the animals to the speciation of AgNPs in the soil pore water, and e) to relate toxicity to Ag body concentrations in the test animals.

6.2 Materials and Methods

6.2.1 Test Organisms

Enchytraeus crypticus (Enchytraeidae; Oligochaeta; Annelida) has been cultured for over 10 years in the laboratory of the Department of Ecological Science, VU University, Amsterdam, The Netherlands. *E. crypticus* were cultured in agar media prepared with soil extract and kept in a climate room at constant temperature (16 °C), relative humidity (75%) and dark conditions. The culture was fed twice a week with a mixture of oatmeal, dried baker's yeast, egg yolk powder, and fish oil. For the experiments, adult *E. crypticus* with white spots in the clitellum region were selected.

6.2.2 Test Compounds

Polyvinyl pyrrolidone (AgNP-PVP) and Cytrate (AgNP-Cit) coated silver nanoparticles and ionic silver in the form of $AgNO_3$ were used in this study. $AgNO_3$ also served as a positive

control to distinguish possible effects originating from the nanoparticles from those of the released Ag^+ ions. AgNP-Cit and AgNP-PVP were purchased from NanoSys GmBH (Switzerland) and obtained as suspensions in water at concentrations of 1 g/L and 10 g/L AgNP, respectively. $AgNO_3$ (Sigma-Aldrich, >99%) was dissolved in deionized water (Milli-Q). Nominal size of AgNP-Cit and AgNP-PVP (1 g/L) provided by the manufacturer was around 25 nm; detailed characterization of the AgNPs used has been reported in Topuz et al. (2014).

6.2.3 Test Soils

The toxicity experiments were conducted in three natural standard soils, namely, Lufa 2.2, Lufa 2.3 and Lufa 5M. Lufa 2.2 is a standard soil type widely used for toxicity studies. Lufa 2.3 and Lufa 5M were selected because of their lower organic carbon content and higher pH, respectively, compared to Lufa 2.2. The properties of these soils are summarized in Table D1 in the Appendices. Soils were air dried at 40 °C before being used in the experiments.

6.2.4 Toxicity Experiments

Effects of the three silver forms on the survival and reproduction of *E. crypticus* were determined according to ISO (2004) and OECD (2004), but using an exposure duration of 3 weeks as recommended by Castro-Ferreira et al. (2012). Test concentrations were selected based on LC50/EC50 values previously reported for earthworms and enchytraeids (Table D2). AgNP-PVP was tested at 25.6, 64, 160, 400, 1000 and 2500 mg Ag/kg dry soil. Applying the same range for AgNP-Cit was not possible as above 160 mg Ag/kg dry soil all animals died, probably because of the high pH of the stock AgNP-Cit solution (10.5±0.152). The pH of soils spiked with AgNP-Cit at 400 mg Ag/kg dry soil was around 8.50 and all organisms died in a few hours. Also 160 mg Ag/kg dry soil led to improper conditions especially for Lufa 5M, which had a higher pH_{CaCl2} (7.14±0.002) than the other soils. Therefore, AgNP-Cit was tested only at concentrations of 25.6 and 64 mg Ag/kg dry soil. $AgNO_3$, which often is more toxic than AgNPs (Table D2), was tested at 10.6, 25.6, 64, 160, 400, 1000 mg Ag/kg dry soil.

The soils were spiked in glass jars with the different Ag forms dissolved in demineralized water, at the same time adjusting soil moisture content to 50% of the water holding capacity (WHC). After spiking, the soils were mixed to achieve an as homogeneous distribution of the Ag as possible. For the 2500 mg Ag/kg concentration of AgNP-PVP, spiking had to be performed in steps, in between which the excess amount of solution was evaporated. Control soils received demineralized water only. Four replicates were prepared for each test

concentration and control. For each replicate, 30 g moist soil was placed in a 100 mL glass jar, which was equilibrated overnight. Toxicity tests started with the introduction of 10 adult *E. crypticus*, with clearly visible clitella and approximately 1 cm length, to the jars. A few grains of oat meal were added as food. The jars were covered with perforated aluminum foil for air transfer and incubated at 20±1 °C and 12:12 h light:dark. Water content and food were checked twice a week and adjusted if necessary. After 21 days, the soil from each test jar was transferred to a plastic box (250 mL) to collect the surviving animals, which were kept overnight in petri dishes filled with ISO 6341 dilution solution (ISO, 1996) containing 294 mg/L $CaCl_2.2H_2O$, 123.3 $MgSO_4.7H_2O$, 5.8 mg/L KCl, and 64.8 mg/L $NaHCO_3$ (Sigma Aldrich, >99%), to empty their guts. Then, they were stored at – 18 °C for metal analysis. The animals remaining in the soil in the glass test jar were fixated by adding 10 mL ethanol (VWR chemicals, 96%). After approximately 2 minutes, the soil was transferred from the glass jar to the plastic box using an additional 100 mL tap water. After that, the samples were stained with 200 µL Bengal rose (Sigma Aldrich, in 1% ethanol). The plastic boxes were closed tightly, agitated and kept overnight at +4 °C to obtain efficient staining of the animals. To separate soil particles from the juveniles, the stained samples were washed with tap water and passed through a 160 µm sieve. The stained juveniles were then transferred to a special white tray (80x50 cm^2) to be counted under a magnifier glass (x2.5).

Separate soil samples were mixed with 0.01 M $CaCl_2$ (Sigma Aldrich, >99%) solution (5:1, w/v) and shaken at 200 rpm for 2 hours. After settling of the soil particles (overnight) pH was measured in the overlying solution using a pH meter (WTW, Inolab pH7110).

6.2.5 Silver Analysis

Spiked soils, pore water and surviving animals collected from the test jars were analyzed for total Ag content. Animals tissues were freeze dried overnight, dry weights determined using an analytical balance (Mettler Toledo GmbH, 1998), and transferred into pre-cleaned pyrex tubes. The animals were digested on a hotplate in 300 µL of a mixture of HNO_3 (Mallbaker Ultrex Ultra Pure, 65%) and $HClO_4$ (Mallbaker Ultrex Ultra Pure, 70%) (7:1), applying different heating steps. Afterwards the digestion mixture was completely evaporated at 180 °C. Residues were taken up in 1 M HCl for Ag measurements. Analyses were conducted by graphite furnace atomic absorption spectrophotometry (Perkin Elmer, PinAAcle 900Z).

Soil was digested with the method described in Waalewijn-Kool et al. (2014). After drying at 40 °C for 24 hours, about 0.130 g soil samples were digested in teflon bombs with 2 mL of a

mixture of HCl (Sigma Aldrich, 37%) and HNO_3 (Sigma Aldrich, %65) (4:1). The bombs were closed tightly and heated at 140 °C for 7 hours in an oven (Binder FD). After cooling down, 8 mL demineralized water was added and the solution was analyzed for Ag by flame AAS (Perkin Elmer Analyst 100). To check for the accuracy of the method, reference material (ISE sample 989 of River Clay from Wageningen, The Netherlands) was also digested with the samples and the recoveries of Ag in the reference material (2.8 mg Ag/kg dry soil) were 88-94%.

For porewater extraction, 30 g dry weight equivalent of the test soil was moistened to 100% WHC by adding deionized water and equilibrated for 3 days. Cellulose nitrate membrane filters (0.45 µm) (S&S Ø 47 mm) were placed in between 2 round paper filters (S&S 597 Ø, 47 mm, pore size 11 µm) at the bottom of Teflon bombs. All filters were pre-conditioned with 0.1 M $Cu(NO_3)_2$ to prevent loss of Ag. After introduction of the soil, they were centrifuged at 2000 g at 15 °C for 45 minutes (Centrifuge Falcon 6/300). Approximately 5 mL of pore water was collected, which was analyzed for total Ag by flame AAS (Perkin Elmer Analyst 100).

6.2.6 Statistical Analysis

Lethal concentrations killing 50% of the test organisms (LC50) and corresponding 95% confidence intervals were calculated with the trimmed Spearman–Karber method (Hamilton et al., 1977). Effective concentrations that caused 10% and 50% reduction in the number of juveniles (EC10 and EC50, respectively) and their 95% confidence intervals were obtained by fitting a 3- parameter logistic dose-response model (Haanstra et al., 1985). To estimate No-observed effect concentrations (NOEC), juvenile numbers in the different treatments were compared with the control (without test compound) with one way analysis of variance (ANOVA) followed by Dunnett's post-hoc test ($p<0.05$). EC50s for the toxicity of $AgNO_3$ and AgNP-PVP and in the three test soils (Lufa 2.2, Lufa 2.3 and Lufa 5M) were compared with a generalized likelihood-ratio test (Sokal and Rohlf, 1995). All analysis were run in SPSS 21 for Windows.

6.3 Results

6.3.1 Soil pH, total Ag and porewater Ag concentrations

The test soils were loamy (Lufa 2.2 and Lufa 5M) and silty sand (Lufa 2.3) having different pH, Organic Carbon (OC) content and Water Holding Capacity (WHC) (Table C1). Soil pH_{CaCl2} (mean, n=2) measured in this study (5.46, 5.47 and 7.14 for Lufa 2.2, Lufa 2.3 and

Lufa 5M, respectively, with relative standard deviation (RSD)<1%) were compatible with the values reported by the provider (Table D1 in Appendix D). The highest OC content was measured in Lufa 2.2 (1.61±0.151 % OC), the lowest in Lufa 2.3.

In the toxicity tests, pH of the spiked soils was close to that of the control soil except for AgNP-Cit (Tables D3-D5). Tables D3-D5 show the measured Ag concentrations in the test soils. For all Ag compounds, recoveries of Ag were 80-120%. Total Ag concentrations in pore water strongly depended on Ag form and soil type, but in all cases increased with increasing soil concentrations (Tables D3-D5). The lowest Ag porewater concentrations were measured in Lufa 5M, while they were highest in Lufa 2.3.

The total Ag concentrations in soil and pore water were quite well fitted with Freundlich isotherms (Figure D1-D2), with R^2 >0.96 (Table 6.1).

Table 6. 1: Freundlich model parameters relating total Ag concentrations with porewater concentrations in three soils spiked with $AgNO_3$ and PVP-coated AgNPs (see Figure D3 and D4 for the corresponding Freundlich isotherms). Letters indicate significant differences between K_f values for the different soils and Ag compounds obtained with a generalized likelihood ratio test ($p<0.05$).

Compound	Soil	K_f	n	R^2
$AgNO_3$	Lufa 2.2	127A	0.433	0.973
	Lufa 2.3	34.9B	0.677	0.985
	Lufa 5M	118C	0.552	0.996
AgNP-PVP	Lufa 2.2	645D	0.345	0.967
	Lufa 2.3	576D	0.391	0.957
	Lufa 5M	758D	0.513	0.989

Freundlich adsorption coefficients (Kf) were significantly higher for AgNP-PVP than for $AgNO_3$ in all soils (Table 6.2). Kf values were significantly different among the three soils (likelihood ratio test; $p<0.05$). The lowest Kf value was obtained for Lufa 2.3, the highest for Lufa 5M. The Kf values for AgNP-PVP showed the same trend, but the difference between soils was not significant. In all soils, the shape parameter (n) of the Freundlich isotherm was below 1 for both $AgNO_3$ and AgNP-PVP.

6.3.2 Toxicity Tests

The control performance of the enchytraeids satisfied the validity criteria of the test guidelines (ISO, 2004; OECD, 2004), with adult mortality <5%, on average 393-584 juveniles and a coefficient of variation of juvenile numbers of 3.3-10.3%.

Dose-response curves for the toxicity of $AgNO_3$ and AgNPs to *E. crypticus* are shown in Figures 1 and S3 for effects on reproduction and survival, respectively. Corresponding LC50, EC50, EC10 and NOEC values are presented in Table 6.2.

LC50 was Ag source dependent (Table 6.2), and for the same Ag compound LC50s did not significantly differ between the test soils (Table D6). LC50 values based on total soil concentrations were significantly higher for AgNP-PVP than for $AgNO_3$ in all three soils ($\chi^2_{(1)}$ =26.2-67.8, $p<0.05$). LC50 values based on Ag concentrations in pore water (Table 6.1; Figure D4) did not differ between soils or Ag source (Table D6). EC50 values for the effect of $AgNO_3$ and EC50 and EC10 for the effect of AgNP-PVP on *E. crypticus* reproduction differed significantly between different soil types (Table 6.2; Figures 6.1 and D5).

For $AgNO_3$, EC50 was significantly higher in Lufa 2.2 (75.2 mg Ag/kg dry soil) than in Lufa 2.3 (26.9 mg Ag/kg dry soil) and Lufa 5M (45.8 mg Ag/kg dry soil), while EC50 in Lufa 5M also was higher than in Lufa 2.3 ($\chi^2_{(1)}$ =25.6-37.3 ($p<0.05$). EC50s for $AgNO_3$ and AgNP-PVP spiked to the same soil type did not significantly differ, but in Lufa 2.3 the EC10 of AgNP-PVP was significantly lower than that of $AgNO_3$. EC50 and EC10 values for the toxicity of $AgNO_3$ and EC50 for the toxicity of AgNP-PVP increased with increasing organic carbon content of the test soils (Figure 6.2). According to EC50 values based on porewater concentrations (Table 6.2; Figures 6.3 and D6), $AgNO_3$ was significantly more toxic than AgNP-PVP in Lufa 2.3 and Lufa 5M ($\chi^2_{(1)}$ =37.8-88.5, $p<0.05$), but not in Lufa 2.2 soil. In Lufa 2.3, EC50 values for $AgNO_3$ were significantly higher than in Lufa 2.2 and Lufa 5M ($\chi^2_{(1)}$ =16.3-19.1, $p<0.05$) (Table 6.2).

NOEC values were independent of Ag source and soil types; only exception was NOEC for $AgNO_3$ which was higher in Lufa 2.2 (30.8 mg Ag/kg dry soil) than in the other soils (Table 6.2). For AgNP-PVP, NOEC values were lower than the lowest tested concentration in all three soils.

Table 6. 2: LC50 (concentration killing 50% of the animals), EC50 and EC10 (concentrations reducing reproduction by 50 and 10% compared to the control) and No-observed Effect Concentration for the toxicity of $AgNO_3$ and two differently coated AgNPs to *Enchytraeus crypticus* after 21 days exposure in three different test soils. LC50 was calculated with the trimmed Spearman–Karber method, EC50 and EC10 with a logistic dose-response model; 95% confidence intervals are reported in brackets. NOEC was estimated by one-way ANOVA and Dunnet's post hoc test ($p<0.05$). Toxicity is related to total soil concentrations in mg Ag/kg dry soil, porewater concentrations in mg Ag/L and body concentrations in the surviving animals in µg/g dry body weight.

Soil type	Ag type	LC50		EC50			EC10	NOEC
		Soil concentration	Porewater concentration	Soil concentration	Porewater concentration	Body concentration	Soil concentration	Soil concentration
Lufa 2.2	$AgNO_3$	111 (89.2-139)	0.132 (0.094-0.170)	75.2 (60.8-90.0)	0.101 (0.077-0.125)	>137	18.3 (10.8-25.8)	30.8
	AgNP-PVP	335 (267-422)	0.217 (0.086-0.348)	92.3 (76.9-108)	0.074 (0.066-0.081)	180 (53.5-304)	25.5 (16.1-34.8)	<20.3
	AgNP-Cit	>102**	>3.41**	51.3-102*	>3.41	>191	-	-
Lufa 2.3	$AgNO_3$	112 (87.0-144)	1.86 (0.077-3.64)	26.9 (22.3-31.5)	0.139 (0.133-0.145)	26.6 (4.38-48.8)	8.29 (5.21-11.4)	9.90
	AgNP-PVP	340 (271-428)	0.498 (0.297-0.70)	28.2 (19.5-36.8)	0.014 (0-0.042)	13.9 (0-41.8)	2.70 (0.320-5.07)	<28.9
	AgNP-Cit	>58.7**	>0.449**	30.5-58.7*	0.13-0.449*	47.3-104*	-	-
Lufa 5M	$AgNO_3$	92.1 (73.3-116)	0.348 (0.094-0.603)	46.0 (39.0-52.6)	0.084 (0.074-0.095)	9.76 (3.0-16.4)	11.7 (7.64-15.7)	9.90
	AgNP-PVP	425 (361-501)	0.364 (0.318-0.411)	55.3 (50.5-60.0)	0.016 (0.01-0.022)	6.39 (6.02-6.76)	12.8 (10.2-15.4)	<24.1
	AgNP-Cit	>63.8**	>0.037**	27.4-63.8*	0.011-0.037*	4.84-6.58*	-	-

*Toxicity test conducted with 3 (Lufa 2.2) and 2 (Lufa 2.3 and Lufa 5M) concentration levels; juvenile numbers were reduced by more than 50% between this ranges
****** Less than 50% mortality at the highest concentration tested.

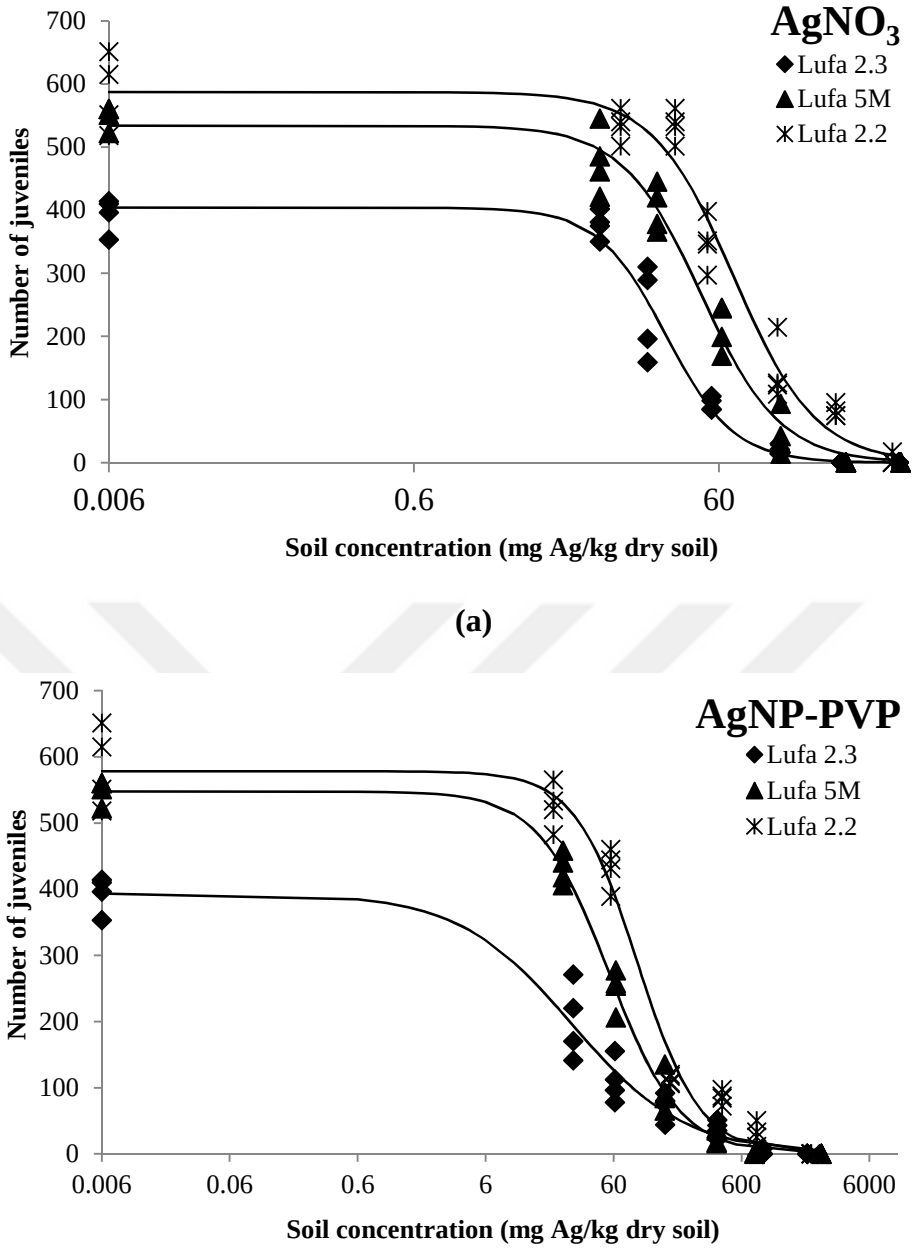


Figure 6. 1: Effects of a) $AgNO_3$ and b) AgNP-PVP on the reproduction of *Enchytraeus crypticus* exposed for 21 days in Lufa 2.2, Lufa 2.3 and Lufa 5M soils. Data points show the number of juveniles produced in the four replicates of each concentration. Solid lines show the fit of a 3-parameter logistic dose-response model.

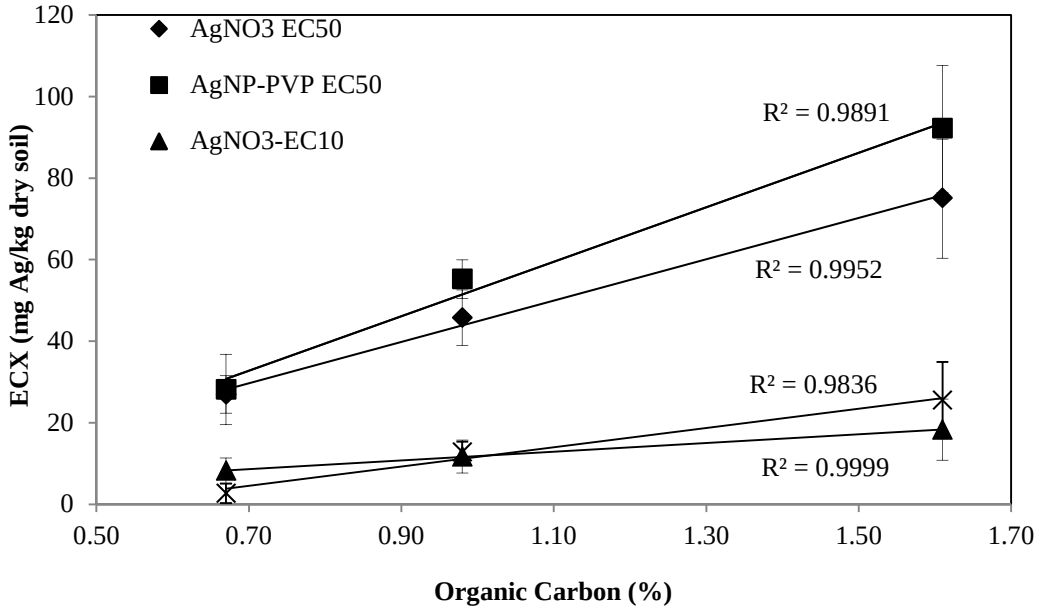


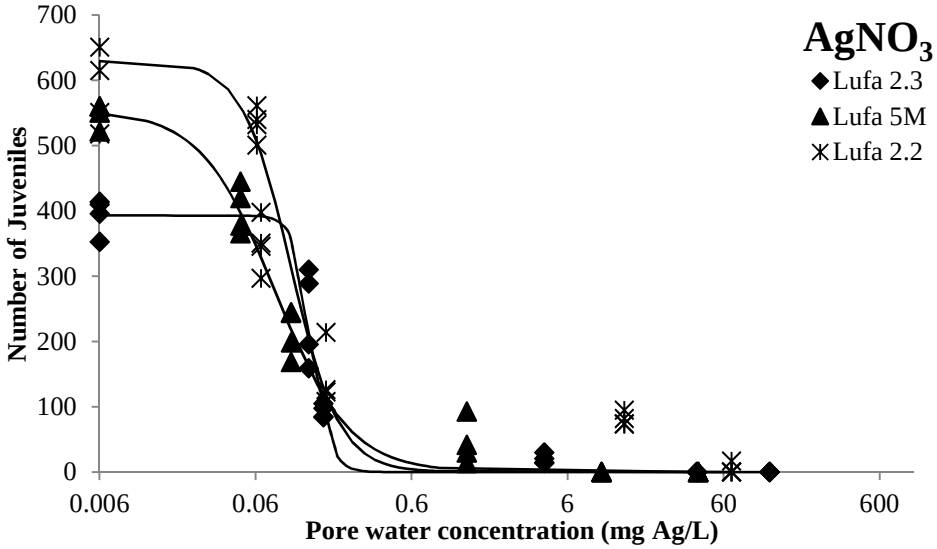
Figure 6. 2: Effect of soil organic carbon content on toxicity of $AgNO_3$ and AgNP-PVP to *Enchytraeus crypticus* after 21 days exposure in three different soils. Data points show the EC50 and EC10 values for the effects on reproduction calculated with a 3-parameter logistic dose-response model. Solid lines represent trend lines relating EC50 and EC10 values to organic carbon content of the soils and R^2 shows the goodness of fit. Error bars represent the 95% confidence intervals for EC50 and EC10 values.

Measured Ag concentrations in *E. crypticus* increased with increasing exposure concentrations for both $AgNO_3$ and AgNP-PVP, regardless of the soil expect for $AgNO_3$ in Lufa 2.2 (Table D7). *E. crypticus* exposed to $AgNO_3$ or AgNP-PVP accumulated higher Ag concentrations in Lufa 2.2 soil than in the other soils, with the lowest internal concentrations found upon exposure in Lufa 5M soil. Ag concentrations were higher in *E. crypticus* exposed to AgNP-PVP than to $AgNO_3$. For *E. crypticus* exposed to AgNP-Cit in all soil types, measured body Ag concentrations were similar to those in animals exposed to similar $AgNO_3$ and AgNP-PVP concentrations.

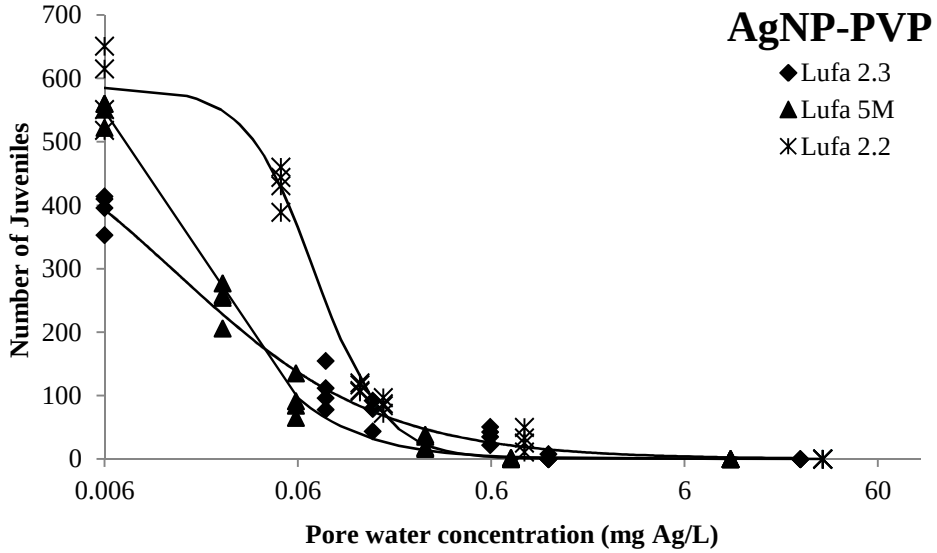
EC50 values relating effects on the reproduction of *E. crypticus* to body Ag concentrations are shown in Table 6.2 and corresponding dose-response curves in Figure 4. For $AgNO_3$ in Lufa 2.2, EC50 could not be calculated. No significant difference in internal EC50 values was observed between both Ag forms and soil types.

Toxicity of AgNP-Cit to *E. crypticus* had to be evaluated without dose-response curves as only few concentrations could be tested. Based on this, it may be concluded that LC50 was higher than 58.7-102 mg Ag/kg dry soil (survival >80% in all soils), while no accurate EC50s could be derived. Table 5.2 reports the concentrations in between which 50% effect must have

occurred; fitting the logistic model to the data of two concentrations gave EC50 estimates of 53.1, 40.2 and 57.8 mg Ag/kg dry soil for Lufa 2.2, Lufa 2.3 and Lufa 5M, respectively.

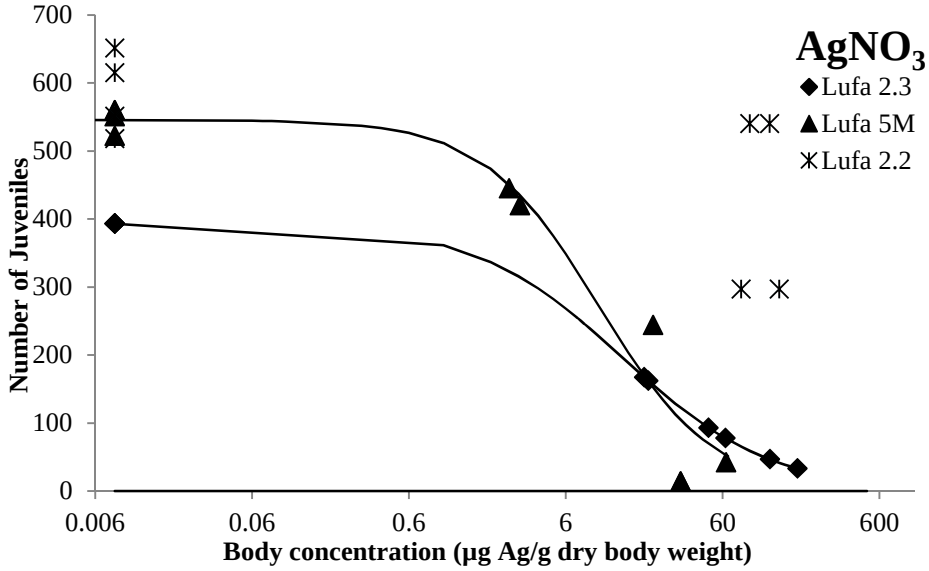


(a)

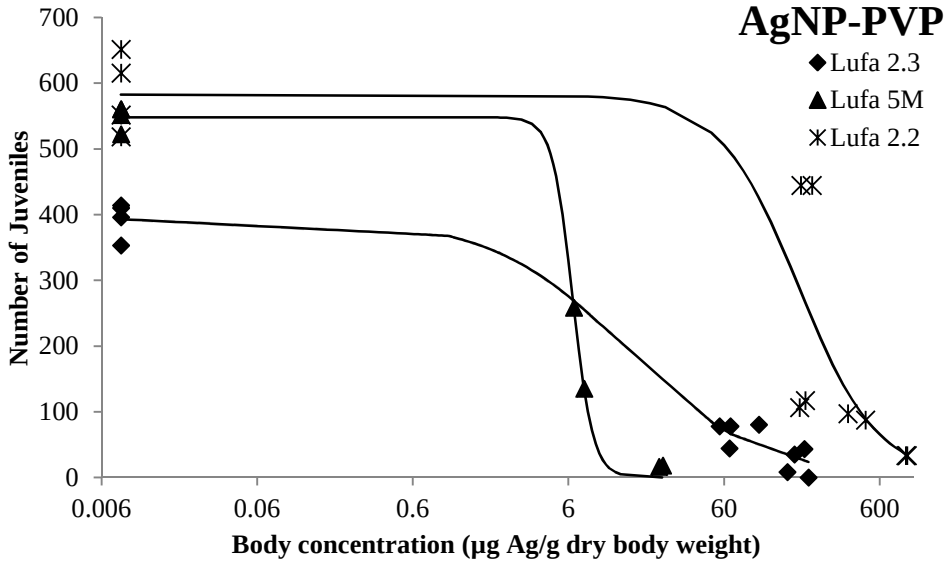


(b)

Figure 6. 3: Effects of a) $AgNO_3$ and b) AgNP-PVP on the reproduction of *Enchytraeus crypticus* exposed for 21 days in a) Lufa 2.2, b) Lufa 2.3, and c) Lufa 5M soils. Effects are related to Ag concentrations measured in pore water at the end of the exposures. Data points show the number of juveniles produced in the four replicates of each concentration. Solid lines show the fit of a 3-parameter logistic dose-response model.



(a)



(b)

Figure 6. 4: Effects of a) $AgNO_3$ and b) AgNP-PVP on the reproduction of *Enchytraeus crypticus* exposed for 21 days in a) Lufa 2.2, b) Lufa 2.3, and c) Lufa 5M soils. Effects are related to Ag concentrations measured in surviving animals. Data points show the number of juveniles produced in the four replicates of each concentration. Solid lines show the fit of a 3-parameter log-logistic dose-response model.

6.4 Discussion

$AgNO_3$ and AgNPs were toxic to the survival and reproduction of *E. crypticus* after 21 days of exposure in different test soils (Table 6.2). Survival was less sensitive than reproduction and toxicity was dependent on the Ag source rather than on soil type. Soil type did affect

reproduction toxicity, and $AgNO_3$ and AgNP-PVP were equally toxic to the reproduction of *E. crypticus*. Both Ag sources may cause negative reproduction effects on *E. crypticus* at fairly low concentrations with NOEC mostly <20 mg Ag/kg dry soil.

6.4.1 Interactions of AgNPs with the test soils

Soil pH was affected by the concentration and the coating material of the AgNPs. Sterically coated AgNP-PVP, which basically is uncharged, increased soil pH slightly while the electrostatically stabilized AgNP-Cit, which is negatively charged, caused a strong increase of soil pH. The latter effect was so strong that it was not possible to test very high concentrations as the pH increase by itself was already sufficient to kill the test animals.

Ag concentrations in pore water were highly dependent on the source of Ag. The lower Ag concentrations in pore water (Tables D3-D5) and higher Freundlich adsorption coefficient (Kf; Table 6.1) showed that AgNP-PVP binds stronger to the soil than $AgNO_3$. This confirms the results of our previous study using an aqueous solution embedded in an inert sand medium, where we also found higher fractions of Ag in the aqueous phase for $AgNO_3$ than for AgNP-PVP. In addition to the Ag source, coating material of AgNPs may have an impact on the speciation of Ag in the soil due to difference in adsorption, release of Ag ions or agglomeration depending on stabilization effects. Although porewater concentrations were available for only two different soil concentrations of AgNP-Cit, the higher percentages of Ag in pore water (2.0-3.5%) for AgNP-Cit than for AgNP-PVP also supports the hypothesis of the coating material effect. The higher porewater concentration of AgNP-Cit could be explained with the results of Navarro et al. (2014) who found a high release of Ag ions in the presence of citrate in the soil due to the complexation reaction between citrate-carboxylate groups and AgNPs (leaving 2 or 3 negatively charged sites). Porewater Ag concentrations in Lufa 2.2 soil were also lower for paraffin-coated AgNP (Waalewijn-Kool et al., 2014) than for AgNP-PVP in this study. However, in both studies the Ag concentrations in pore water were below 1.5% of the soil concentration which is comparable with Whitley et al. (2013) and Schlic et al. (2013).

Although less pronounced in this study, soil pH and organic matter content also have an impact on Ag concentrations in pore water. The solubility of metals is much higher at low pH (Spurgeon and Hopkin, 1996; Li and Hurt, 2010), therefore the lowest porewater concentrations would be expected for the soil with the highest pH. This effect was indeed found for $AgNO_3$ and AgNPs in Lufa 5M soil, which had the highest pH. Lufa 5M also had

the highest CEC which indicates a higher availability of binding sites. Although organic matter content of this soil is close to that of Lufa 2.3 soil, the Ag concentrations in pore water confirm the dominant effect of high soil pH. A higher organic matter content generally leads to lower Ag concentrations in pore water (Liu and Hurt, 2010), which was confirmed by the difference in porewater concentrations between Lufa 2.2 and Lufa 2.3, having similar soil pH. Organic matter can inhibit the oxidation of Ag ions which may bind to the AgNP surface and are released to the soil solution (Borm et al., 2006). However, the Kf values for AgNP-PVP did not differ between the test soils, while for $AgNO_3$ they were significantly lower for Lufa 2.3 (Table 6.2). This suggests that sorption of AgNP-PVP was still dominated by particle interactions with the soil rather than by sorption of ionic Ag. It can be speculated that organic matter content of the soil is more effective in affecting the speciation of Ag in pore water unless the pH is high enough to limit dissolution.

6.4.2 Toxicity of Ag to *Enchytraeus crypticus*

To the best of our knowledge, this study is the first in the literature to report on the toxicity of $AgNO_3$ and AgNPs to *E. crypticus* in soil. Reproduction was more sensitive than survival, as also was observed for *L. rubellus* (van der Ploeg et al., 2014) and *E. albidus* (Gomes et al., 2013). Shoults-Wilson et al. (2011b) found that avoidance behaviour of *E. fetida* exposed to $AgNO_3$ and AgNPs was even more sensitive than mortality or reproduction, with an EC50 <10 mg Ag/kg soil dry weight.

In our study, effects of $AgNO_3$ and AgNP-PVP on the reproduction of *E. crypticus* became effective at 20-30 mg Ag/kg dry soil. NOECs obtained were in line with Schlic et al. (2013) and van der Ploeg et al. (2014), who reported values of ≤15 and 1.5 mg Ag/kg dry soil for $AgNO_3$ and AgNP-PVP, respectively. Total Ag concentrations in sewage sludge are in the range of 2-195 and 3-14 mg Ag/kg according to USEPA (2009) and Johnson et al. (2014), respectively, and Gottschalk et al (2009) predicted concentrations in sewage sludge in the range of 1.29-6.24 mg nano-Ag/kg. Comparing our NOECs with these concentrations shows that AgNPs can pose a risk for *E. crypticus* in soils repeatedly dosed with sewage sludge. The use of sewage sludge as fertilizer for agricultural lands should be studied further to properly assess the potential risk of exposure of soil organisms to AgNPs and other NPs.

The data on $AgNO_3$ and AgNPs in soils with different characteristics show that toxicity may change depending on the Ag source (ionic or nano) and on soil characteristics.

Effect of Ag source

One of the biggest questions about AgNPs is whether their toxicity is due to ionic silver released or caused by nano-specific characteristics. The results of our study show that the source of the toxicity may depend both on the endpoint and on soil characteristics such as pH and organic matter content. The LC50s for the effect on the survival of *E. crypticus* were lower for $AgNO_3$ (source of ionic Ag) than for AgNP-PVP (Table 6.2), while no difference was found in the EC50 or EC10 values for the effect on reproduction (Table 6.2). Significant differences between $AgNO_3$ and AgNP-PVP in terms of LC50 were observed in studies on *E. albidus* (Gomes et al., 2013) and *E. fetida* (Heckmann et al., 2011). Van der Ploeg et al.(2014) also point at the effect of Ag source on the bioavailability of Ag to earthworms.

The higher Ag concentrations in the pore water of $AgNO_3$ spiked soils (Tables D3-D5) explain the higher toxicity of ionic Ag on survival. Although ionic Ag also seems to be the major source of toxicity, it may be speculated that the nano-specific characteristics of AgNP-PVP also contributed to its toxicity. Shoults-Wilson et al. (2011a) came to a similar conclusion. Since the dissolution of the AgNP-PVP used in our study was reported to be up to 12% by Odzak et al. (2014), the LC50 of AgNP-PVP was expected to be 10% of that of $AgNO_3$; in our study, this was however, around 25%. In our previous study, where *E. crypticus* were exposed to same AgNP-PVP in an aqueous solution embedded in an inert sand medium, the LC50 for AgNP-PVP was 50% of that for $AgNO_3$ (Topuz and Van Gestel, *submitted*), supporting this assumption. The lower LC50 (in % of the one for $AgNO_3$) in this study compared to our previous study is consistent with the literature since the sand medium is known to cause higher toxicity for AgNPs because of their lower affinity to bind to the sand (Navarro et al., 2014). The LC50s for $AgNO_3$ and AgNP-PVP based on porewater concentrations (Table 6.2) suggest similar toxicity of nano Ag and ionic Ag, which may be due to the higher availability of AgNPs in pore water or the presence of ionic Ag resulting from its dissolution. The latter however, seems less likely since AgNP-PVP was highly stable in synthetic and natural aquatic media with various water chemistry characteristics (Topuz et al., 2014; Topuz et al., *submitted*). Ag concentrations measured in *E. crypticus* were also similar for $AgNO_3$ and AgNP-PVP except for Lufa 2.2 soil with the highest organic matter content. The latter is in line with the differences between LC50 values, which were also higher for Lufa 2.2 soil than for other soils. The contradiction for the effect of Ag source between LC50s based on soil and porewater concentration does not provide a consistent answer to the question which dose is more representative for toxicity: soil or porewater concentration? It is beyond the scope of this study to answer this question.

Dose-response curves for the effects of $AgNO_3$ and AgNP-PVP on reproduction, based on soil concentrations (Figure D5), overlap perfectly suggesting that toxicity is similar for ionic and nano forms in all soil types. Ribeiro et al. (2014) and Schlic et al. (2013) also found similar toxicity of $AgNO_3$ and AgNPs to *Pseudokirchneriella subcapitata* and *E. andrei*, respectively. This may suggest that toxicity is increased due to the indirect, nano-specific effects of AgNPs (Ribeiro et al., 2014). Differences in toxicity mechanisms towards survival and reproduction therefore could be the reason for the finding of nano-specific effects on reproduction and not for survival. It is known that reproduction can be affected by reactive oxygen species production, DNA damage and protein denaturation (Zhao and Wang, 2011). Ahn et al.(2014) demonstrated ROS damage for the nematode *Caenorhabditis elegans* upon exposure to AgNP-PVP which is not observed for $AgNO_3$. On the other hand, dose-response curves and EC50s for the effects of $AgNO_3$ and AgNP-PVP on reproduction based on porewater concentrations (Figure D6; Tables 6.2 and D6) demonstrate that AgNP-PVP may be more toxic than $AgNO_3$ in Lufa 2.3 and Lufa 5M soils. This is line with the survival toxicity based on porewater and total Ag concentrations in *E. crypticus* (Table D7). It seems that AgNP-PVP can be mobile in all soil types; however, their toxicity may be reduced but their bioaccumulation increased due to the presence of higher organic matter content in the pore water of Lufa 2.2 soil.

Effect of organic matter and pH

The dose-response curves for the effects of $AgNO_3$ and AgNP-PVP (Figure D7) were almost overlapping and LC50s did not significantly differ between soil types (Table 6.2). This suggests that LC50 is not sensitive to organic matter content (0.6-1.6%) and pH (5.5-7.5) at least in the range used in our study. Also EC50 values related to porewater concentrations did not differ (Figure D8; Table 6.2).

Dose-response curves for the effect of $AgNO_3$ and AgNP-PVP on reproduction based on soil concentrations suggest a major effect of organic matter (Figure 6.1), corresponding with the increase of the Kf values with increasing organic matter content of the soils (Table 6.2). Ag ions are known to have high affinity to organic matter (Gao et al. 2012). In addition to the clear shifts in the dose-response curves, EC50s for $AgNO_3$ and AgNP-PVP were significantly different between soils (Table D7). EC50 and EC10 values strongly correlated with organic carbon content of the soils (Figure 6.2), for both Ag forms. EC50s for the toxicity of $AgNO_3$ and AgNP-PVP to *E. albidus*, AgNP-Cit to *E. fetida* and $AgNO_3$ to *L. rubellus* reported in other studies (Gomes et al., 2013; Kwak et al., 2014; van der Ploeg et al., 2014) were higher

than the values found in our study, which could be due to the higher organic carbon contents of the soils used by these authors. The EC50s of 46.9 and 80 mg Ag/kg dry soil for $AgNO_3$ and AgNP-PVP, respectively found for *E. fetida* in RefSoil (Table D2; Schilic et al., 2013) are comparable with the values obtained in our study for Lufa 5M (Table 6.2) which had the same organic carbon content (Table D1). Although the dose-response curves of AgNP-PVP based on porewater concentrations (Figure 6.3) did not show an as clear pattern as the curves based on soil concentrations, EC50s showed the similar trend of higher toxicity of AgNP-PVP in the soil with the lower organic matter content. The presence of organic matter, such as low molecular weight organic thiols in pore water, may block the active sites of AgNPs, leading to lower toxicity (Yang et al., 2014). Higher Ag concentrations in *E. crypticus* (Table D7) and the lower toxicity in Lufa 2.2 soil support this hypothesis. Yang et al. (2014) also reported that organic matter diminished the toxicity of Ag compounds to *C. elegans.* According to the EC50s based on porewater concentrations, $AgNO_3$ had the lowest toxicity in Lufa 2.3 with the lowest organic matter content which is difficult to explain with the scope of this study and existing studies. Yang et al. (2014) showed that a type of fulvic acid decreased toxicity to *C. elegans* upon exposure to $AgNO_3$. Therefore, the structure of organic matter rather than its quantity may be speculated to decrease the toxicity depending on the Ag source.

Dose-response curves for the effects of Ag compounds on enchytraeid reproduction based on body concentrations (Figure 6.4) also suggest toxicity was lowest in Lufa 2.2 soil. The higher organic matter content in the pore water of Lufa 2.2 soil may also bind Ag ions with complexation reactions and deactivates the toxicity despite of the rather high concentration taken up into the body. The body Ag concentration for animals exposed in Lufa 2.2 soil seems fairly high compared to the other soils, and remains hard to explain.

It may be speculated that there are different pathways for the bioavailability of ionic Ag and AgNPs in addition to pore water since the uptake was higher in the soil with the lower Ag concentration in the pore water. However, uptake from pore water could become more dominant for AgNPs in soils with higher organic matter content, which increases their mobility as a result of stabilization. Nevertheless, organic matter may decrease toxicity by occupying active sites which leads to bioaccumulation. Therefore, the effect of organic matter content on the toxicity may not be as effective for ionic Ag as for AgNPs.

Most of the significant differences in toxicity between the test soils seem related to the difference in organic matter content. The Lufa 5M soil did not show any specific differences which could be related to its high pH value even though Ag speciation in the soil and the pore

water should also be highly affected by the difference in pH. Soil pH also was not as effective as organic matter content in influencing the avoidance behaviour of *E. fetida* to AgNPs in the study of Shoults-Wilson et al. (2011b).

6.5 Conclusions

NOEC values for the reproduction toxicity of AgNPs to *Enchytraeus crypticus* were as low as the predicted environmental concentrations for sewage sludge, emphasizing the need for environmental risk assessment studies. Reproduction toxicity of both $AgNO_3$ and AgNP-PVP negatively correlated with soil organic matter content, but no such correlation was found for effects on survival. Soil pH seemed not to affect toxicity of both Ag forms. Survival seems especially affected by ionic Ag, although in case of AgNP-PVP a (minor) contribution of nano-specific properties to toxicity could not be excluded. Reproduction may be as sensitive to effect of nano-Ag as to ionic Ag. Therefore, further study on the mechanisms of reproduction toxicity of nano-Ag is needed.

APPENDIX D: Chapter 6

Table D.1: Characterization of the Lufa soil types used for the toxicity test with silver nanoparticles and silver nitrate. Characterization is provided by the supplier (LUFA-Speyer, Germany, 2009) (for more detailed information, see: http://www.lufa-speyer.de/).

Parameters	Lufa 2.2	Lufa 2.3	Lufa 5M
organic carbon in % C	1.61±0.15	0.67 ± 0.04	0.98 ± 0.05
pH-value (0.01 M $CaCl_2$)	5.5 ± 0.1	5.8 ±0.7	7.3 ±0.1
cation exchange capacity (meq / 100g)	10.0 ± 0.7	7.3 ±1.0	16.1 ± 4.2
maximum water holding capacity (g/100g)	43.3 ±2.6	35.6± 1.7	39.8 ± 2.1
weight per volume (g/1000ml)	1236 ±32	1334 ±6	1291 ± 45
soil type (German DIN)	Loamy sand	Silty sand	Loamy sand
soil type (USDA)	Sandy loam	Loamy sand	Sandy loam

Table D.2: Reported data for the toxicity of $AgNO_3$ and PVP-coated Ag nanoparticles (AgNP-PVP) to different soil organisms in different test matrices and using different endpoints.

Compound	Organism	Endpoint	Matrix	LC50/EC50 (mg Ag/ kg dry soil)	Reference
$AgNO_3$	*Eisenia andrei*	Survival	OECD soil*	530	Kwak et al. (2014)
$AgNO_3$	*Eisenia fetida*	Reproduction	RefeSol 01A†	42	Schlich et al. (2013)
$AgNO_3$	*Enchytraeus albidus*	Reproduction	OECD soil*	<50	Gomes et al. (2013)
AgNP-Cit	*Eisenia andrei*	Survival	OECD soil*	>2000	Kwak et al. (2014)
AgNP-PVP	*Eisenia fetida*	Survival	Askov‡	>1000	Heckmann et al. (2011)
AgNP-PVP	*Enchytraeus. albidus*	Reproduction	OECD soil*	225	Gomes et al. (2013)
AgNP-PVP	*Eisenia fetida*	Reproduction	RefeSol 01A†	146	Schlich et al. (2013)
AgNP-PVP (10 nm)	*Eisenia fetida*	Avoidance	Yeager¶	8.39	Shoults-Wilson et al. (2011b)
AgNP-PVP (30-50 nm)	*Eisenia fetida*	Avoidance	Sandy Loam	4.26	

*7% sphagnum peat, 20% kaolin clay and 73% of sand

† a loamy, medium-acidic, and lightly humic sand (pH 5.67; Corg 0.93%, sand 71%, silt 24%, clay 5%)

‡ a sandy loam soil with pH of 5.8, total organic carbon 1.36%, clay 11.6%, silt 21.4%, and sand 64.7%

¶ The OECD artificial soil consisted of 69.58% dry mass quartz sand, 0.43% dry mass crushed limestone, 20% dry mass kaolin clay, and 10% dry mass sphagnum peat moss (sieved to < 2 mm)

Table D.3: Soil pH_{CaCl2} and total soil and porewater Ag concentrations in the Lufa 2.2 soil spiked with $AgNO_3$ and two differently coated AgNPs for the toxicity experiments with *Enchytraeus crypticus*. Also included is the recovery of Ag from the spiked soils. All values are averages (relative standard deviation) (n=2).

Nominal Concentration (mg Ag/kg dry soil)	Measured Concentration (mg Ag/kg dry soil)	Recovery (%)	pH_{CaCl2}	Porewater Concentration (mg Ag/L)
0	<LOD	-	5.46(01.43)	<LOD
		AgNO3		
10.2	13.5 (14.7)	132	5.31 (0.386)	-
25.6	30.8 (0.07)	120	5.31 (0.040)	0.061 (18.5)
64	50.2 (11.9)	78.5	5.35 (0.714)	0.065 (8.7)
160	144 (5.49)	90.2	5.48 (0.633)	0.169 (4.18)
400	349 (5.43)	87.3	5.36 (0.040)	13.8 (6.39)
1000	819 (3.35)	81.9	5.26 (2.03)	67.4 (13.6)
		AgNP-PVP		
25.6	20.3 (6.59)	79.2	5.35 (0.767)	-
64	56.8 (1.43)	88.8	5.41 (0.445)	0.049 (8.66)
160	166 (32.1)	104	5.65 (0.551)	0.126 (1.69)
400	422 (8.42)	105	5.79 (0.757)	0.166 (17.0)
1000	785 (0.573)	78.5	5.90 (0.515)	0.890 (28.5)
2500	2093 (22.2)	83.7	6.45 (0.691)	31.1 (6.15)
		AgNP-Cit		
25.6	21.4 (17.1)	83.7	5.71 (0.941)	0.904 (10.4)
64	51.3 (5.82)	80.2	5.72 (0.210)	3.41 (12.0)
160	102 (1.0)	63.6	6.21 (0.682)	

Table D.4: Soil pH_{CaCl2} and total soil and porewater Ag concentrations in the Lufa 2.3 soil spiked with $AgNO_3$ and two differently coated AgNPs for the toxicity experiments with *Enchytraeus crypticus*. Also included is the recovery of Ag from the spiked soils. All values are averages (relative standard deviation) (n=2).

Nominal Concentration (mg Ag/kg dry soil)	Measured Concentration (mg Ag/kg dry soil)	Recovery (%)	pH_{CaCl2}	Porewater Concentration (mg Ag/L)
0	<LOD	-	5.47(3.25)	<LOD
		$AgNO_3$		
10.2	9.9 (28.0)	96.9	5.64 (0.364)	-
25.6	20.3 (16.3)	79.3	5.66 (0.537)	0.131 (4.32)
64	53.3 (14.3)	83.3	5.65 (0.300)	0.163 (37.3)
160	149 (0.624)	93.3	5.62 (0.365)	4.26 (5.82)
400	379 (21.4)	94.7	5.70 (1.46)	40.3 (13.2)
1000	900 (4.35)	90.0	5.59 (0.771)	118 (0.958)
		AgNP-PVP		
25.6	28.9 (4.13)	113	5.69 (0.310)	-
64	61.5 (20.9)	96.0	5.70 (0.410)	0.084 (4.23)
160	152 (0.89)	94.5	5.77 (0.870)	0.146 (21.3)
400	387 (4.15)	96.8	5.91 (2.24)	0.592 (1.43)
1000	878 (9.04)	87.8	6.31 (1.36)	1.19 (34.6)
2500	1957 (3.65)	78.3	6.68 (0.074)	23.8 (12.2)
		AgNP-Cit		
25.6	30.5 (28.4)	119	5.74 (2.75)	0.13 (43.9)
64	58.7 (3.37)	91.7	6.08 (1.26)	0.449 (12.0)

Table D.5: Soil pH_{CaCl2} and total soil and porewater Ag concentrations in the Lufa 5M soil spiked with $AgNO_3$ and two differently coated AgNPs for the toxicity experiments with *Enchytraeus crypticus*. Also included is the recovery of Ag from the spiked soils. All values are averages (relative standard deviation) (n=2).

Nominal Concentration (mg Ag/kg drysoil)	Measured Concentration (mg Ag/kg drysoil)	Recovery (%)	pH_{CaCl2}	Porewater Concentration (mg Ag/L)
0	<LOD	-	7.14(0.069)	<LOD
		$AgNO_3$		
10.2	9.9 (7.79)	96.6	7.19 (0.098)	-
25.6	23.5 (18.0)	91.8	7.27 (0.399)	0.048 (2.95)
64	43.0 (3.62)	67.1	7.35 (0.491)	0.101 (95.2)
160	148 (3.88)	92.5	7.35 (0.154)	1.36 (1.57)
400	407 (5.57)	102	7.41 (0.382)	9.88 (26.5)
1000	927 (4.80)	92.7	7.44 (0.276)	41.2 (0.72)
		AgNP-PVP		
25.6	24.1 (13.6)	94.1	7.28 (0.515)	-
64	62.3 (15.7)	97.4	7.34 (0.540)	0.025 (20.2)
160	150 (2.14)	93.9	7.38 (0.374)	0.059 (52.0)
400	383 (0.812)	95.7	7.51 (0.198)	0.273 (28.5)
1000	762 (29.7)	76.2	7.68 (0.534)	0.758 (4.2)
2500	2511 (2.81)	100	7.91 (0.215)	10.4 (11.2)
		AgNP-Cit		
25.6	27.4 (9.69)	107	7.25 (0.205)	0.011 (9.1)
64	63.7 (1.97)	100	7.70 (0.092)	0.037 (21.7)

Table D.6: Comparison of LC50 and EC50 values for the effects of $AgNO_3$ and AgNP-PVP on the survival and reproduction of *Enchytraeus crypticus* after 21 days exposure in Lufa 2.2, Lufa 2.3 and Lufa 5M soils. Effect concentrations are based on soil, porewater and body concentrations and compared using a generalized likelihood ratio test. The significance of differences is reported as using $\chi^2_{(n=1)}$ values ($p < 0.05$). When there is no difference, χ^2 were reported as <3.84 ($p<0.05$).

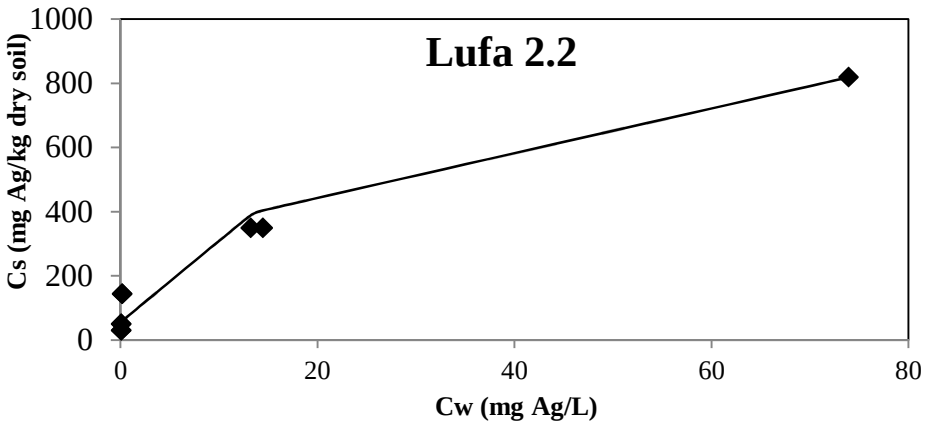
Type	Comparison	LC50		EC50		
		Soil	Pore water	Soil	Pore water	Body
		$\chi^2_{(n=1)}$; $p<0.05$				
Lufa 2.2	$AgNO_3$-AgNP-PVP	32.7	<3.84	<3.84	7.60	*
Lufa2.3	$AgNO_3$-AgNP-PVP	26.2	6.37	<3.84	37.8	<3.84
Lufa 5M	$AgNO_3$-AgNP-PVP	67.8	<3.84	<3.84	88.5	<3.84
$AgNO_3$	Lufa 2.2-Lufa 2.3	<3.84	<3.84	40.7	10.1	*
	Lufa 2.2-Lufa 5M	<3.84	<3.84	16.3	<3.84	*
	Lufa 2.3-Lufa 5M	<3.84	<3.84	19.1	21.1	<3.84
AgNP-PVP	Lufa 2.2-Lufa 2.3	<3.84	<3.84	37.3	<3.84	6.33
	Lufa 2.2-Lufa 5M	<3.84	<3.84	25.6	76.5	<3.84
	Lufa 2.3-Lufa 5M	<3.84	<3.84	27.1	<3.84	<3.84

*Data can not fit to the model

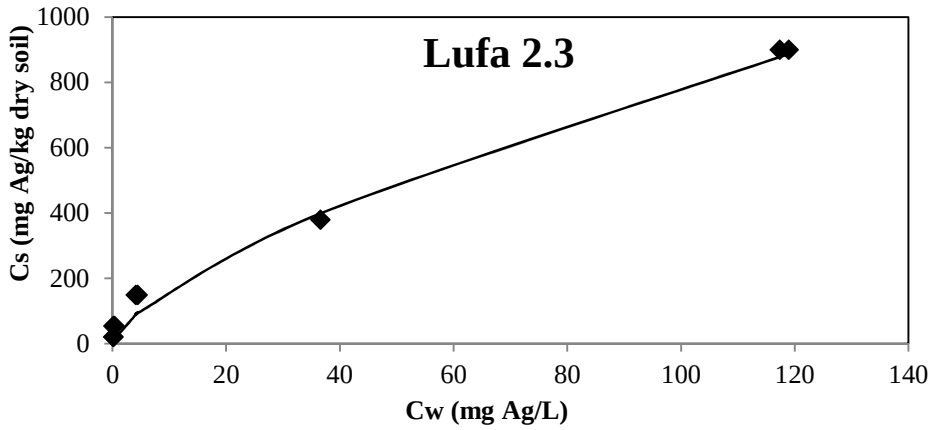
Table D.7: Total Ag concentrations in *Enchytraeus crypticus* exposed for 21 days to different concentration levels of $AgNO_3$ and two differently coated AgNPs spiked to Lufa 2.2, Lufa 2.3 and Lufa 5M soils. Results are given as the mean (relative standard deviation) for 2 replicates of test jars.

Nominal Concentration (mg Ag/kg drysoil)	Animal concentrations (µg Ag/mg dry body weight)		
	Lufa 2.2	Lufa 2.3	Lufa 5M
	$AgNO_3$		
10.2	103 (8.41)	22.8 (16.1)	5.67 (41.6)
25.6	105 (20.6)	19.6 (4.37)	2.83 (11.1)
64	108 (38.7)	55.6 (17.3)	16.6 (42.9)
160		150 (28.1)	47.8 (45.4)
400			
1000			
	AgNP-PVP		
25.6	115 (25.6)	19.8 (27.9)	6.51 (n.a.)*
64	204 (11.7)	61.0 (11.5)	7.56 (n.a.)
160	192 (38.7)	82.7 (30.3)	8.14 (10.1)
400	430 (18.1)	184 (10.4)	-
1000	892 (1.21)	182 (22.0)	23.6 (n.a.)
2500			
	AgNP-Cit		
25.6	38.5 (6.21)	47.3 (35.4)	6.58 (12.5)
64	191 (20.2)	104 (39.6)	4.84 (2.21)

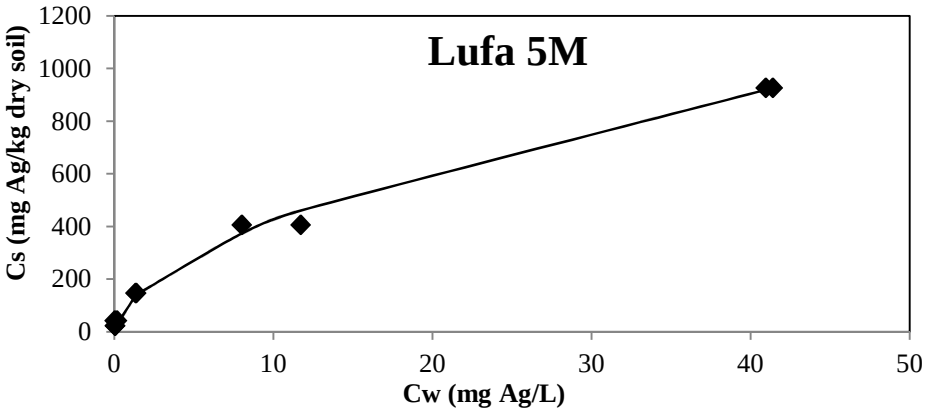
*n.a.=not avaliable



(a)

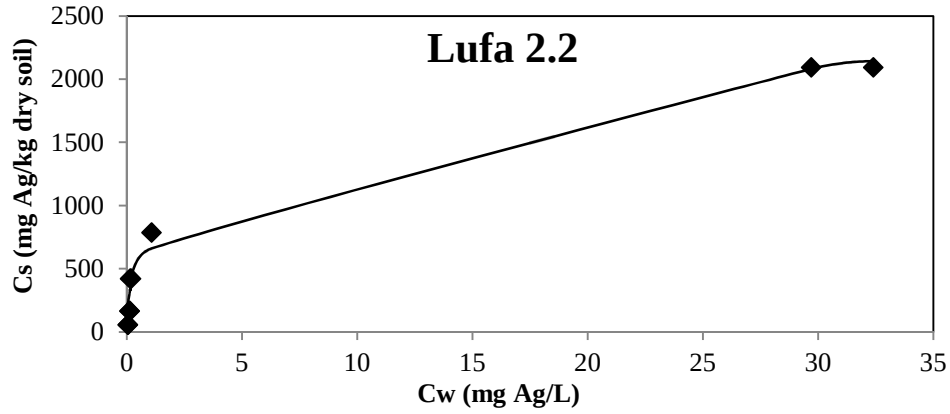


(b)

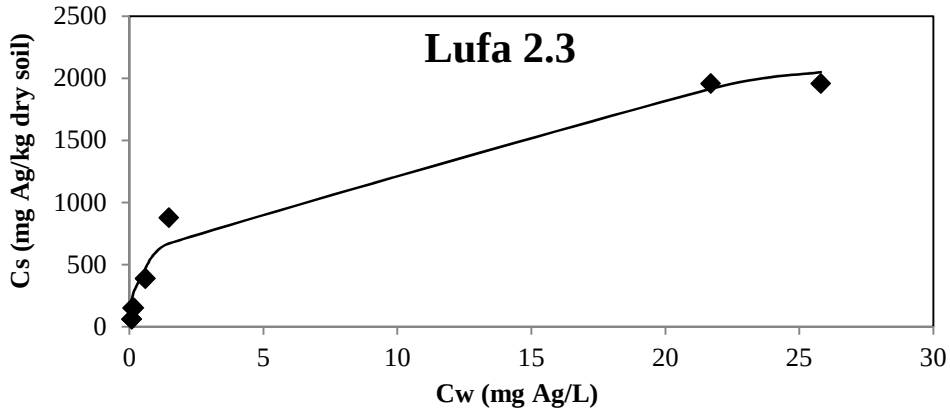


(c)

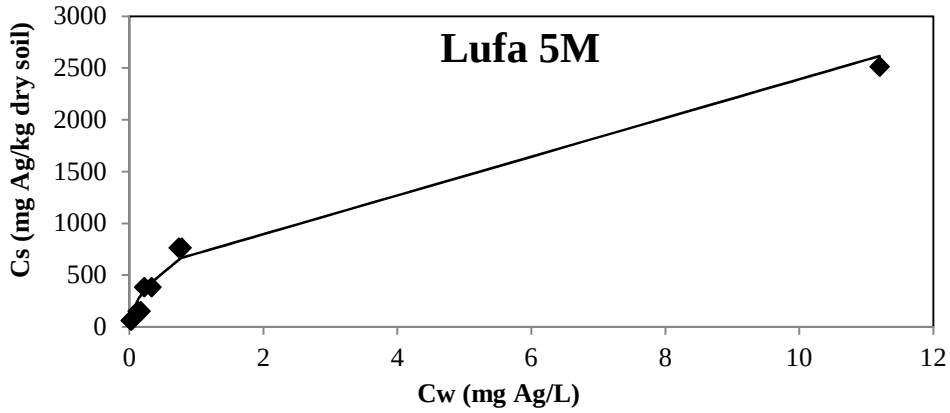
Figure D.1: The relation between total Ag concentration in soil and the concentration in pore water of a) Lufa 2.2, b) Lufa 2.3, and c) Lufa 5M soils spiked with $AgNO_3$. Data points show the measured concentrations. Solid lines represent the fit of a Freundlich isotherm to the data.



(a)

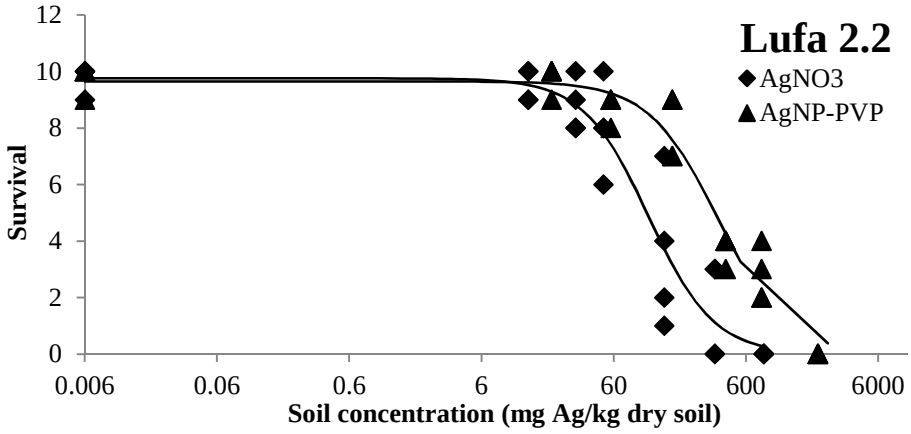


(b)

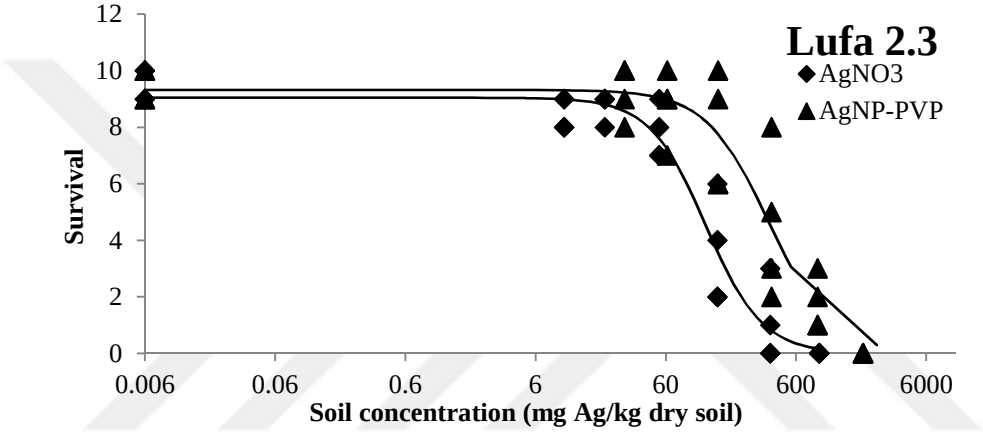


(c)

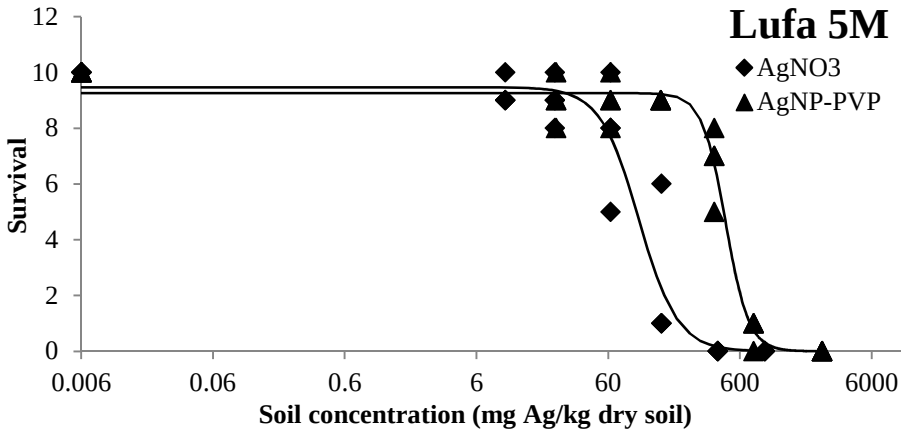
Figure D.2: The relation between total Ag concentration in soil and the concentration in pore water of a) Lufa 2.2, b) Lufa 2.3, and c) Lufa 5M soils spiked with AgNP-PVP. Data points show the measured concentrations. Solid lines represent the fit of a Langmuir isotherm to the data.



(a)

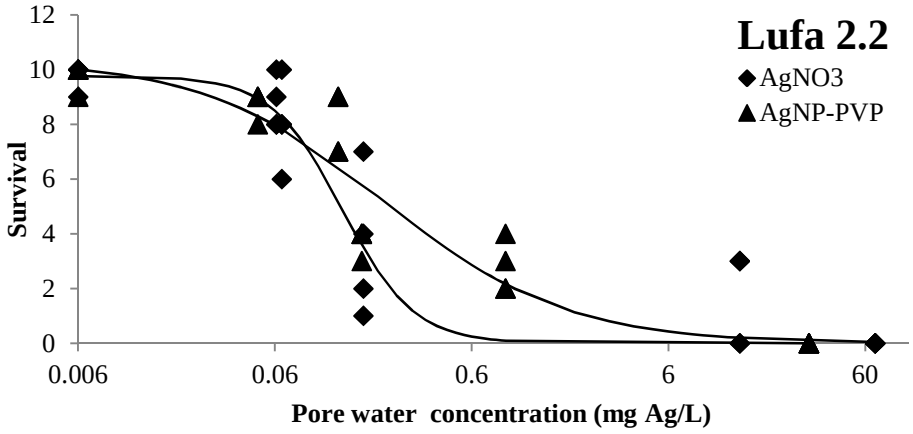


(b)

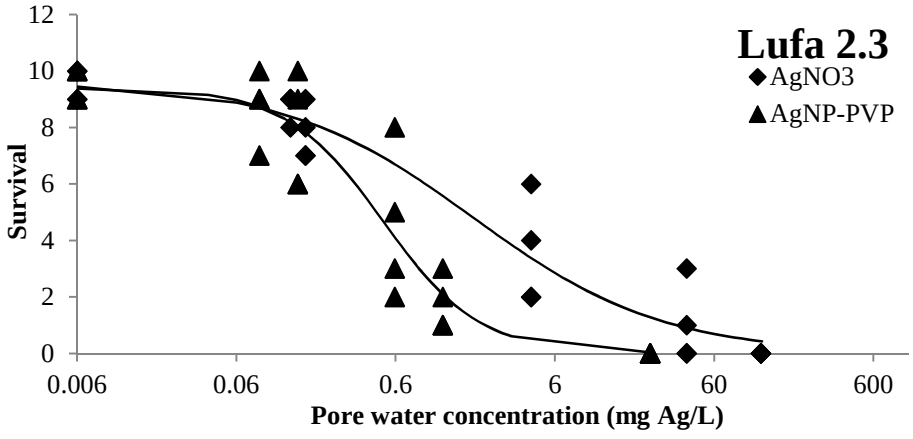


(c)

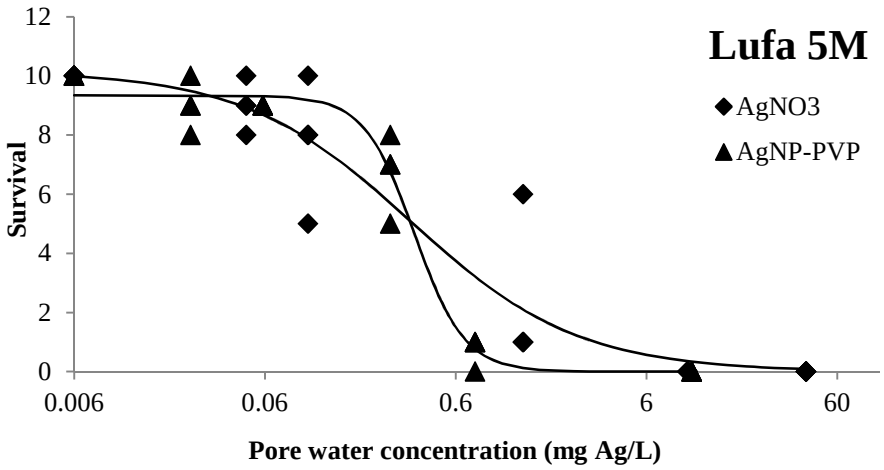
Figure D.3: Comparison of dose-response curves for the effect of on $AgNO_3$ and AgNP-PVP the survival of *Enchytraeus crypticus* exposed for 21 days in a) Lufa 2.2, b) Lufa 2.3, and c) Lufa 5M soils. Effects are related to total Ag concentration in the test soils. Data points show survival (out of 10 individuals) in the four replicates of each concentration. Solid lines show the fit of a 3-parameter logistic dose-response model.



(a)

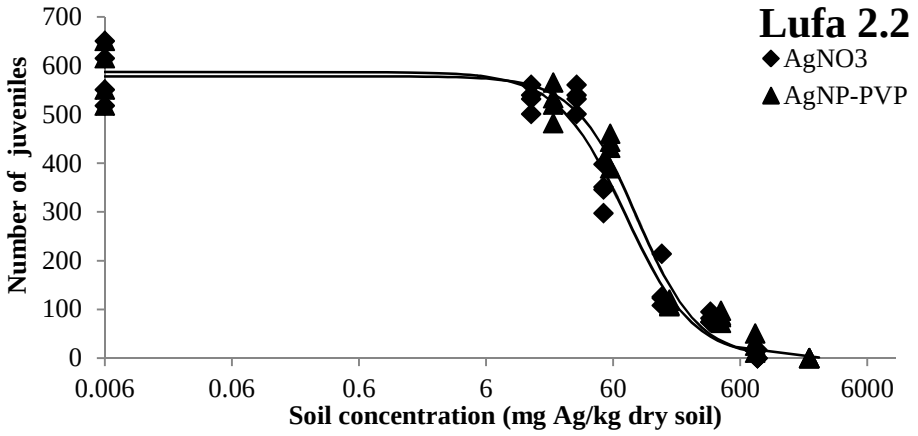


(b)

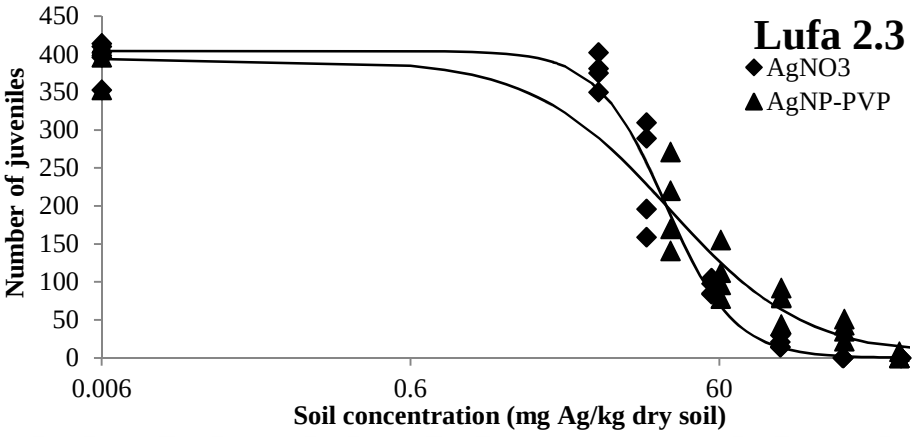


(c)

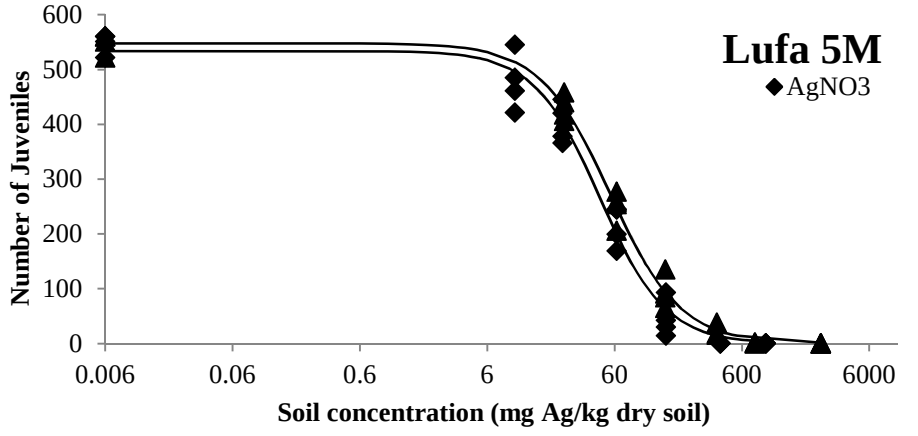
Figure D.4: Comparison of dose-response curves for the effect of $AgNO_3$ and AgNP-PVP on the survival of *Enchytraeus crypticus* exposed for 21 days in a) Lufa 2.2, b) Lufa 2.3, and c) Lufa 5M soils. Effects are related to porewater Ag concentrations measured at the end of the exposures. Data points show survival (out of 10 individuals) in the four replicates of each concentration. Solid lines show the fit of a 3-parameter logistic dose-response model.



(a)

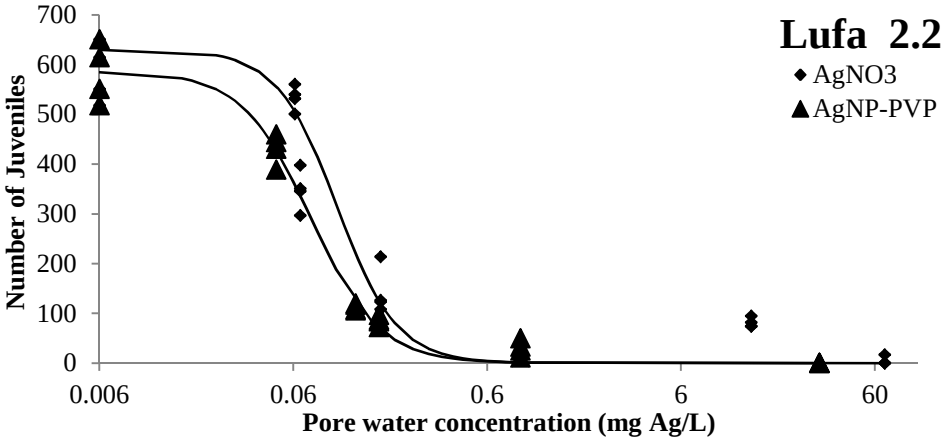


(b)

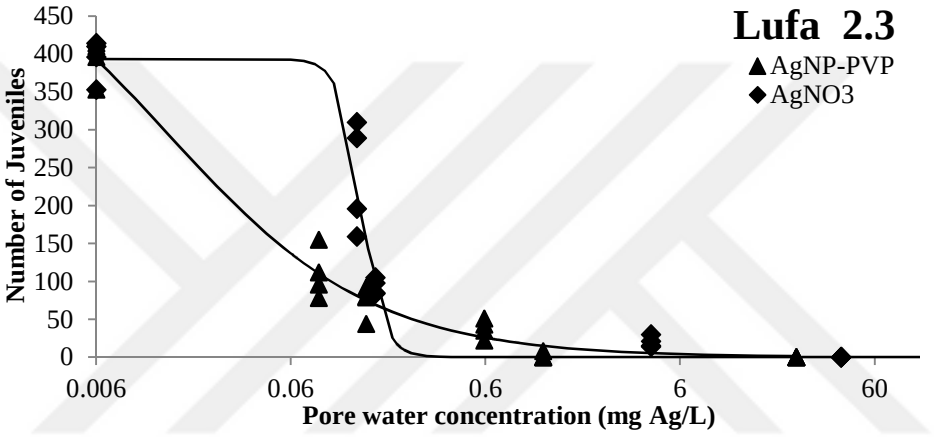


(c)

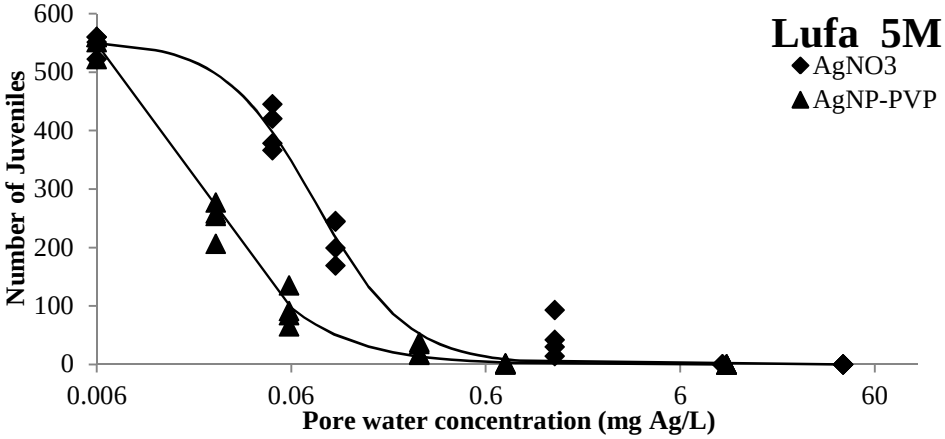
Figure D.5: Comparison of dose-response curves for the effect of $AgNO_3$ and AgNP-PVP on the reproduction of *Enchytraeus crypticus* exposed for 21 days in a) Lufa 2.2, b) Lufa 2.3, and c) Lufa 5M soils. Effects are related to total Ag concentration in the test soils. Data points show the number of juveniles produced in the four replicates for each concentration. Solid lines show the fit of a 3-parameter logistic dose-response model



(a)

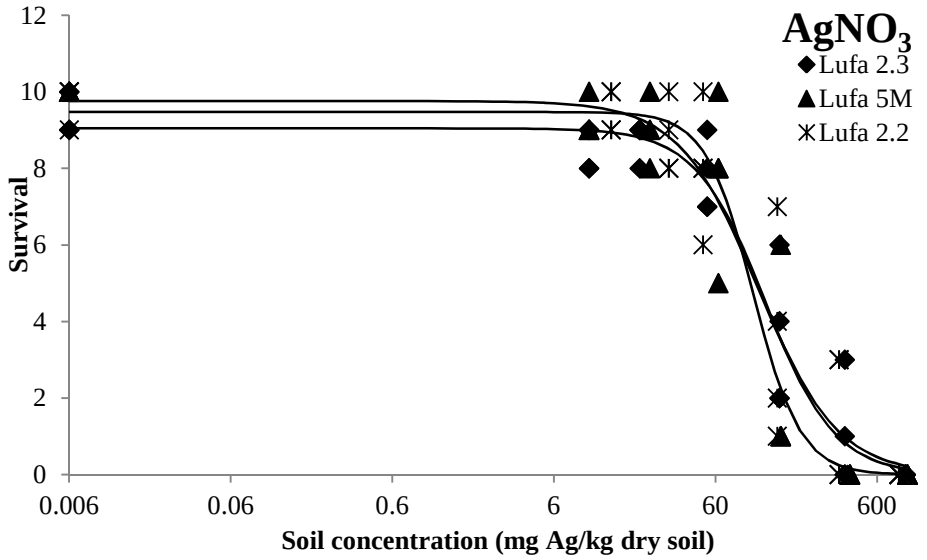


(b)

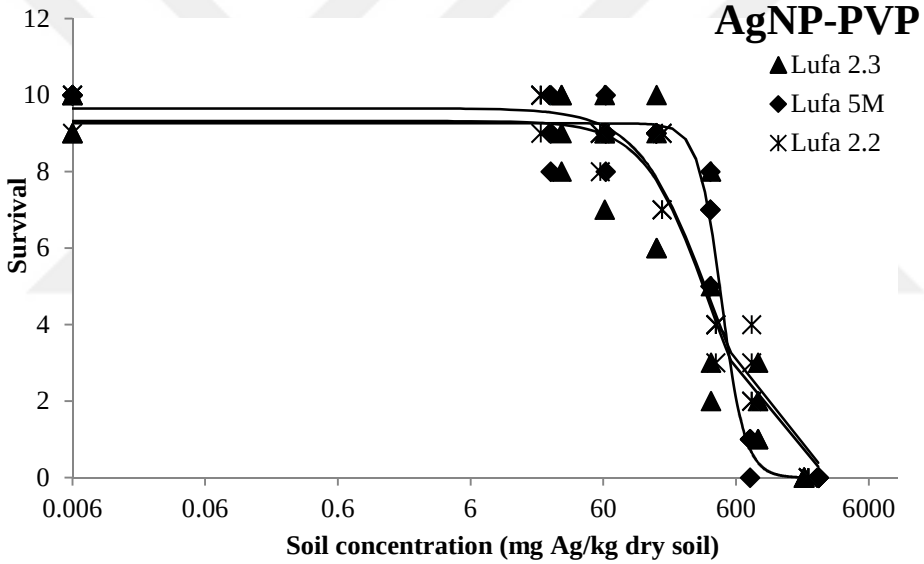


(c)

Figure D.6: Comparison of dose-response curves for effect of $AgNO_3$ and AgNP-PVP on the reproduction of *Enchytraeus crypticus* exposed for 21 days in a) Lufa 2.2, b) Lufa 2.3, and c) Lufa 5M soils. Effects are related to porewater Ag concentrations measured at the end of the exposures. Data points show number of juveniles produced in the four replicates of each concentration. Solid lines show the fit of a 3-parameter logistic dose-response model.

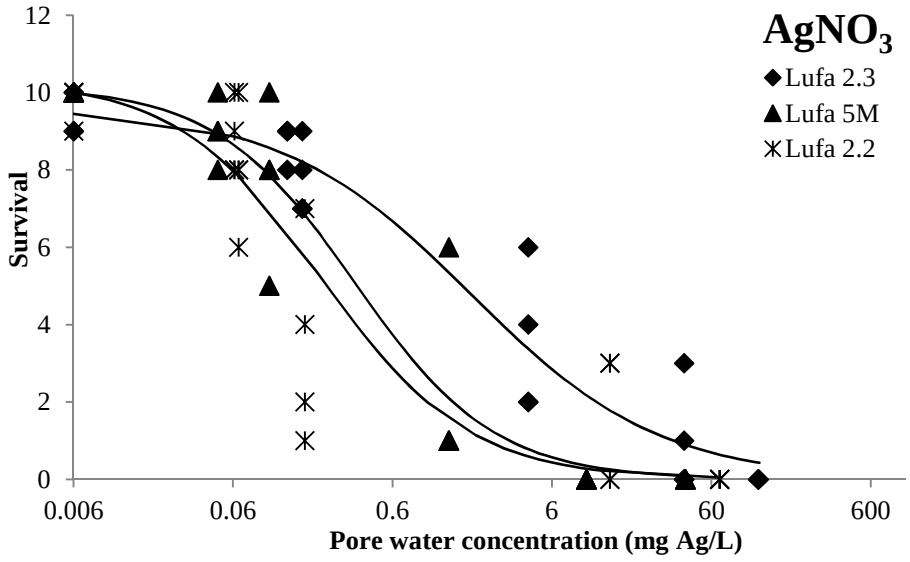


(a)

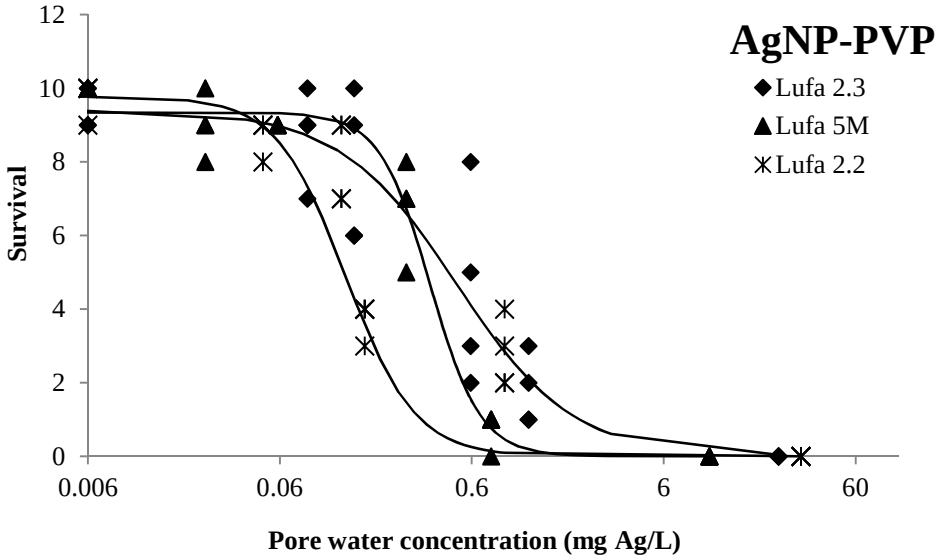


(b)

Figure D.7: Effects of a) $AgNO_3$ and b) AgNP-PVP on the survival of *Enchytraeus crypticus* exposed for 21 days in Lufa 2.2, Lufa 2.3 and Lufa 5M soils. Data points show survival (out of 10 individuals) in the four replicates for each concentration. Solid lines show the fit to the data of a 3-parameter logistic dose-response model



(a)



(b)

Figure D.8: Effects of a) $AgNO_3$ and b) AgNP-PVP on the survival of *Enchytraeus crypticus* after 21 days exposure in Lufa 2.2, Lufa 2.3 and Lufa 5M soils, related to porewater concentrations measured in the soils at the end of the exposures. Data points show survival (out of 10 individuals) in the four replicates for each test concentration. Solid line shows the fit to the data of a 3-parameter logistic dose-response model

References

LUFA-Speyer, Germany, 2009. www.lufa-speyer.de/

Gomes SIL, Amadeu AMVM, Soares AMVM, Scott-Fordsmand JJ, Amorim MJB. 2013. Mechanisms of response to silver nanoparticles on *Enchytraeus albidus* (Oligochaeta): Survival, reproduction and gene expression profile. J Haz Mat 254-255: 336-344.

Heckmann LH, Hovgaard MB, Sutherland DS, Autrup H, Besenbacher F, Scott-Fordsmand JJ. 2011. Limit-test toxicity screening of selected inorganic nanoparticles to the earthworm *Eisenia fetida*. Ecotoxicology 20:226-233.

Kwak JI, Lee WM, Kim SW, An YJ. 2014. Interaction of citrate-coated silver nanoparticles with earthworm coelomic fluid and related cytotoxicity in *Eisenia andrei*. J Appl Toxicol.

Schlich K, Klawonn T, Terytze K, Hund-Rinke K. 2013. Effects of silver nanoparticles and silver nitrate in the earthworm reproduction test. Environ Toxicol Chem 32:181–188.

Shoults-Wilson WA, Zhurbich OI, McNear DH, Tsyusko OV, Bertsch PM, Unrine JM. 2011b. Evidence for avoidance of Ag nanoparticles by earthworms (*Eisenia fetida*). Ecotoxicology 20: 385–396.

CHAPTER 7
AN APPROACH FOR ENVIRONMENTAL RISK ASSESSMENT OF ENGINEERED NANOMATERIALS USING ANALYTICAL HIERARCHY PROCESS (AHP) AND FUZZY INFERENCE RULES

The usage of Engineered Nanoparticles (ENPs) in consumer products is relatively new and there is a need to conduct environmental risk assessment (ERA) to evaluate their impacts on the environment. However, alternative approaches is required for ERA of ENPs because of the huge gap in data and knowledge compared to conventional pollutants and their unique properties that make it difficult to apply existing approaches. This study aims to propose an ERA approach for ENPs by integrating Analytic Hierarchy Process (AHP) and fuzzy inference model which provide a systematic evaluation of risk factors and reducing uncertainty about the data and information, respectively. Risk is assumed to be the combinantion of occurrence likelihood, exposure potential and toxic effects in the environment. A hierarchy was established to evaluate the sub factors of these components. Evaluation was made with fuzzy numbers to reduce uncertainty and incorporate the expert judgements. Overall score of each component was combined with fuzzy inference rules by using expert judgements. Proposed approach reports the risk class and its membership degree such as Minor (0.7). Therefore, results are precise and helpful to determine the risk management strategies. Moreover, priority weights calculated by comparing the risk factors based on their importance for the risk enable user to understand which factor is effective on the risk. Proposed approach was applied for Ag (two nanoparticle with different coating) and TiO_2 nanoparticles for different case studies. Results verified the proposed benefits of the approach.

7.1 Introduction

Nanomaterials are defined by the European Commission as “natural, incidental or manufactured materials 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 nm-100 nm” (EU 2011). Since about one decade, the usage of nanomaterials has been offering more and more promising applications for a wide category of products including cosmetics, electronics, building materials etc. (Nanotechproject, 2013). According to the nanotech project (Nanotechproject, 2013), Ag and TiO_2 nanoparticles are first and second, respectively, in the product inventory based on the number of products. (Royce et al., 2014) reported that AgNPs were used in 410 consumer products in 2014 with an almost 20 fold increase in 8 years. While AgNPs are mainly used for antimicrobial purposes, TiO_2 NPs are used for the antimicrobial, self-cleaning, water repellent, moisture absorbent, and UV protection purposes (BMBF 2011). (Piccinno et al., 2012) reported the usage and the distribution based on product category for Ag and TiO_2 nanoparticles as follows:

- Production amount is lower for Ag (<10 ton/year) than for TiO_2 (10000 ton/year).
- Most of the Ag and TiO_2 were used in Switzerland (3.1 and 435 ton/year, respectively).
- AgNPs were mostly used in textiles (30-50%) and $Ti0_2$ NPs in cosmetics (70-80%).

Although the products containing these nanomaterials were a reasonable choice at the beginning, the consumers became concerned about the usage of these products because of the many debates raised by environmental and human health scientists (Benn et al., 2010). ENPs can be released during the production, manufacturing processes of the products they are used in and during the usage of these products in matrix-bound, single or dispensed forms (Gottschalk and Nowack, 2009; Brar et al., 2010). Keller and Lazareva (2013) reported that 66,000 tons of ENPs end up in freshwater every year. Recent studies showed that nanoparticles may be released from consumer products such as antibacterial agents in textiles to washing (detergent) solutions and surface water (Benn and Westerhoff, 2008; Geranio et al., 2009; Windler et al., 2012; von Goetz et al., 2013; Hedberg et al., 2014), from biocidal plastics and textiles to surface water and agricultural land through the wastewater treatment plants (Blaser et al., 2008), from fabric and cleaning toys for children to sweat and tap water

(Quadros et al., 2013), and from sunscreens to freshwater due to swimming activities (Gondikas et al., 2014). However, local variations in their usage lead to uncertainties in the predictions of the amount of ENPs that end up in wastewater treatment plant (WWTP) effluent and biosolids (Lazareva and Keller, 2014). The amount of money that the countries allocate for research and development and income levels (Inequality-Adjusted Human Development Index-IHDI) can be a good indicator of the ENP production and the usage of ENP products, respectively. In addition to ENP production and usage, release of ENPs can be influenced by the fraction of wastewater that goes to WWTPs and the technologies applied for water purification because of the critical processes that occurs in WWTPs such as dissolution, sorption to organic matter and sulfidation (Lazareva and Keller, 2014). Released ENPs end up in receiving environmental bodies via direct and indirect pathways. ENPs in biosolids can end up on land via the application to the land, disposal in landfills and incineration.

The dramatic increase in the usage of ENPs in consumer products and concerns raised by release studies about the occurrence of ENPs in the environment require assessment of their environmental impact. ENPs start to be included in regulatory guidelines where environmental risk assessment needs are mentioned. According to BMBF (2011), the EU Biocidal Products Directive 528/2012 is the first regulation that mentions the new EU definition of nanomaterials. It is required to separately evaluate the risks of biocidal products including nanomaterials to human, animal and environment health and to label the biocidal products to contain nanomaterials (BMBF 2011). More discussions are going on about whether there is a need and if so, how to include ENPs in different regulatory frameworks (Justo-Hanani and Dayan, 2015). The risk assessment of ENPs is however, still receiving little attention in the literature (Museljic and Olsen, 2014).

7.2 Environmental Risk Assessment for ENPs

Risk is defined as the combination of the probability of a hazard to occur and the magnitude of the consequences of this hazard (Wessberg et al., 2007). Achour et al. (2005) describe the characteristics of environmental risk as follows: (i) the probability of the occurrence of an event such as the discharge of hazardous material to the environment, (ii) the probability of the transport of this material in the environment and exposure of organisms, and (iii) the effects caused by the exposure. One of the most common approaches to characterize the risk is taking the ratio (RQ) of the Predicted Exposure Concentration (PEC) and the Predicted No-Effect Concentration (PNEC), which shows that there is risk when RQ>1 (Saouter et al.,

2001). However, this approach is not adequate for ERA of ENPs because of their relatively short history of usage in products, which results in limited and uncertain data.

Most studies agree that there are still many uncertainties and lots of complicated aspects to be addressed, making it impossible to proceed with a robust ERA (Miseljic and Olsen, 2014, Schauman et al., 2014, Gajewicz, et al., 2012, Gotschalk et al., 2011, Grieger et al., 2011, Wiesner and Bottero, 2011, Sorensen et al., 2010). Moreover, the PEC/PNEC approach does not give precise information about the level of risk and is not sufficiently informative for risk management strategies. Therefore, researchers are encouraged to propose different risk characterization methods for use in risk assessment studies (Miseljic and Olsen, 2014, Schauman et al., 2014, Gajewicz, et al., 2012, Gotschalk et al., 2011) and to identify urgent research needs (Linkov et al., 2009). Although different methods have been proposed, they are lacking either in providing a holistic approach that considers all aspects related with risk or in quantitative and informative risk characterization.

Worst-case definition models are one of the alternative approaches applied for ERA, however, risk characterization was not quantitative which could hamper risk communication with stakeholders (Kühnel et al., 2014). Grieger et al. (2011) identified protected units (PU) and causes of risk (CR), gave priority scores to PU and PU-CR relations (1-3, from low to high) and afterwards they determined ‘worst-case scenarios’ which they gave a score of 3. Their PU-CR diagrams are very detailed to include all possible combinations for each ecosystem component and the modes of action of ENPs. However, developing more diagrams, which represent the release and fate of ENPs, and incorporating to PU-CR relations would provide a more holistic approach for determining worst case scenarios. Sorensen et al. (2010) suggested a Worst Case Definition Model to describe risk-contributing factors, which aids to handle uncertainty by applying knowledge mapping principles. After defining Worst Case Composition (WCC) using Protection Specification (PS) and Cause of Risk Decomposition (CRD) models, they employed a worst case positioning model which aims at reducing the uncertainties of the WCC model.

Quantitative risk characterization was applied in a few studies. O’Brien and Cummins et al. (2011) proposed a risk assessment framework for metallic nanomaterials based on aquatic exposure and behavior. They developed semi-quantitative exposure assessment frameworks consisting of 3 steps, which are surface water quantities, aquatic behavior and material comparison/ranking. Their results consist of likelihood ratios for aquatic exposures; however, toxicological threshold limits were not included for the overall risk estimation. Money et al.

(2012) established a baseline model called FINE with expert commentary, which has a Bayesian nature. The model has 4 basic components for particle behavior, exposure potential, general organism hazard and risk. Experts assign probability functions to the factors of compartments and after Bayesian calculations, risk quotients are estimated by using the no observed effect concentrations (sediment and water). Although expert judgements were incorporated into the model with probabilistic approaches, risk characterization would also make use of expert interferences. Moreover, for estimating the exposure potential, a separate component would be provided to analyze release of ENPs from products which is also an essential component of environmental risk.

The uncertain nature of ERA for ENPs, its dependence on multiple and complex factors and the need for informative quantified risk characterization have triggered us to propose an approach which may compensate for the limitations listed for the existing approaches. According to a literature review of LCA studies (Miseljic and Olsen, 2014), future studies should focus on characterizing the impact of ENPs including their release, fate and toxic effects on ecosystems. Therefore, the aim of this study is to propose an approach for the ERA of ENPs, including their release from products (likelihood of occurrence), fate in the environment (exposure potential) and toxic effects (strength of the hazard), that provides a holistic approach and quantified risk characterization with efficient risk communication.

Decision making tools are also promising methods to use in the ERA for ENPs (Linkov et al., 2009, Godwin et al., 2015, Justo-Hanani and Dayan, 2015). AHP (Analytical Hierarchy Process), a multi criteria decision making tool, can be used to solve a problem by decomposing it into related factors in a hierarchy, evaluating the factors in terms of contribution to the risk and analyzing the priorities of these factors (Saaty, 1994). Therefore, AHP may identify the factors related with the environmental risk of ENPs and define the relation between these factors. This may lead to a holistic approach for ERA. Not only the evaluation of the factors that contribute to the risk, but also the combination of the main factors should make use of expert judgements to compensate for the uncertain and limited data about the release, fate and effects of ENPs in the environment. Expert judgement can be incorporated into risk assessment frameworks by using fuzzy scales and fuzzy inference rules (Zhen et al., 2007).

7.3 Proposed Approach for the ERA of ENPs

Based on the above, our proposed approach has three major aims: i) to identify in a systematic manner indicator sub-factors for the likelihood of the occurrence of ENPs in the environment, their exposure potential and the toxic effects which are contributing to their risk, ii) to evaluate each factor in terms of its contribution to the risk and to determine priority weights for these factors using expert judgements which can compensate for the uncertainty in knowledge and for vague data, iii) to combine all factors, using expert judgement, for risk characterization to estimate risk magnitude and risk class by using a fuzzy inference rule base. The approach that was proposed previously by Topuz et al. (2011) to conduct ERA for industrial hazardous materials is quite convenient for our purpose and so may meet the requirements of the ERA for ENPs. They integrated two different methodologies, namely, Analytical Hierarchy Process (AHP) and fuzzy inference system and adapted the usage of fuzzy numbers for the scoring of factors. AHP is able to take into account all the factors within a hierarchic style, which enables to arrange them systematically and to elucidate their contribution to the environmental risks with priority weights. Moreover, expert judgement can easily be incorporated into such an ERA approach, owing to the scoring of the factors in a hierarchy. Scoring the factors with fuzzy numbers has the advantage of reducing uncertainty, since fuzzy numbers are used to convert linguistic variables of expert judgements into quantitative scores providing the flexibility of membership functions to this process. The fuzzy inference system, with "if…then…" rules, gives the opportunity of combining risk components with expert judgement instead of formulating several risk factors into one equation and relying on several assumptions to estimate a risk magnitude.

The proposed approach, which is adapted from Topuz et al. (2011), is outlined in Figure 7.1. There are 3 basic steps, including the preliminary, scoring and fuzzy inference. Before proceeding with these steps, an expert group, which is composed of different disciplines related with the risk factors proposed in the hierarchy, should be established. Taking the risk factors into consideration, research and development engineers for ENPs and ENP-based products, environmental engineers, ecotoxicologists, biologists and chemists could be included in the expert group. The mission of this group is to run the proposed framework from the beginning to the end to obtain the risk magnitude.

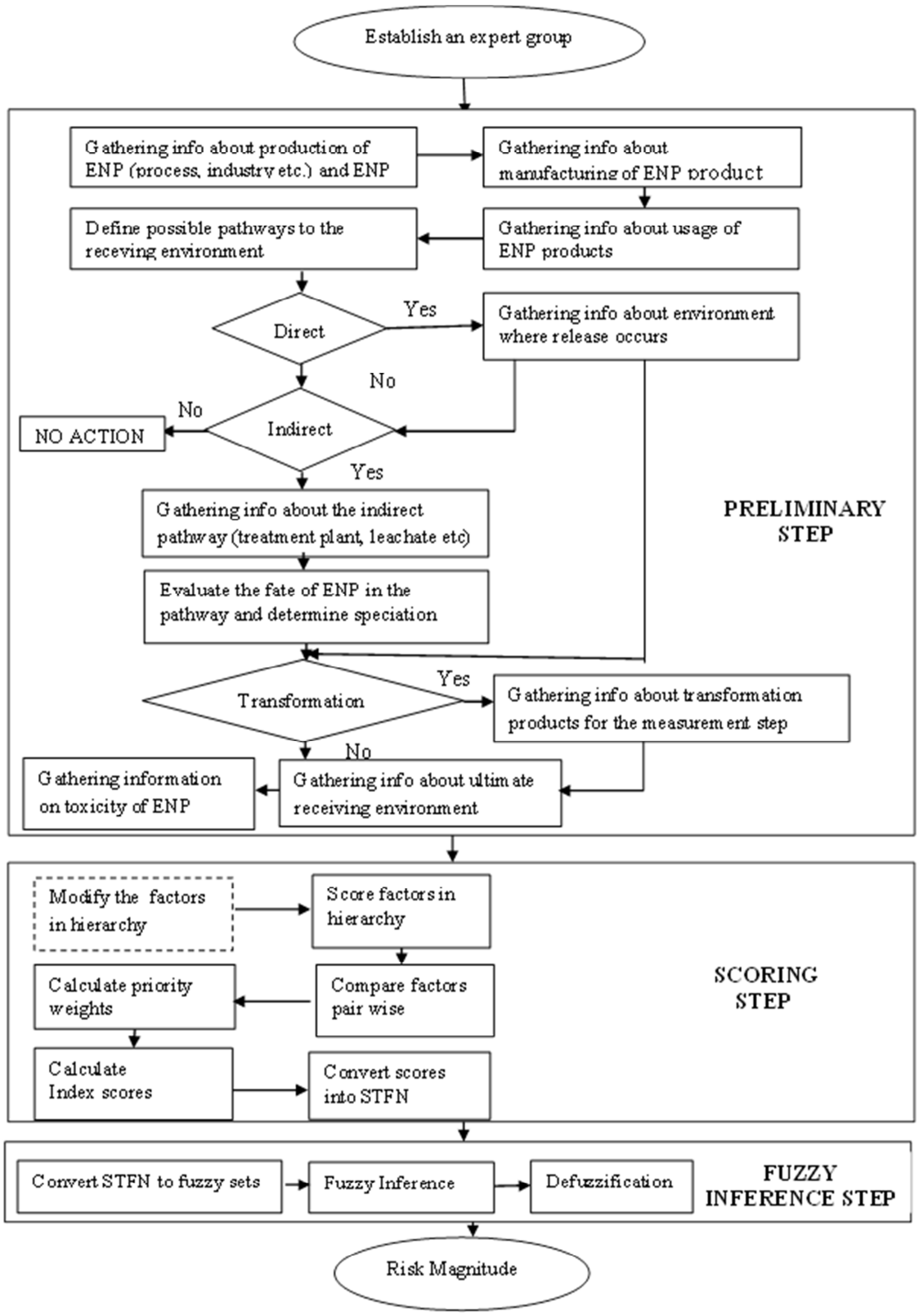


Figure 7. 1: Proposed environmental risk assessment approach for engineered nanomaterials. STFN means Standard Trapezoidal Fuzzy Numbers.

7.3.1 Preliminary step

The preliminary step starts with gathering data and information on the production of ENPs, manufacturing processes of ENP products and the usage purposes and habits for these products. The expert group should decide which kind of information and data are required for an accurate assessment before proceeding with gathering data. There are two milestones in the preliminary step, which are to determine the possible release pathways to the environment and to estimate the possible transformation products that could occur in each pathway. First, all data about the production of ENPs, manufacturing of ENP products, their usage purposes and the usage habits of the consumers have to be evaluated critically by the experts to get consensus on the release of ENPs. The life-cycle of the product has to be considered from production to the end-of life to address all possible release pathways. Afterwards, the experts should categorize the release pathways as direct and indirect and follow the instructions given in the framework.

Direct release means that the ENP product is being used directly in the environmental receiving medium and the release happens directly in the environment, such as TiO_2 NPs release from sunscreens during swimming in the sea or freshwater. If the release is direct, the data gathering step has to be extended to about the environment where the release occurs. Indirect release implies that ENPs pass through a pathway (treatment plants, agricultural lands etc.) before ending up in the ultimate receiving environmental medium. For instance, AgNPs released from textile washing and passing through the municipal sewage collectors and WWTP before being discharged/disposed to the receiving environmental body. If the release is indirect, information about the release pathways to the receiving environment has to be gathered. Especially, data concerning the speciation of ENPs is highly crucial to address the forms of ENPs and in which environmental compartments they will end up. Upon completion of the data, the second milestone question has to be answered. If ENPs are transformed into other products, data are needed about these transformation products. The preliminary step is ended with gathering data about the possible environmental compartments to which the ENP will be transported and the ecotoxicological data for the ENP and its transformation products.

7.3.2 Scoring step

In Figure 7.2, a basic hierarchy is proposed for the ERA of ENPs. The calculation steps of the methodology are quite flexible, which enables users to modify this hierarchy depending on their case and the information they gathered during the preliminary step. The first level of the

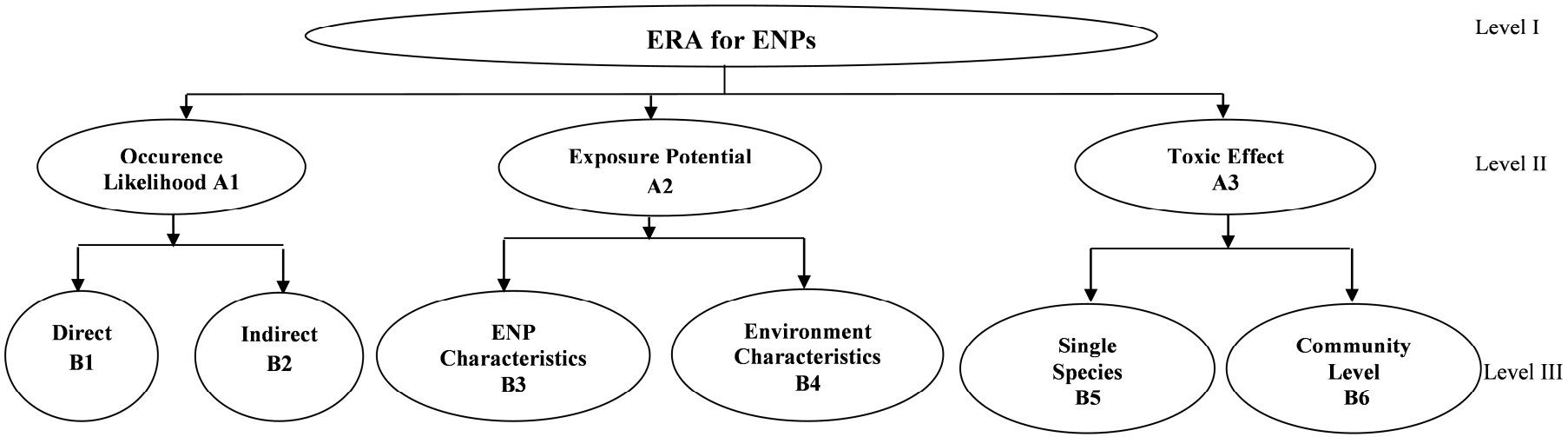


Figure 7. 2: Overall hierarchy for the environmental risk assessment of engineered nanoparticles including the 3 main indices (A1, A2 and A3) and their sub-factors.

hierarchy reveals the purpose, which for this study is the ERA of ENPs. The second level addresses the main factors that are composing the possible environmental risk of ENPs. If there is likelihood for ENPs to reach environmental bodies, there could be exposure potential for the inhabiting organisms. And if ENPs are toxic to single species or the community, then environmental risk is possible. Therefore, ERA for ENPs definitely requires 3 indices: likelihood of occurrence (OL) in the environment due to their release from products, the exposurepotential (EP) of environmental components and the toxic effects (TE) exerted on these components. The sublevels of the hierarchy are constructed taking into account the principle of placing factors that are comparable in a logic way and at the same level.

For OL (Figure 7.3), the key questions are in which form and at what amount ENPs will arrive in the environment. Transformation of ENPs may take place in the medium they pass before ending up in the environment (Ma et al., 2014, Levard et al., 2013, Gottschalk and Nowack et al., 2011, Kaegi et al., 2011 and Lowry et al., 2011), so the environmental medium may receive either the intact ENP or its transformation products. Therefore, `direct` (ENP directly released in the environmental body) and `indirect` (released ENPs end up in environmental body via a pathway) release are the main factors for OL. For both factors, the `frequency` and the `amount` of product usage and the factors related with `release process` are critical to estimate the released concentration and form of the ENP. To clarify the factor of `release process`, the released form and amount of ENP are mostly dependent on the production technology of the ENP (Windler et al. 2012; Quadros et al., 2013) which gives an idea about `product properties` and `medium properties` in which they are released (Gondikas et al., 2014, Al-Kattan et al., 2012, Tejamaya et al., 2012). For example, AgNP based textile may release AgNPs in high or low amounts depending on how strongly they are embedded in the textile (Ben and Westerhoff, 2008) and the dissolved Ag release is higher in the presence of bleaching agents in the washing solution (Geranio et al., 2009). Further sub factors are not given in this study since there are huge numbers of various ENP products, with the properties of their production technology and medium in which they are released varying significantly. Therefore, the users of this approach are highly recommended to adapt their own sub factors to “release process”.

Indirect releases of ENPs to the environmental medium can occur via `treatment plants`, `run off from agriculture` and `leachate from agricultures` depending on the pathway they will take after being released from products. Treatment plants can be categorized into 3 groups since their waste characteristics and treatment technology can be significantly different. `Industrial

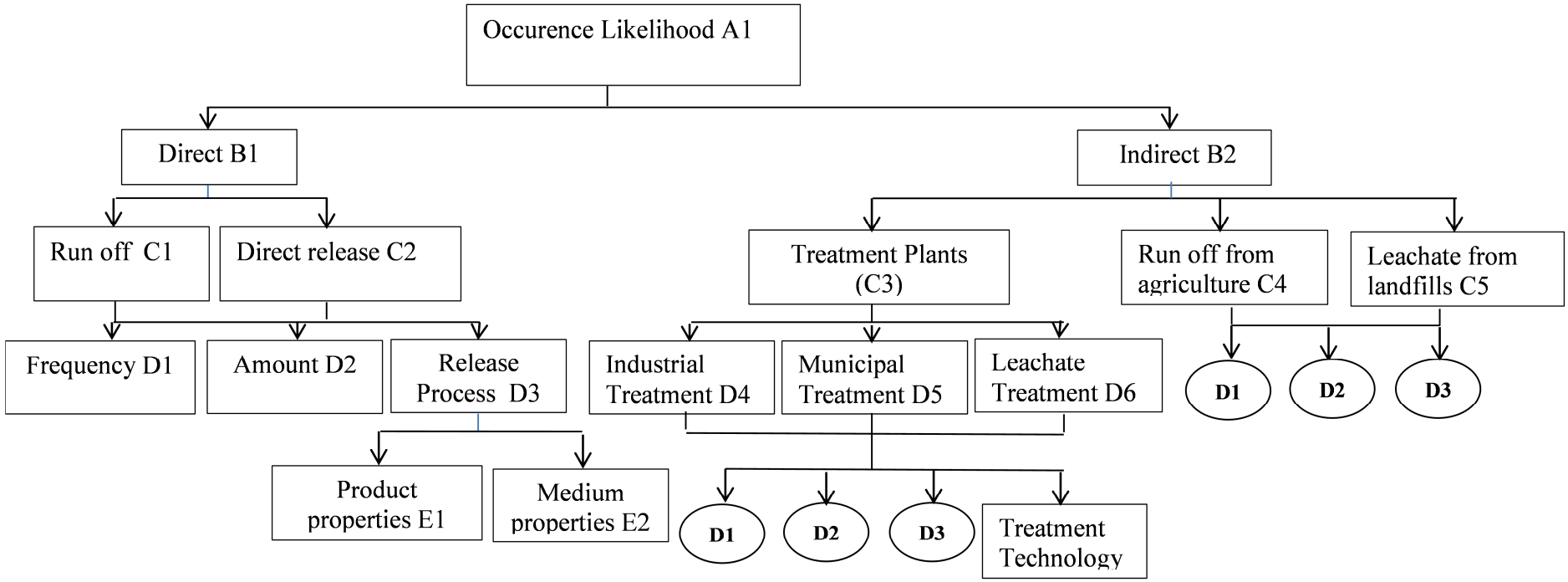


Figure 7. 3: Hierarchy to evaluate sub-factors of the occurrence likelihood in terms of their contribution to the risk of ENPs.

treatment plants` can receive ENP residues from the process units. Most of the ENPs are used in daily life products and they end up in `municipal wastewater treatment plants` (Barton et al., 2015, Lazareva et al., 2014, Keller et al., 2013, Westerhoff et al., 2011). ENP products or wastewater treatment sludge that contain ENPs can be disposed to landfills at the end of their life (Keller et al., 2014), therefore ENPs may also be transported to the `landfill leachate treatment plants` through leachate collection networks. Although leachate is collected in landfills, soil and aquatic media close to the landfill area have the possibility to receive ENPs with `leachate from landfills` (Boldrin et al., 2014, Marcoux et al., 2013). It is known that most of the ENPs and their transformation products (TPs) partition in treatment sludge (Kaegi et al., 2011 and Keller et al., 2013), so that usage of treatment sludge for agricultural applications can be a sink of ENPs and its TPs in environmental media (Barton et al., 2015, Kirkegaard et al., 2015 and Hendren et al., 2013). All sub-level factors under the indirect release can be evaluated with `frequency`, `amount` and `release process` factors as they have been explained for the case of `direct release`. In addition to these factors, `treatment technology` also has to be evaluated as a sub-level of `treatment plants` factor, since the treatment processes that have as anaerobic stage may lead to complete sulfidization (Kaegi et al., 2011) and therefore can have great effect on the fate of ENPs .

Environmental compartments into which ENPs will partition, their bioavailability and the exposure potential (EP) of organisms to ENPs are mostly influenced by the processes including agglomeration (Topuz et al., 2015), dissolution (Loosli et al., 2015, Mitrano et al., 2014) and adsorption (Quik et al., 2014, Garner et al., 2014), and by transformation processes (Ma et al., 2014, Kaegi et al., 2011). Fundamentally, these processes are configured by the `ENP characteristics` and Environment characteristics` (Figure 7.4).

Nano-specific characteristics of ENPs greatly influence their transportation/transformation, such as `size` (Albanese et al., 2012, Bokand et al., 2012), `coating` (Topuz et al., 2014) and `surface charge` (Kendall and Holgate, 2012). In addition to the nano-specific characteristics, `dissolution` is critical to evaluate the bioavailability of ENPs in aquatic and soil environments (Loosli et al., 2015, Garner et al., 2014, Mitrano et al., 2014). Homoaggregation and heteroaggregation are expected to be effective in natural water (Topuz et al., 2015 and Quik et al., 2013). `Attachment efficiency` is used to evaluate homoaggregation of ENPs in natural water and can be related to heteroaggregation (Quik et al., 2014).

`Environment Characteristics` are represented by `soil` and `water` compartments (Figure 7.4). Air is not included since the processes in air are different and independent from both these

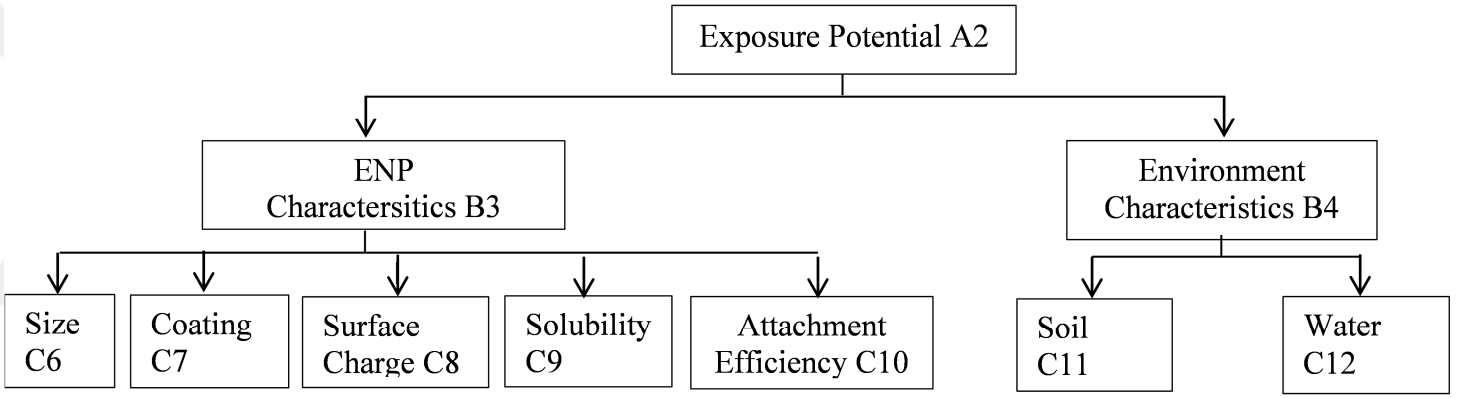


Figure 7. 4: Hierarchy to evaluate sub-factors of the exposure potential in terms of their contribution to the risk of ENPs.

bodies and require significantly different knowledge compared to soil and water. However, users can easily add their own hierarchy for air and include it in the calculations based on the results of the preliminary step. The fate of an ENP in soil is determined by its speciation in `stationary phase` or `pore water` (Figure 7.5), because agglomeration, attachment and dissolution and transformation processes have to be evaluated separately due to the different textures of these media. For the stationary phase crucial factors are `pH` (Fang et al. 2009), `natural organic matter (NOM) content`, WHC` (Vandevert et al., 2014) and 'CEC' for agglomeration, `clay` content (Cornelis et al., 2010) for attachment and `redox potential` (Vandevert et al., 2014) for transformation. Background concentration may impact the fate of ENPs, with some studies proving that the change in their behavior may depend on concentration (Praetorius et al., 2013) and that exposure potential may be enhanced. Pore-water characteristics are similar to the aquatic medium characteristics, `ionic strength`, `pH`, `NOM content` are related with agglomeration (Vandevert et al., 2014), `chlorides ($Cl^-$)` are related with dissolution (Zhang et al., 2015) and `sulfides` are related with transformation processes (Vandevert et al., 2014) and they mostly determine the mobility and exposure potential of ENPs. `Water` bodies are considered in 2 main factors including `water chemistry` and `hydrological properties` (Figure 7.6), which relate to the probable chemical and physical transportation/transformation processes. Water chemistry is highly related to the fate of ENPs in the aquatic environment (Behra et al., 2013). `pH` (Garner and Keller et al., 2010), `SS` (suspended solids) (Quik et al., 2014), `$Ca^{2+}$/$Mg^{2+}$` (Topuz et al., 2014, French et al., 2009, Handy et al., 2008) and `NOM` (Loosli et al., 2013, Fabrega et al., 2009) have influence on the ENP agglomeration/aggregation processes in the aquatic environment. `Dissolved oxygen (DO)` and excessive `$Cl^-$` concentration (Chambers et al., 2014) may affect the dissolution process `Hydrological characteristics` need further sub-levels since they are related to the physical conditions based on the type of water body. `Depth` and `water velocity` are used for `flowing water 'due to their influence on the time of travel for ENPs and in this way, to which extend they can be available for organisms. `Mixing depth`, `dispersion coefficients`, `temperature change` and `discharge point` are necessary to predict the mixing conditions which show the availability of ENPs through the factor `stagnant water`. `Mixing points` in `sea` where they are fed with freshwater are crucial to evaluate the transport of ENPs. In the case of infiltration to `groundwater`, the slope and distance of the aquifer shows the possibility to reach water sources.

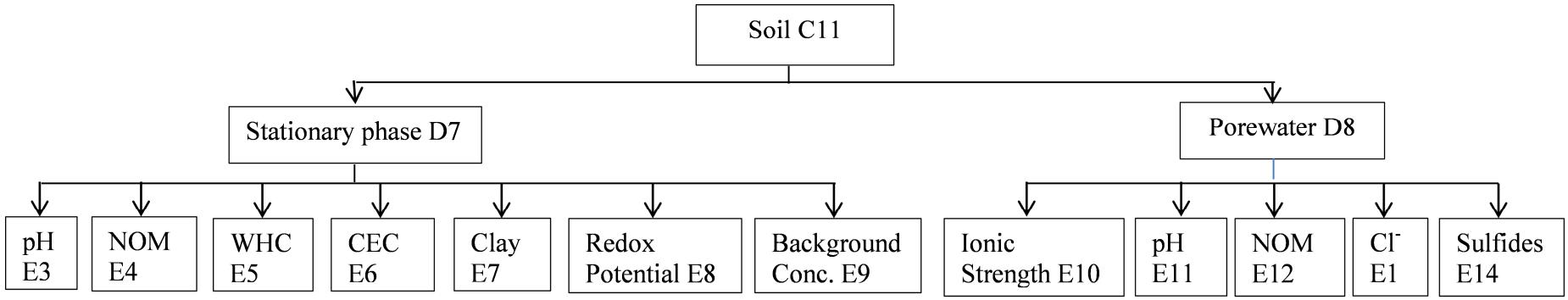


Figure 7. 5: Hierarchy to evaluate sub-factors of the soil in terms of their contribution to the risk of ENPs.

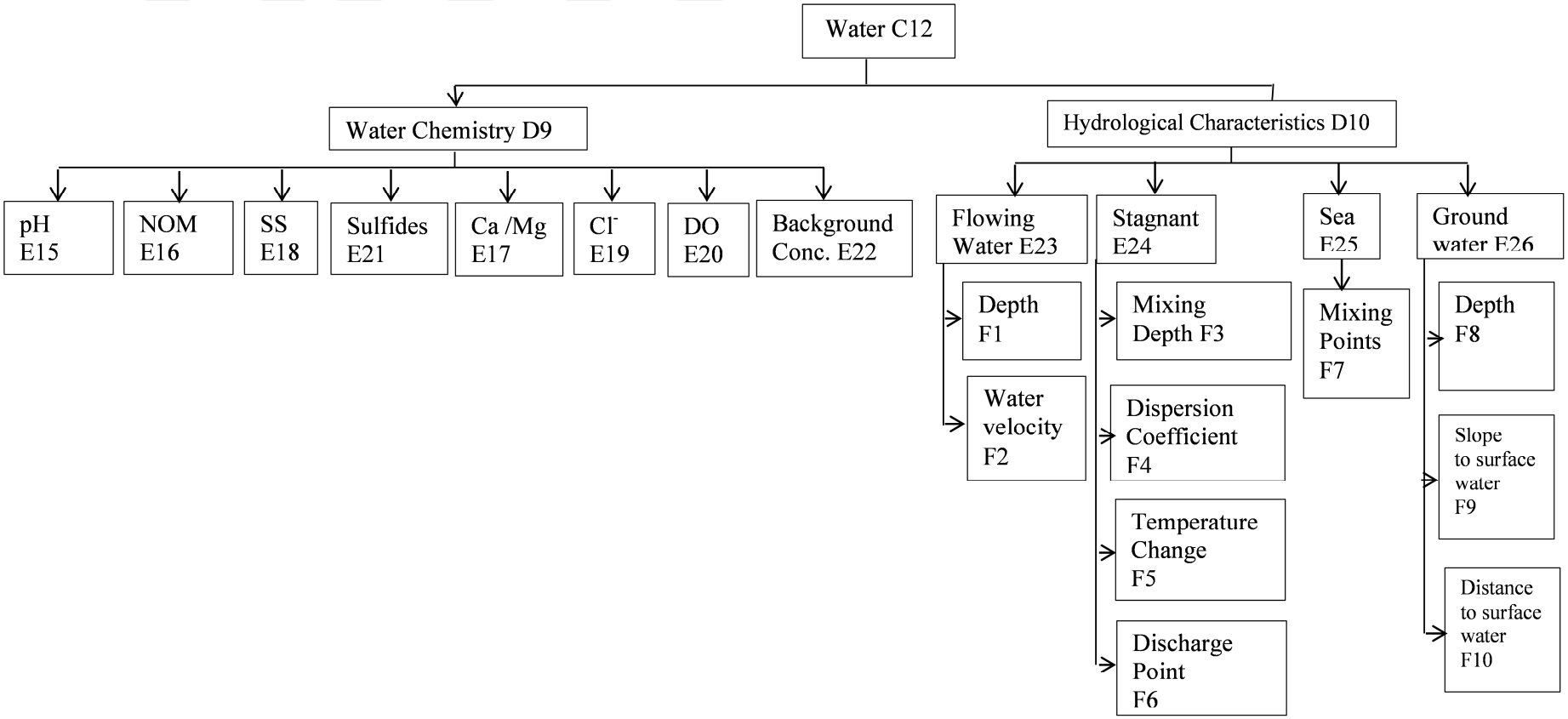


Figure 7. 6: Hierarchy to evaluate sub-factors of the soil in terms of their contribution to the risk of ENPs

`Toxic effect` factors are organized based on the endpoints considering the high variability in sensitivity of the organism levels and the direct relation between the effects and endpoints. The risk magnitude can vary according to the level where the effect is observed: `single species` or `community level` (Figure 7.7). Toxic effects should be considered separately depending on whether they are `acute` or `chronic` for single species. For `community levels`, `functional` and `structural` effects can be significant. Most representative and most commonly known endpoints are placed under these main factors (Figure 7.7).

After revising the hierarchy based on the needs of the case, the calculation steps can be followed from "FI Measurement Step" given in Topuz et al. (2011). For convenience, the calculation steps are also quoted in Appendice E. All factors that are at the bottom level of the hierarchy are scored based on the scale given in Table 7.1, which is helpful for altering the linguistic scores by the consensus of the expert group about the numbers.

Table 7.1: The fuzzy scale used for the scoring of factors at the bottom level of the hierarchy used for assessing the environmental risk of ENPs (OL: occurrence Likelihood, EP: Exposure Potential, TE: Toxic Effects

Definition of OL	**Explanation**	**OL Fuzzy Number**
very high (VH)	Very high contribution to occurrence	(0.0, 0.0, 2.5)
high (H)	Significant contribution to occurrence	(0.0, 2.5, 5.0)
medium (M)	No critical contribution to occurrence	(2.5, 5.0, 7.5)
low (L)	Hardly no contribution to occurrence	(5.0, 7.5, 10.0)
very low (VL)	Exactly no contribution to occurrence	(7.5, 10.0, 10.0)
Definition of EP	**Explanation**	**EP Fuzzy Number**
very low (VL)	Very low probability	(0.0, 0.0, 2.5)
low (L)	Low probability	(0.0, 2.5, 5.0)
medium (M)	There is possibility	(2.5, 5.0, 7.5)
high (H)	There is high possibility	(5.0, 7.5, 10.0)
very high (VH)	Inevitable to occur	(7.5, 10.0, 10.0)
Definition of TE	**Explanation**	**TE Fuzzy Number**
very low (VL)	Uncertain effect	(0.0, 0.0, 2.5)
low (L)	Low effect	(0.0, 2.5, 5.0)
medium (M)	Medium effect	(2.5, 5.0, 7.5)
high (H)	High effect	(5.0, 7.5, 10.0)
very high (VH)	Intensive and inevitable effect	(7.5, 10.0, 10.0)
Definition of RM	**Explanation**	**RM Fuzzy Number**
negligible (N)	Risk can be accepted	(0.0, 0.0, 1.0, 3.0)
minor (Mi)	Risk can be tolerated but precautions must be taken	(1.0, 3.0, 4.0, 6.0)
major (Ma)	Risk must be reduced	(4.0, 6.0, 7.0, 9.0)
critical (C)	Risk cannot be accepted	(7.0, 9.0, 10.0, 10.0)

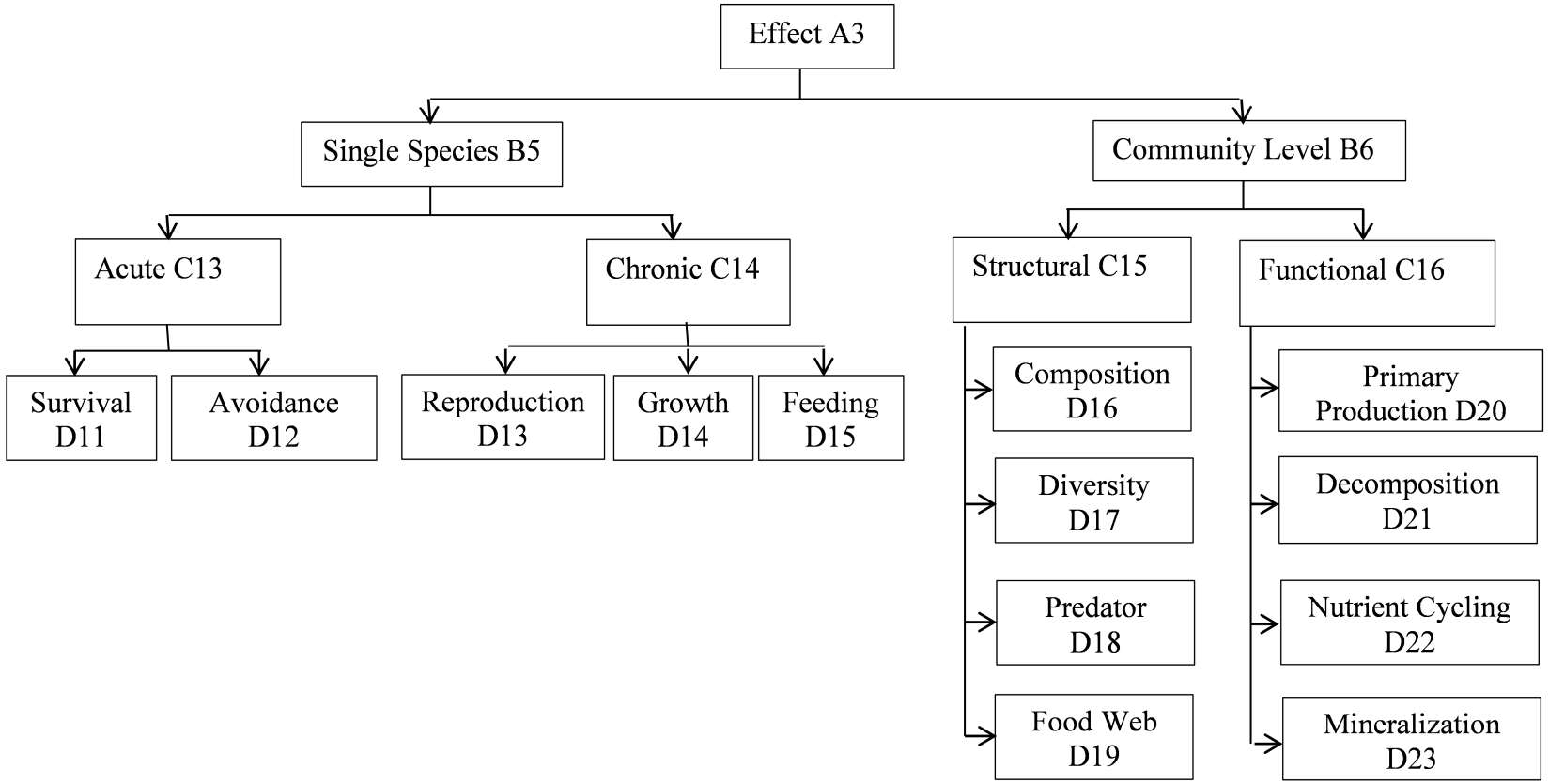


Figure 7. 7: Hierarchy to evaluate sub-factors of the toxic effect in terms of their contribution to the risk of ENP

Then the factors which are at the same level are compared based on their importance for the risk to obtain priority weights. Comparison was made by using Chang's fuzzy scale (see Appendice E) and the priority weights of the factors at their own levels (Equation E(2)) and at the hierarchical level (w') were calculated (Equation E(3)).

Then, index scores for OL, EP and TE were obtained by multiplying the scores of their bottom level factors with their priority weights (w') in the hierarchy (Equation E(4)). The last step was to convert the scores given for sub-levels to Standard Trapezoidal Fuzzy Numbers (STFN) for homogeneity.

7.3.3Fuzzy Inference Step

Risk magnitude was obtained from the if..then… rule base using a fuzzy inference model and expert judgement. All calculation steps are explained in detail in the Supplementary Information and below in Section 7.4.1.

7.4 Implementation of the Proposed Approach

7.4.1 Case Studies I and II: Use of T-shirts with antimicrobial functionality of AgNP

For Case Study I, the scenario was based on the usage of T-shirts, which have antimicrobial property because of the application of AgNP-Cit, in Duebendorf, Switzerland. AgNPs are commonly used in textiles. Our previous studies addressed the agglomeration behavior of AgNPs in surface water and wastewater treatment plants (WWTPs) in this region and there are more studies on the usage of ENPs in Switzerland. Since the usage of ENP products is relatively new, and there is not so much real-case information about the OL, EP or TE for environmental risk, case studies for which data is relatively easily available in the literature may serve as a kind of validation of the proposed risk assessment method. Case study I is summarized in Table 7.2. Case study II assumes that AgNP-PVP is used for the manufacturing of T-shirts, but all other conditions in Case Study I are also valid for Case Study II.

Table 7.2: Summary of the conditions assumed for Case Study I. T-shirts, which contain citrate coated AgNPs (AgNP-Cit) to have antimicrobial properties, are manufactured by a factory and used by people in Duebendorf, Switzerland. AgNP-Cit is also produced by the same company. Case Study II has the same conditions except for the type of AgNP.

General Information				
Production capacity	400,000 pieces/year			
Product Information	83% polyester, 17% wool; 89/64 g textile per T-shirt for male/female, 183±10 mg Ag/kg textile (von Goetz et al., 2013)			
ENP Characteristics				
Surface Coating	Citrate	**Surface Charge**	Negative	
Size	61 nm (Topuz et al., 2014)	**Solubility**	1-12% (Odzak et al., 2014)	
Attachment Efficiency	~$2.5*^{10-2}$ L/mg/day (predicted from Quik et al. 2014)			
Transformation	High tendency to transform into Ag_2S in WWTP, soil			
Environmental Characteristics				
Soil	Standard Lufa 2.2 soil properties (Topuz et al., 2015; Waalewijn-Kool et al., 2014)			
Water	Glatt-Rheinsfelden (Topuz et al., 2015)			
	Depth	498 m	**Length**	41.1 km
	Surface Area	416 m^2	**Flow rate**	8.75 m^3/s
	Water chemistry characterization in Appendince E Table E.1			
Release Characterization				
Release way	Production process, washing solution and end of life disposal			
Usage habits	Bleaching agents, 1 washing/week			
Possible emission sources	AgNP-Cit and T-shirt Manufacturing Industry Treatment Plant, Municipal WWTP, Incineration Plants, Landfill			
WWTP	Duebendorf WWTP (Topuz et al., 2015; Kaegi et al., 2011)			
Landfill characterization	Detailed information in Mueller et al. (2013)			
Receiving bodies	Glatt-Rheinsfelden via WWTP discharges Soil (around Duebendorf) via landfilling of end-of-life T-shirts and of WWTP sludge and bottom ash of incineration plants			
Effect Characterization				
Single Species	Bondarenko et al. (2013)			
Community Level	Lowry et al. (2013)			

Preliminary Step

Required data and information for release pathways, ENP and environmental characteristics and data on the toxicity of ENPs were gathered and are presented in Table 7.2. Although expert evaluations considering ENP transformations were always considered during the detailed scoring of all factors, assumptions were made roughly to have an idea about the mass of Ag to have more robust data and knowledge in mind. Half of the total amount of AgNP-Cit is assumed to end up in landfill facilities (Mueller and Nowack, 2008) due to the end of life

disposal to municipal solid waste. Half of the total Ag was assumed to be sulfidized in the soil environment (Lowry et al., 2013). Approx. 10% of the AgNP-Cit is transported to the landfill leachate treatment plant. Less than 1% will end up in aquatic media via the leachate treatment plant and the rest is sent back to the landfill with treatment sludge. AgNP-Cit is released in washing solution, transported to the municipal WWTP, 90% partitions in the sludge (Barton et al., 2015), effluent is discharged to Glatt-Rheinsfelden and the WWTP sludge is incinerated. It is known that most of the AgNPs are transformed into Ag_2S by sulfidation during the transport in sewage collectors and during wastewater treatment; however, sulfidation may depend on the size, sulfide availability and residence times (Kaegi et al., 2011). Therefore, sulfidation was assumed to be around 75% in WWTP sludge considering the variabilities in WWTPs. In the incineration plant, 90% AgNP-Cit partitions to bottom ash and the bottom ash is sent to the landfill as an ultimate disposal. Approx. 1% of AgNP-Cit will end up in an Industrial WWTP and the disposal pathways will be the same as for the municipal WWTP.

Scoring step

Environmental risk was calculated separately for the aquatic and the soil environment. For calculating aquatic and soil environmental risks, environmental characteristics of each compartment in the hierarchy are not included in the calculations. Based on the preliminary step, there was no possibility for direct release in the environment and no indirect release via agricultural run-off; therefore, these factors and their sub-level factors were not scored. All related sub-factors at the bottom of the hierarchy were listed and scored with fuzzy numbers according to the scale in Table 7.2 (see Tables 7.3-5). Then, all actors at the same level in the hierarchy were compared based on their importance for the risk. For example, Table 7.6 shows the comparison matrix for the factors under the “treatment plants”, where comparison scores are in fuzzy numbers which then were converted to STFN values.

The row of the matrix in Table 6.6 is compared with its column by using Chang’s 1-9 scale (See Appenice E). If the row is more important than the column in terms of risk, a score is given from 1 to 9, in increasing order. In case columns are more important than rows in terms of risk, reciprocal numbers are used from 1 to 1/9. For example, since the municipal WWTPs are much more important sinks of AgNP-Cit than Industrial WWTPs, the score (first row, third column in Table 7.6) was given as (1/6-1/8).

Table 7. 1: Scores of Case Study I (usage of T-shirts embedded with AgNP-Cit in Duebendorf, Switzerland) for all of the sub-factors at the bottom level of the hierarchy of occurrence likelihood considering the aquatic medium, STFN numbers (A,B,C,D) corresponding to the scores, priority of the factors at the hierarchy level (w') (Equation S3) and final Index Score of occurrence likelihood (Equation S4).

Factor	Sub Factor	Score		STFN				w'	A* w'	B* w'	C* w'	D* w'
				A	B	C	D					
Ind. Treatment plants	Frequency	9	10	9	9	10	10	0.001	0.01	0.01	0.01	0.01
	Amount	9	10	9	9	10	10	0.004	0.03	0.03	0.04	0.04
	Product Properties	5	7	5	5	7	7	0.003	0.02	0.02	0.02	0.02
	Medium Properties	9	10	9	9	10	10	0.000	0.00	0.00	0.00	0.00
	Technological	6	7	6	6	7	7	0.003	0.02	0.02	0.02	0.02
Municipal Treatment plants	Frequency	6	7	6	6	7	7	0.020	0.12	0.12	0.14	0.14
	Amount	5	7	5	5	7	7	0.028	0.14	0.14	0.19	0.19
	Product Properties	5	6	5	5	6	6	0.005	0.03	0.03	0.03	0.03
	Medium Properties	4	5	4	4	5	5	0.014	0.06	0.06	0.07	0.07
	Technological	7	8	7	7	8	8	0.003	0.02	0.02	0.02	0.02
Leachate Treatment plants	Frequency	6	8	6	6	8	8	0.024	0.14	0.14	0.19	0.19
	Amount	3	6	3	3	6	6	0.036	0.11	0.11	0.22	0.22
	Product Properties	6	7	6	6	7	7	0.006	0.04	0.04	0.04	0.04
	Medium Properties	4	6	4	4	6	6	0.021	0.08	0.08	0.12	0.12
	Technological	7	8	7	7	8	8	0.003	0.02	0.02	0.02	0.02
Leachate off landfill	Frequency	4	7	4	4	7	7	0.095	0.38	0.38	0.66	0.66
	Amount	4	7	4	4	7	7	0.209	0.84	0.84	1.47	1.47
	Product Properties	5	6	5	5	6	6	0.118	0.59	0.59	0.71	0.71
	Medium Properties	6	7	6	6	7	7	0.407	2.44	2.44	2.85	2.85
Index Score									**5.08**	**5.08**	**6.84**	**6.84**

Table 7. 2: Scores of Case Study I (usage of T-shirts embedded with AgNP-Cit in Duebendorf, Switzerland) for all of the sub-factors at the bottom level of the hierarchy of exposure potential considering the aquatic medium, STFN numbers (A,B,C,D) corresponding to the scores, priority of the factors at the hierarchy level (w') (Equation S3) and final Index Score of exposure potential (Equation S4)

Factor	Sub Factor	Score		STFN				w'	A* w'	B* w'	C*w'	D*w'
				A	B	C	D					
ENP Characteristic	Size	3	5	3	3	5	5	0.237	0.71	0.71	1.18	1.18
	Coating	6	3	6	6	3	3	0.139	0.83	0.83	0.42	0.42
	Surface Charge	6	4	6	6	4	4	0.088	0.53	0.53	0.35	0.35
	Solubility	5	3	5	5	3	3	0.086	0.43	0.43	0.26	0.26
	Attachment Efficiency	3	6	3	3	6	6	0.160	0.48	0.48	0.96	0.96
Soil-Stationary Phase	pH	1	3	1	1	3	3	0.004	0.00	0.00	0.01	0.01
	NOM	6	8	6	6	8	8	0.039	0.23	0.23	0.31	0.31
	SS	4	3	4	4	3	3	0.012	0.05	0.05	0.03	0.03
	Sulfides	4	6	4	4	6	6	0.028	0.11	0.11	0.17	0.17
	Ca/Mg	4	3	4	4	3	3	0.071	0.29	0.29	0.21	0.21
	Chlorides	7	3	7	7	3	3	0.015	0.10	0.10	0.04	0.04
	DO	5	3	5	5	3	3	0.009	0.05	0.05	0.03	0.03
	Background	5	2	5	5	2	2	0.006	0.03	0.03	0.01	0.01
Hydrological	Depth	4	7	4	4	7	7	0.062	0.25	0.25	0.44	0.44
	Water velocity	2	4	2	2	4	4	0.044	0.09	0.09	0.18	0.18
Index Score									**4.18**	**4.18**	**4.61**	**4.61**

Table 7. 3: Scores of Case Study I (usage of T-shirts embedded with AgNP-Cit in Duebendorf, Switzerland) for all of the sub-factors at the bottom level of the hierarchy of toxic effects considering the aquatic medium, STFN numbers (A,B,C,D) corresponding to the scores, priority of the factors at the hierarchy level (w') (Equation S3) and final Index Score of toxic effects (Equation S4)

Factor	Sub-Factor	Score		STFN				w'	A* w'	B* w'	C* w'	D* w'
				A	B	C	D					
Acute	Survival	3	2	3	3	2	2	0.100	0.30	0.30	0.20	0.20
	Avoidance	3	5	3	3	5	5	0.026	0.08	0.08	0.13	0.13
Chronic	Reproduction	5	6	5	5	6	6	0.463	2.31	2.31	2.78	2.78
	Growth	6	5	6	6	5	5	0.092	0.55	0.55	0.46	0.46
	Feeding	4	5	4	4	5	5	0.058	0.23	0.23	0.29	0.29
Structural	Composition	2	4	2	2	4	4	0.033	0.07	0.07	0.13	0.13
	Diversity	1	3	1	1	3	3	0.017	0.02	0.02	0.05	0.05
	Predator	5	3	5	5	3	3	0.012	0.06	0.06	0.04	0.04
	Food web	3	1	3	3	1	1	0.014	0.04	0.04	0.01	0.01
Functional	Primary Production	3	6	3	3	6	6	0.016	0.05	0.05	0.10	0.10
	Decomposition	3	5	3	3	5	5	0.025	0.07	0.07	0.12	0.12
	Nutrient cycling	5	3	5	5	3	3	0.078	0.39	0.39	0.23	0.23
	Minerilization	2	3	2	2	3	3	0.067	0.13	0.13	0.20	0.20
Index Score									**4.31**	**4.31**	**4.74**	**4.74**

Then, STFN numbers (Table 7.6) were defuzzified into crisp numbers (Table 7.7) before calculating the priority weights for Industrial WWTP versus municipal WWTP by using Equation E(2) as follows:

$$\frac{1/6+2*\left(\frac{1}{6}+\frac{1}{8}\right)+1/8}{6}=0.146$$

Weights of the factors at their level and at the hierarchy were calculated by using Equations E(3) and E(4), respectively, as follows:

$$w_{Municipal\ WWTP}=\frac{\frac{7}{1+7+7.5}+\frac{1}{0.146+1+1.5}+\frac{0.75}{0.134+0.75+1}}{3}=0.409$$

$w'_{\text{Municipal WWTP}}=w_{\text{Municipal WWTP}}*w_{\text{Treatment plants}}$ = 0.409*0.17=0.07

These factors have one higher level in the hierarchy, namely, “Treatment Plants”, so the weight of Municipal WWTPs at the hierarchy level is obtained by multiplying its weight in its own hierarchy with the weight of “Treatment Plants”. The value of w’ for the sub-factors of municipal treatment plants (Table 7.3) is then calculated by multiplying w’ for municipal treatment plants with their own w’ at their own levels.

Finally, the scores of the factors for OL were multiplied with their priority weights (w’) in the hierarchy and the overall sum of these values resulted in an Index score (in STFN) for OL (Table 7.3). The same calculations were applied for EP and TE.

Fuzzy Inference Step

STFN values for OL, EP and TE are needed to convert into fuzzy sets for fuzzy inference. The index scores in Table 7.2 were converted into a graph and STFN values were also drawn in this graph. Intersection points of STFN with the index score in Table 7.3 show the fuzzy sets for the corresponding index. Figure 7.8 shows the fuzzy sets for the OL, EP and TE indices of environmental risk for AgNP-Cit in aquatic media.

Table 7.6 : Comparison matrix with fuzzy numbers and their STFN values for the sub-factors of “Treatment Plants” considering the risk for Case Study I (AgNP-Cit in aquatic media)

	Industrial WWTP		Municipal WWTP		Landfill WWTP	
	Score	STFN	Score	STFN	Score	STFN
Industrial WWTP	(1,1)	(1,1,1,1)	(1/6,1/8)	(1/6,1/8, 1/6,1/8)	(7,8)	(7,7,8,8)
Municipal WWTP	(6,8)	(6,6,8,8)	(1,1)	(1,1,1,1)	(1,1/2)	(1,1,1/2,1/2)
Landfill WWTP	(7,8)	(7,7,8,8)	(1,2)	(1,1,2,2)	(1,1)	(1,1,1,1)

Table 7. 4: Comparison matrix with crisp numbers (for the sub-factors of “Treatment Plants” considering the risk for Case Study I (AgNP-Cit in aquatic media)

	Industrial WWTP	Municipal WWTP	Landfill WWTP
Industrial WWTP	1	0.146	0.134
Municipal WWTP	7	1	0.75
Landfill WWTP	7.5	1.5	1

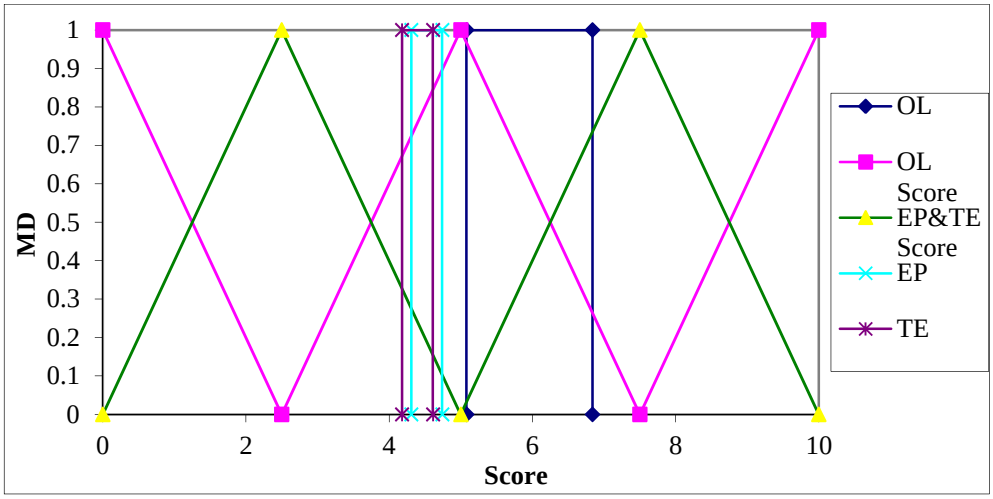


Figure 7.8: Fuzzy set inference for index scores obtained in Tables (7.3-5) for occurrence likelihood (OL), exposure potential (EP) and toxic effects (TE) for Case Study I (AgNP-Cit in aquatic media). Interceptions of green line with EP and TE index score and interceptions of pink line with OL show the membership degrees (MD) for fuzzy sets OL, EP and TE.

For example, the STFN value of OL intersects the scale at low (0.7) and medium (1), which means that the likelihood for AgNP-Cit occurrence in the environment can be low or medium with membership degrees of 0.7 and 1, respectively.

Fuzzy sets of OL, EP and TE are combined by using the "and" operator of fuzzy rule base. Table 7.8 shows the combination of the fuzzy sets of these factors for the environmental risk of AgNP-Cit in aquatic medium. For example, if the OL is low (0.9), EP is low (0.7) and TE is low (0.3), the risk magnitude is judged as Negligible (0.32). The Membership degree of risk magnitude is determined as the minimum membership degree among OL, EP and TE since the "and" operator is being used. Fuzzy union (maximum operator) is used to obtain the overall membership degrees for each risk magnitude class, which are negligible, minor, major and critical. For example, in Table 7.8 (in bold) the membership degrees of these classes are 0.3, 0.7, 0.82 and 0, respectively.

Table 7.8: Combination of fuzzy sets of index scores for occurrence likelihood (OL), exposure potential (EP) and toxic effects (TE) for Case Study I (AgNP-Cit in aquatic media) to obtain corresponding risk classes and membership degrees based on the fuzzy inference rule.

		TE	
OL	EP	Low(0.3)	Medium(0.88)
Low(0.7)	Low(0.32)	**Negligible(0.3)**	Minor(0.32)
	Medium(0.82)	Negligible (0.3)	**Minor(0.7)**
Medıum(1)	Low(0.32)	Negligible (0.3)	Major(0.7)
	Medium(0.82)	Negligible (0.3)	**Major(0.82)**

The membership degree of each risk class that is obtained in Table 7.8 is defuzzified into a crisp number indicating the Risk Magnitude (RM) by using Equation E(5) as follows:

$$RM = \frac{0.3+0.7*4+0.82*7+0*10}{0.3+0.7+0.7} = 4.86$$

Finally, the risk magnitude number is intercepted with the risk magnitude scale in Table 7.2 (Figure7.9) and the intersection points show the risk classes for Case Study I (AgNP-Cit in aquatic media) with their membership degrees (in parenthesis) which are Major (0.45) and Minor (0.55). Since the risk magnitude scale in Table7.2 is a fuzzy scale, the risk classes have membership degrees (between 0-1) which show what extend risk belongs to this class.

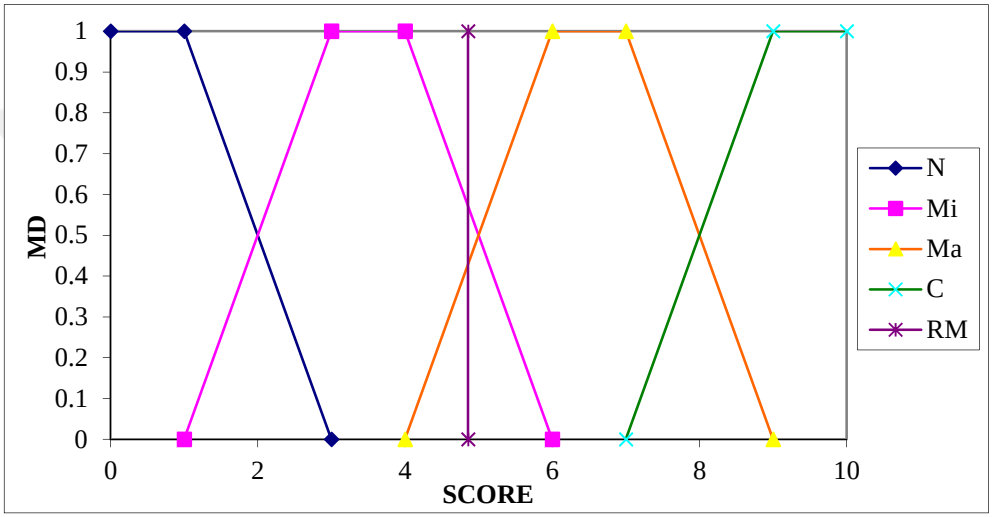


Figure 7.9: Determination of the risk class for Case Study I (AgNP-Cit in aquatic media) and membership degree based on the intersection of RM (Equation E(4)) with the scale in Table 7.2 (MD: Membership degree, N: Negligible, Mi: Minor, Ma: Major, C: Critical, RM: Risk Magnitude)

7.4.2 Case Study III: The usage of paints in which TiO_2 nanoparticles are embedded

Case study III is based on the usage of paints embedded with TiO_2 nanoparticles which are used for the buildings in Duebendorf, in Switzerland. Paints are produced in Duebendorf. It is assumed that half of the total number of buildings in this city is using the paint, with 20% being fresh-painted and the rest containing aged-paintings (for more than 2 years). Production process assumptions are the same as for Case Study I. Approx. 1% of the emission is expected to reache the Industrial WWTP, where the TiO_2 ENPs partition to the sludge and end up in landfills through the bottom ash of incineration plants. Leachate is expected due to the run-off with rain water. The release will be direct through the run-off and indirect through the WWTPs or landfill leachate. No transformation processes of TiO_2 nanoparticles was expected in any stage of release. The information gathered by expert groups is summarized in Table 7.9.

Table 7.9: Summary of the conditions assumed for Case Study III. Building paints, which contain TiO_2 NPs, are manufactured by a factory and used for building painting in Duebendorf, Switzerland. TiO_2 NPs are also produced by the same company.

General Information			
Production capacity	40,000 ton/year		
Product Information	Hombikat UV 100 WP, an aqueous dispersion of 50% nano TiO2 (anatase) stabilized with poly-acrylate (Al-Kattan et al., 2014)		
ENP Characteristics			
Surface Coating	No	**Surface Charge**	-
Size	<10 nm (Al-Kattan et al., 2014)	**Solubility**	-
Attachment Efficiency	-		
Transformation	No transformatıon		
Environmental Characteristics			
Soil	See Table 2		
Water			
Release Characterization			
Release way	Production processes, weathering and end of life disposal		
Usage habits	20% freshly painted, 80% aged paintings		
Possible emission sources	Paint Manufacturing Industry Treatment Plant, Municipal WWTP, Incineration Plants, Landfill, direct run-off		
WWTP	Duebendorf WWTP (Topuz et al., 2015; Kaegi et al., 2013)		
Landfill characterization	Detailed information in Mueller et al. (2013)		
Receiving bodies	Glatt-Rheinsfelden via run off, WWTP discharges Soil (around Duebendorf) via direct release, landfilling of demolition waste, WWTP sludge, bottom ash of incineration plants		
Effect Characterization			
Single Species	Bondarenko et al., 2013		
Community Level	Lowry et al., 2013		

7.5 Results and Discussion

Environmental ENP risks of the defined case studies were evaluated by applying the approach proposed in this study and the risk classes with their membership degrees were determined. Although no distinction was defined in the framework of the approach, separate evaluation of environmental risks for aquatic and soil media were applied without any problem owing to the flexibility of the proposed approach. Overall results are given in Table 7.10.

Table 7.10: Environmental risk classes and their membership degrees for Case Studies I and II (usage of T-shirts embedded with AgNPs in Duebendorf, Switzerland) and Case Study III (usage of building paints embedded with TiO_2 NP in Duebendorf, Switzerland)

		Aquatic		Soil	
		Minor	Major	Minor	Major
Case Study I	AgNP-Cit	0.55	0.45	0.6	0.4
	AgNP-PVP	0.28	0.72	1	0
Case Study II	TiO2	1		1	

Environmental risk of AgNP-Cit in aquatic media

Usage of AgNP-Cit embedded T-shirts (for antimicrobial purposes) in Duebendorf, Switzerland is expected to cause minor and major environmental risk for aquatic media (Table 7.10) with a membership degree of 0.55 and 0.45, respectively.

Compared to the PEC/PNEC approach which only informs whether there is risk or not, usage of fuzzy sets for scaling the risk magnitude in different classes enables the user to present the results in more detail, for instance that the risk can vary from minor to major for AgNP-Cit in aquatic media. This kind of detail can facilitate decision making to determine the content of risk management strategies. According to the scale given in Table 7.2, minor and major risks in the environment require to take precautions and reduce the risks, respectively. Since the environmental risk assessment is the step preceding risk management applications, risk magnitude needs to be properly addressed to be able to decide what to do afterwards. Classifying the risk magnitudes (Table 7.2) also can give useful feedbacks for risk management strategies.

According to the priority weights of the factors at hierarchy level, landfill leachates (0.83), AgNP-Cit characteristics (0.71) and single species toxicity (0.74) are the main reasons for the occurrence of AgNP-Cit in the environment, exposure potential and toxic effects to aquatic organisms, respectively, that are contributing to the aquatic risk. Evaluation of OL was supported with relatively rigid data available for the scenario and knowledge about the fate of these compounds in the treatment plants especially in municipal WWTP. However, the release potential and form of AgNP-Cit are related to the usage habits that can affect the release significantly, for instance usage of bleaching detergents, and this may lead to uncertainties. Nevertheless, the release of AgNP-Cit and its transformation products via TP discharge to aquatic media is shown to be low (as much as <10% of influent) in many studies (Kaegi et al., 2013). As explained in the preliminary step (Section 7.4.1), most of AgNP-Cit and its

transformation products end up in landfill which becomes major sink for Ag. The lack of studies on the release of AgNPs by leaching (amount, form and release potential to water bodies) leads to vague and uncertain data which also increase the score for the contribution to risk.

Among the factors related to leachate of landfills, the medium properties (priority weight at its own level: 0.41) are highly contributive to the risk because of its highly concentrated water chemistry properties. Due to the high concentration of Ca^{2+}/Mg^{2+}, the agglomeration potential of AgNP-Cit is very high despite of the high organic matter content (Topuz et al., 2014). However, the uncertainty about the effect of organic matter remains due to the possible presence of biopolymers, which are very effective for long-term stabilization of the ENPs (Topuz et al., 2015). In addition to the effect of biopolymers, the presence of high concentrations of chloride can lead to higher Ag solubility (Quadros et al., 2013) which results in higher mobility and the potential to reach water bodies. Usage of hierarchy to organize all of these factors systematically and expert opinions to reflect the scientific facts that can be not be formulated (because of gaps in the literature and complexity of the problem) facilitate the evaluation of the factors contribution to the risk and may help to get more precise results.

AgNP-Cit characteristics were determined to be more important for the risk of these nanoparticles in the aquatic environment compared to the other factors related to EP. Most distinguished characteristic of AgNP-Cit is its vulnerability to size changes and reactions that can result in unexpected dissolution properties. Therefore size (0.24), coating size (0.14) and solubility (0.09) are among the most effective factors for the risk. Although water chemistry parameters have lower contributions because of the dominant role of AgNP-Cit, the most important factors were determined to be NOM and Ca^{2+}/Mg^{2+} ions. This is in agreement with our previous study and provides a general classification of surface waters for agglomeration process based on Ca^{2+}/Mg^{2+} and NOM concentrations (verified with natural water samples) (Topuz et al., 2015). The scoring of these parameters was made based on this suggested classification.

Toxic effects of AgNP-Cit seem to be more critical for single species. This especially concerns chronic effects, which were observed in most of the species tested. Effects on reproduction and growth were observed for various species, such as green algae, crustaceans and fısh. Therefore, reproduction (0.46) and growth (0.1) endpoints have high importance in terms of risk. Unfortunately, the data about the effects of AgNPs at the community level is

very limited. Although strong effects are not expected due to the fact that toxic concentrations observed for single species were higher than environmentally realistic concentrations, the uncertainty of the situation was reflected by the fuzzy scores. In general, lower scores were assigned to these factors because of the possible lower environmental concentrations of AgNP-Cit.

Environmental risk of AgNP-Cit in soil

Usage of AgNP-Cit embedded T-shirts (for antimicrobial purposes) in Duebendorf, Switzerland is expected to cause minor (0.6) and major (0.4) environmental risk for the soil compartment. The contributions of the main factors of OL, EP and TE to the risk were similar for the soil environment. For the evaluation of OL, the factor of release potential under the treatment plants was determined to be more influential on the risk for soil than for aquatic media since sewage sludge is a sink for the AgNP-Cit that partition to solid media. The effects of environmental factors on EP were mostly depending on the stationary phase rather than on pore water, because AgNP-Cit was expected to attach to soil due to its agglomeration potential. Among environmental factors, organic matter and clay content were determined to have the biggest influence on exposure potential. Our previous study (Topuz et al., 2015) showed that organic matter in the soil can limit the exposure to AgNPs possibly due to the higher binding capacity for AgNPs. This study can provide a prediction of the extent of risk reduction depending on the organic matter fraction in the soil. Soil pH was shown to have the least influence on risk. Clay content of the soil was shown to have significant influence by keeping AgNP-Cit in the stationary phase as shown by the study of Cornelis et al. (2010). Like for the effect of water chemistry on aquatic risk, the organic matter content and the concentration of divalent cations in pore water have the biggest importance for the EP due to their strong effects on the agglomeration of AgNP-Cit. Toxic effects had more or less the same importance in soil as in the aquatic medium. Most studies focused on the effects on single species, with effects on reproduction, growth and avoidance observed at relatively low but still not environmentally relevant concentrations. Lowest observed effect concentrations were lower than 20 mg Ag/kg dry soil in our study assessing effects on the reproduction of *E. crypticus* (Topuz et al., 2015). The same conclusion regarding uncertainty of the data made for the aquatic medium can also be made for soil, but uncertainty was much more pronounced in the latter case.

Environmental risk of AgNP-PVP in aquatic and soil media

While the environmental risk of AgNP-PVP in the aquatic environment was minor and major, it was only minor for soil (Table 7.10). Evaluating the environmental risk separately seems to provide more precise results. The overall environmental risk would be minor if a combined assessment were applied, and would lead to inappropriate risk management strategies such as just taking precautions instead of risk reduction. Therefore, the modular structures of the risk assessment approaches might be critical to yield more sensitive results. Environmental risks of AgNP-PVP in the aquatic and soil medium were higher and lower than the risks of AgNP-Cit, respectively (Table 7.10). AgNP-PVP is much more stable due to steric stabilization of the coating material and its neutral surface. Exposure potential to AgNP-PVP is considered to be low due to the its stability resulting in a tendency to partition in the aquatic phase. Therefore, scoring of EP leads to lower STFN numbers and lower fuzzy sets as an input to risk magnitude inference. Similarly, the higher risk in the aquatic environment is stemming from the higher occurrence and exposure potential of AgNP-PVP because of its coating properties. So, the scores are higher for OL, for instance due to the amount releasing from landfills, and for EP due to for instance its solubility and coating, which results in higher risk magnitudes.

Environmental risk of TiO_2 in aquatic and soil medium

Environmental risk of TiO_2 releasing from the building paintings in Duebendorf, Switzerland in aquatic and soil environments is minor (Table 7.10). TiO_2 are used in relatively high amounts and release also is higher than for AgNPs, therefore their OL score was higher than for AgNPs. In this case study, direct release of TiO_2 to soil and run-off to water bodies were also possible. However, indirect release (0.63) was expected to be higher due to the rainwater collection networks and end-of life disposal of demolition waste to landfills. For EP, especially size and attachment had higher weights since TiO_2 has the tendency to change its size depending on the environmental conditions and to attach to solid phases due to its negligible solubility. Organic matter and Ca^{2+}/Mg^{2+} had higher importance in terms of environmental conditions, which is supported by our previous study providing detailed information and classification of the agglomeration potential in Glatt-Rheinfelden (Topuz et al., 2015). For TE, the importance weights of the factors were similar to those for the AgNPs, however, the scores were much lower since the known toxic concentrations were far above the environmentally realistic ones. Therefore, only taking precautions can be suggested to decision makers by considering increasing the stability of TiO_2, embedding TiO_2 nanoparticles

more tightly in the products and conducting more toxicity studies to assess effects at the community level.

Overall, this approach provides answers to key questions in terms of risk assessment by identifying which environmental compartment is most at risk, what are the reasons for the occurrence of risk, how important the role of different factors is for the risk, what is the class and magnitude of the risk and what strategies should be followed for risk management.

7.6 Conclusion

The usage of hierarchical structure to analyze the environmental risk of ENPs provides a systematic overview to the critical factors that cause the risk when they interact and not to miss important aspects contributing to the risk. The modular structure of the approach enables the user to easily revise the hierarchy and it can be easily adapted to other studies as an independent compartment, such as Life Cycle Assessment (LCA). Scoring the factors with fuzzy numbers offers flexibility to the user but also provides insight into the uncertainty related with the risk. Fuzzy inference provides the flexibility of combining all factors related to the risk due to the use of expert judgments instead of formulations. Quantification of risk magnitude might be useful for the decision making processes since different alternatives or cases can be ranked. Since the class of risk points out the necessity of taking precautions or measure to reduce risks, not only magnitude, but also classification of the risk based on management strategies is expected to be beneficial for the risk management step. In addition to the risk management, risk communication also is expected to improve especially through the stakeholders such as industries, public and scientist, because the risk characterization is more tangible with clearly quantified results. Comparison of the factors in terms of their importance with priority weight numbers directly points out the role of factors in the risk. This should be very handy for risk managers to establish their strategies to reduce the risk or for the scientists to focus on more detailed studies. In addition, industries can use the results for product research-and-development projects to manufacture more environmental-friendly products and regulators can benefit from the results for the decision making actions related with legislative issues.

From the perspective of the case studies, there is a critical need for toxicity studies especially for assessing toxicity at the level of communities. There are relatively few studies on the fate of AgNPs and TiO_2 in landfills, especially on their dispersion in leachate from the field to the environment or their behavior during and after leachate treatment. More studies are needed on

the correlation of the fate and toxicity of ENPs with environmental parameters or nano-QSAR studies to find indicators of chemical properties for the effects observed. According to the up-to-date studies, TiO_2 seems to be safe, at least in terms of usage for paintings. The knowledge on AgNPs is rapidly developing, with transformation of AgNPs to Ag_2S compounds being one of the most recent critical findings. Therefore, there is an urgent need for critical reviews about the environmental behavior and effects of AgNPs. Moreover, there is a need for representative experiments with standard Ag_2S NPs.

APPENDIX E: Chapter 7

Calculation Steps

Calculation steps of this study is referred to the methodology proposed in Topuz et al. (2011). Therefore, the scales for scoring and the equations were quoted from Topuz et al. (2011) as follows. (Note that: Equations are re-numbered for this study (in red) and the modified terminology were re-written (in BOLD))

Compare factors pair wise

Each member in a level is compared with other factors in the same level under the same group based on their relative contribution to ER. Chang's 1-9 scale is used for pair wise comparison. According to that scale, the scale of 1 is given for factors that have equal importance. 3,5,7 and 9 denote weakly, strongly, very strongly and absolutely more important, respectively. If there are slight differences between factors, even scales (2, 4, 6, 8) are used (Saaty, 2001). Experts can give their scores in fuzzy scale if it is needed.

Convert scores into STFN

Since the scores for the factor measurement and pair wise comparisons are in different formats, it is needed to convert them into a common form in order to precede calculations. Standard trapezoidal fuzzy numbers (STFN), which is also used in study of Zeng et al. (2007) and its conversion equation is shown, is preferred for that study. A trapezoidal membership function can be expressed as $A=(a^l, a^m, a^n, a^u)$. For example, triangular fuzzy numbers are converted into STFN as $a^m=a^n$, a numerical range corresponds to $a^l= a^m$ and $a^n= a^u$.

Calculate priority weights

Arithmetic averaging method given in **equation E1** is employed in order to calculate priority weights of factors in comparison matrix. a_{ij} is the defuzzified form of score that is given for the comparison of **factor$_{(i)}$ and factor$_{(j)}$** factor in the same level in which there are n factors. If total STFN is shown as $a_{ij}=(a^l_{ij} , a^m_{ij} , a^n_{ij} , a^u_{ij})$, the crisp value of a_{ij} can be calculated by using defuzzification equation 2.

$$w_i=\frac{1}{n}\sum_{j=1}^{n}\frac{a_{ij}}{\sum_{k=1}^{n} a_{kj}} \qquad i,j= 1,2,\ldots., n \qquad \textbf{(E1)}$$

$$a_{ij} = \frac{a^1{}_{ij} + 2*(a^m{}_{ij} + a^n{}_{ij}) + a^u{}_{ij}}{6} \qquad \textbf{(E2)}$$

While w_i is the weight of **factor$_{(i)}$** in its own level, w'_i which is given in **equation E3** shows the weight of factor in the hierarchy. $w^{(i)}_{section}$ indicates the priority weight of i. section that is above this factor in the case of being t level above it.

$$w'_i \times \prod_{i=1}^{t} w^{(i)}\,section \qquad \textbf{(E3)}$$

Calculate Occurrence Likelihood, Exposure Potential and Toxic Effects

Instead of FI, 3 different indexes were used in this study, namely, Occurrence Likelihood (OL), Exposure Potential (EP) and Toxic Effects (TE). Therefore, index scores calculated separately for 3 indexes. Index scores can be calculated by using **equation E4** where P_i is the STFN form of the score that is given by experts to the bottom level risk factors in the hierarchy. n indicates the number of bottom level risk factors in the hierarchy which belongs to a specific main risk source.

$$\textbf{Index Score} = \sum_{i=1}^{n} P^{*}_{i} \times w'_i \qquad i = 1,2,\ldots,n \qquad \textbf{E(4)}$$

Fuzzy Inference

This step provides user to achieve ultimate risk magnitude by using fuzzy sets of risk components with the fuzzy inference engine.

Convert STFNs to fuzzy sets

It is needed to convert **OL, EP and TE index scores** to fuzzy sets to be able to use in fuzzy inference system in which linguistic variables are used during fuzzy rule construction. Intersection between STFN forms of **OL, EP and TE index scores** and their respective membership functions give the membership degrees of those factors in corresponding fuzzy set which is shown in **Figure E1** for illustration of scenario implementation.

Fuzzy inference

If-then rules are used in order to achieve RM by combining **OL, EP and TE** components. Fuzzy intersection (minimum) operation provides combining the OL, EP and TE parameters with “and” operator which leads to getting truncated fuzzy RM results. Therefore, fuzzy union (maximum) operation is used for getting a single fuzzy membership function.

Defuzzification

RM can be expressed in terms of a numerical value that matches fuzzy result achieved in fuzzy inference system. For that purpose, centre-average method is used as shown in **equation E5** where Y_i indicates the center of the i^{th} fuzzy set of RM and $\mu_{RM}(y_i)$ is the membership function of the i^{th} fuzzy set of RM

$$RM = \frac{\sum_{i=1}^{q} Y_i \mu_{RM*}(y_i)}{(\sum_{i=1}^{q} \mu_{RM*}(y_i)} \qquad i=1,2,..,q$$

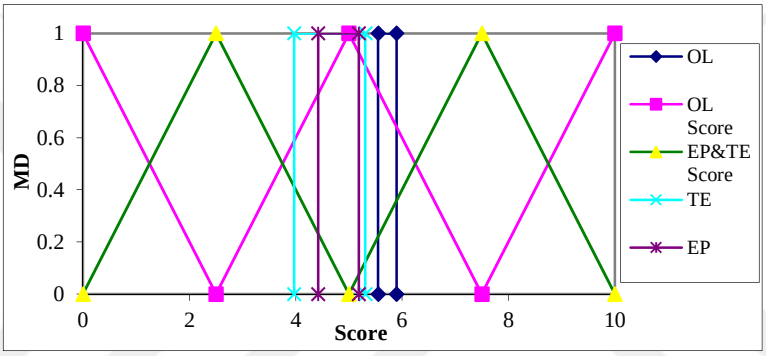


Figure D.1: Fuzzy set inference for index scores of occurrence likelihood (OL), exposure potential (EP) and toxic effects (TE) for Case Study I (AgNP-Cit in soil media). Interceptions of green line with EP and TE index score and interceptions of pink line with OL show the membership degrees (MD) for fuzzy sets OL, EP and TE.

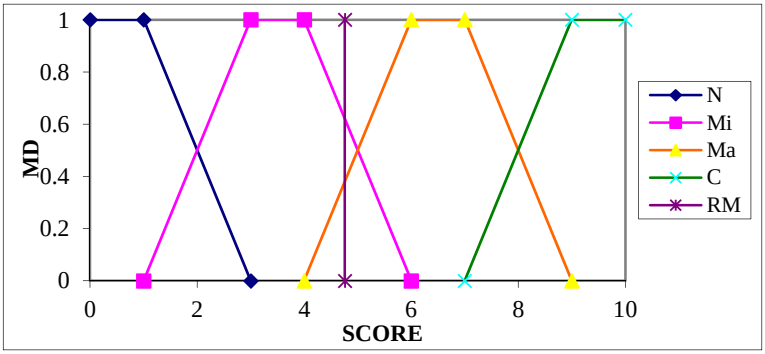


Figure D.2: Determination of risk class for Case Study I (AgNP-Cit in soil media) and membership degree based on the intersection of RM with the scale in Table 6.2 (MD: Membership degree, N: Negligible, Mi: Minor, Ma: Major, C: Critical, RM: Risk Magnitude)

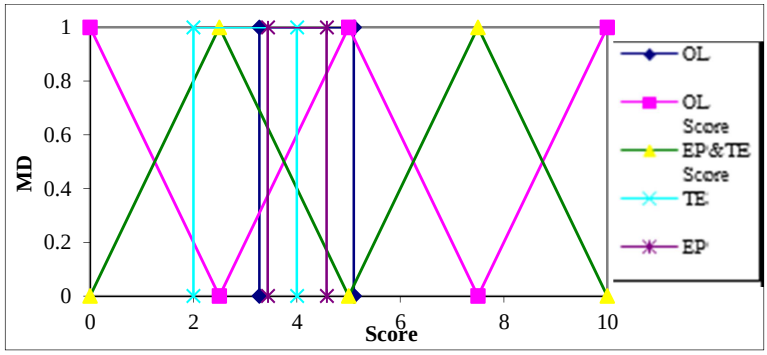


Figure D.3: Fuzzy set inference for index scores of occurrence likelihood (OL), exposure potential (EP) and toxic effects (TE) for Case Study II (AgNP-PVP in aquatic media). Interceptions of green line with EP and TE index score and interceptions of pink line with OL show the membership degrees (MD) for fuzzy sets OL, EP and TE.

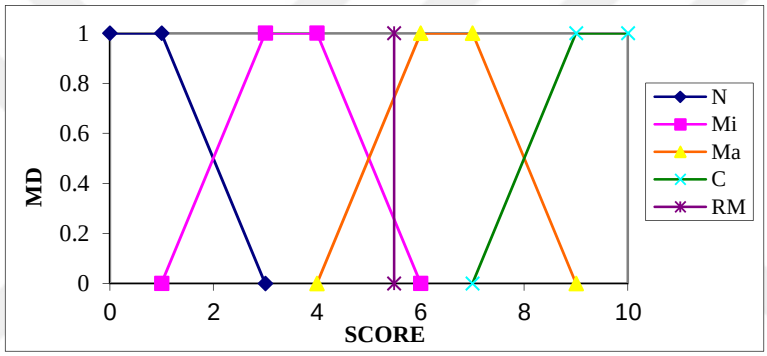


Figure D.4: Determination of risk class for Case Study II (AgNP-PVP in aquatic media) and membership degree based on the intersection of RM with the scale in Table 6.2 (MD: Membership degree, N: Negligible, Mi: Minor, Ma: Major, C: Critical, RM: Risk Magnitude)

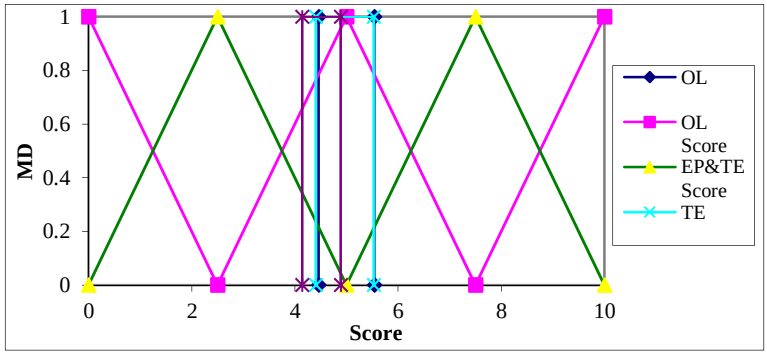


Figure D.5: Fuzzy set inference for index scores of occurrence likelihood (OL), exposure potential (EP) and toxic effects (TE) for Case Study II (AgNP-PVP in soil media). Interceptions of green line with EP and TE index score and interceptions of pink line with OL show the membership degrees (MD) for fuzzy sets OL, EP and TE.

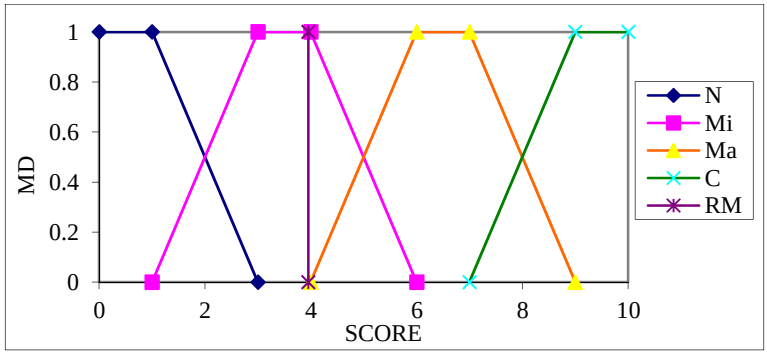


Figure D.6: Determination of risk class for Case Study II (AgNP-PVP in soil media) and membership degree based on the intersection of RM with the scale in Table 6.2 (MD: Membership degree, N: Negligible, Mi: Minor, Ma: Major, C: Critical, RM: Risk Magnitude)

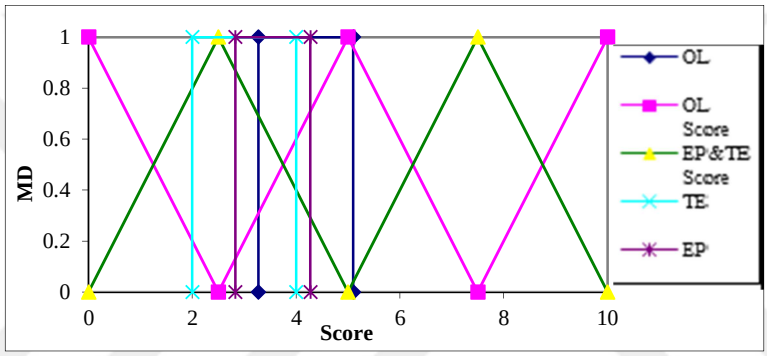


Figure D.7: Fuzzy set inference for index scores of occurrence likelihood (OL), exposure potential (EP) and toxic effects (TE) for Case Study III (TiO_2 in aquatic media). Interceptions of green line with EP and TE index score and interceptions of pink line with OL show the membership degrees (MD) for fuzzy sets OL, EP and TE.

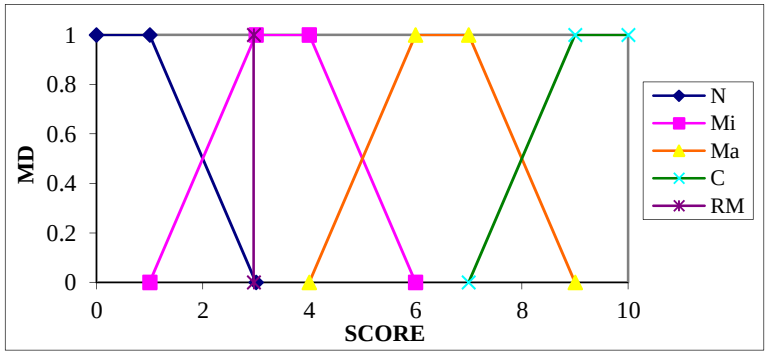


Figure D.8: Determination of risk class for Case Study III (TiO_2 in aquatic media) and membership degree based on the intersection of RM with the scale in Table 6.2 (MD: Membership degree, N: Negligible, Mi: Minor, Ma: Major, C: Critical, RM: Risk Magnitude)

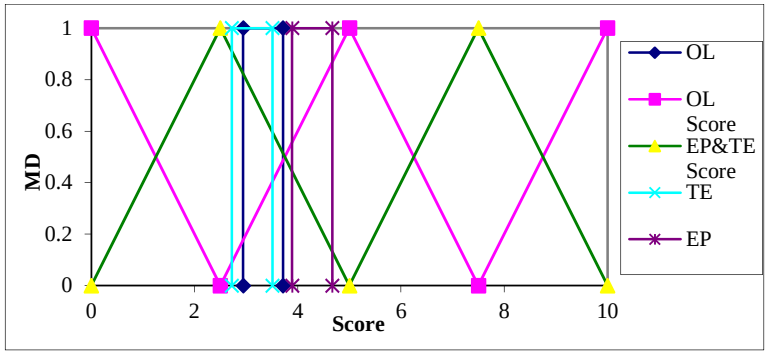


Figure D.9: Fuzzy set inference for index scores of occurrence likelihood (OL), exposure potential (EP) and toxic effects (TE) for Case Study III (TiO_2 in soil media). Interceptions of green line with EP and TE index score and interceptions of pink line with OL show the membership degrees (MD) for fuzzy sets OL, EP andTE.

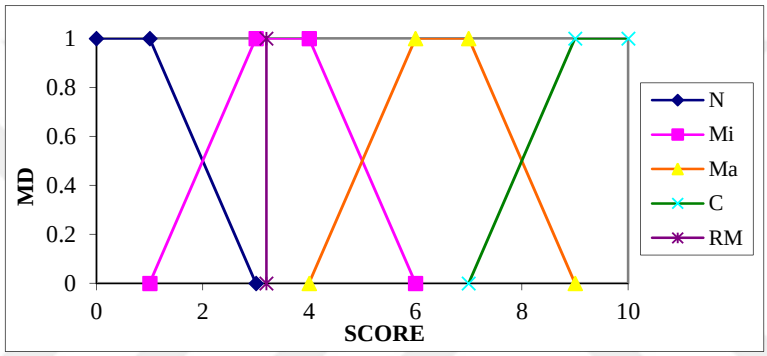


Figure D.10: Determination of risk class for Case Study III (TiO_2 in soil media) and membership degree based on the intersection of RM with the scale in Table 6.2 (MD: Membership degree, N: Negligible, Mi: Minor, Ma: Major, C: Critical, RM: Risk Magnitude

CHAPTER 8
DISCUSSION AND CONCLUSION

Since 2006 engineered nano-particles (ENPs) are widely applied in many different products, including both consumer and industrial products. Due to their large-scale application and limited removal from waste water, ENPs are released into the environment. Due to the fact that they manufactured to be applied in goods of human interest, they are new to the environment. Therefore, ENPs are categorized as emerging pollutants. Hence, there is a increasing need for environmental risk assessment (ERA) of ENPs. There is a huge gap in data and knowledge on ENP occurrence, fate and effects in the environment while unique properties such as their nanometer size (1 – 100 nm) resulting in great surface reactivity may lead to unexpected properties. This restricts the usage of conventional, retrospective, risk assessment approaches and calls for the application of prospective approaches.

Prospective risk assessment approaches are designed to evaluate all factors that might be related to environmental risk. These approaches apply tools that enable a systematic evaluation of risk factors, reduce uncertainty and incorporate expert judgment into the risk assessment process to ensure a reliable and convenient ERA. The initial motivation of this thesis is to propose an ERA approach for ENPs by using an analytical hierarchy process (AHP) and fuzzy inference tools (Chapter 7). To develop a clear and pragmatic approach, silver (AgNPs) and titanium dioxide (TiO_2 NPs) nanoparticles were selected as model compounds as they are among the most commonly used ENPs in daily life products.

For a systematic evaluation of risk factors, there is a need to clarify the major relations not only in terms of mechanistic understanding but also in terms of correlations with surrogate parameters. Conventional risk assessment methods do not sufficiently take into account the unique properties of ENPs. Agglomeration is the most relevant fate process for ENPs, due to their unique size and surface properties. I determined the most relevant chemical parameters for ENP agglomeration in aquatic ecosystems using a systematic experimental design (Chapter 3) and making correlations with the results of experiments conducted on real environment samples (Chapter 4). These studies filled a gap in the literature and improved the overal rioska ssessment framework, allowing predictions for the case studies (Chapter 7).

Another significant point that was lacking in the literature was the understanding of the uptake and bioaccumulation rates of ENPs and the relationship between ENP toxicity and environmental chemistry parameters. These relations were relatively clear for aquatic organisms, but few data were available for soil organisms. Integration of toxicokinetic and toxicodynamic analysis led to an improved understanding of the relationship between uptake and toxicity of AgNPs in a model organism, the potworm *Enchytraeus crypticus* (Chapter 5), and improved the predictions of effects at the community level (Chapter 7). Determination of the effects of AgNPs on the survival and reproduction of *E. crypticus* were done in different soil types (Chapter 6).

8.1 Determination of water chemistry parameters related with agglomeration

Because agglomeration is the most important process driving the fate and effects of nano-particles, it is of utmost importance to understand the conditions that promote or prevent agglomerastion. A systematic experimental design was planned to determine the most relevant water chemistry parameters (Chapter 3). After testing the effect of pH for each ENP, selected cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), anions (Cl^-, NO_3^-, SO_4^{2-}, CO_3^{2-}), natural organic compounds (humic acid, fulvic acid) and synthetic compounds (sodium dodecyl sulphate, alkyl ethoxylate) were used as test media separately and in dual combinations in agglomeration experiments. Dynamic Light Scattering (DLS) and Nanosight (NTA) were used to characterize size distributions of ENPs. In addition to the determination of most relevant parameters for the agglomeration over time, environmentally relevant concentrations of ions and organic matters commonly found in the environment were mixed to simulate natural freshwater. This medium was validated for the agglomeration behavior of ENPs with natural water samples. Therefore, a relatively large number of real environmental samples could be analyzed for the interpretation of agglomeration data.

8.1.1 Specific roles of water chemistry parameters in ENP agglomeration

Specific roles of commonly observed ions and organic matters on the agglomeration of ENPs in aquatic environment (**first part of the second research question**) were defined under this heading.

AgNP-Cit

Citrate-coated AgNPs (AgNP-Cit) appeared to be stable at pH valuses between 4 and 10, which is the most common range of pH levels observed in natural waters and wastewater.

AgNP-Cit particles maintain their original size (50 nm) under these conditions. AgNP-Cit tends to agglomerate at pH values below 4, which is probably due to neutralization of surface charges resulting in electrostatic double layer (EDL) compression. The same trend for the agglomeration of AgNP-Cit (at pH< 4) was also observed by (Badawy et al., 2010) and Cumberland and Lead (2009).

Agglomeration is prevented by the large surface charge in NPs, which represent an energy barrier. Monovalent cations could not decrease this energy barrier for agglomeration, but divalent cations could, most likely by shielding the surface charge of AgNP-Cit and compress the electrochemical double layer around the particle. This leads to agglomeration from an average particle size of 60 nm up to 1 µm in 1 week. The effect of Ca^{2+} was more pronounced than that of Mg^{2+}, which would be attributed to the higher affinity of Ca^{2+} to complex with citrate as reported by Akaighe et al., (2011), Chinnapongse et al. (2011), Hyunh et al. (2011) and Badawy et al., (2010). For the anions, only NO_3^- and SO^{2-} (10 mM) showed slight agglomeration inducing potential after 1 week. Critical coagulation concentrations were determined to be around 50 mM for $NaNO_3$ (Hyunh and Chen, 2011; Lie et al., 2010; Gondikas et al. 2012) and Na_2SO_4 (Chinnapongse et al., 2011). Our results for the ion combinations also showed that their agglomeration potential can be neglected when they are co-present with divalent cations.

Humic acid (15 mg/L) caused a slight increase in average size and Gao et al. (2012) also highlighted the agglomeration effect of humic acid when it is present at high concentrations due to the bridging effect between ENPs. Fulvic acid showed stabilizing effects for AgNP-Cit in this study, although Chinnapongse et al. (2011) found slight agglomeration in the presence of fulvic acid. Surfactants caused lower average sizes than the original size of AgNP-Cit over 1 week, however, it is difficult to draw conclusions on the effect of surface charge due to less negative zeta potential measurements which could be attributed to steric stabilization (Kvitek et al., 2008).

AgNP-PVP

Polyvinylpyrrolidone (PVP) is an amphiphilic compound, which has a very high molecular weight and makes very strong N bonds with Ag (Thio et al., 2012; Zhang et al., 2012; El Badawy et al., 2010; Cumberland and Lead, 2009). This should provide a strong steric stabilization. Z-average size of the AgNP-PVP tested was stabile around 50 nm at pH 4-12 due to its non-charged polymer capping, which was compatible with the studies of El Badawy (2010) and Thio (2012). AgNP-PVP was stable in the presence of $NaNO_3$, NaCl, $CaCl_2$ and

humic acid, which was also observed by Gondikas et al., (2012), Hyunh (2011), El-Badawy (2010), and Hyunh (2012), respectively.

In addition to being compatible with these studies on ions and humic acid, AgNP-PVP suspensions were found to be stable in most of the individual media including KCl, Na_2SO_4, Na_2CO_3, fulvic acid, sodium dodecyl sulphate and alkyl ethoxylate (up to 15 mg/L) even for more than 1 week in this study. The only critical parameter in terms of agglomeration of AgNP-PVP were Mg^{2+} ions as 3.3 mM of Mg^{2+} led to a Z-average size of 200 nm in 1 hour. However, 3.3 mM of Ca^{2+} led to a slight agglomeration, which was under the critical coagulation concentration of Ca^{2+} (4.9 mM) (Hyunh et al., 2011). The effect of Mg^{2+} cannot be explained from EDL compression since the zeta potential did not change. There is a gap in the literature about the effect of Mg^{2+} ions on the agglomeration of AgNP-PVP since most authors tested Ca^{2+} as a representative of divalent cations. Thio (2011) and Zhang (2012) reported high agglomeration of AgNP-PVP in seawater, which has a very high concentration of ions. Hence, it is not clear what causes such the high degree of agglomeration of AgNP-PVP in the presence of Mg^{2+}.

TiO_2

TiO_2 studies presented more uncertain results due to the effect of pH. Although TiO_2 was also stable at higher and neutral pH values, especially media prepared with different kinds of ions resulted in different mean particle sizes at different pH levels. Therefore, pH was always adjusted to around 8.5 for our agglomeration tests. Lower pH values with ionic media caused a higher mean particle size. Therefore, pH could be the most critical factor for the agglomeration of TiO_2 in aquatic environments and interactions of pH and ions are important. Especially for toxicity studies, the test medium should be adjusted to an optimum pH. A further study should focus on searching for the effects of different ions on the agglomeration of TiO_2 at different pH levels.

TiO_2 behaves very similar to AgNP-Cit in aqueous media, including similarity in response to ions. Divalent cations led to significant agglomeration in 1 day and even increased particle size to over 1 µm in 1 week due to compressing of the EDL (Shih et al., 2012; Ottofuelling et al., 2011; Domingos et al., 2010; Keller et al., 2010; Mukherjee and Weaver, 2010; Chowdhury et al., 2009). Both Ca^{2+} and Mg^{2+} had the same effect, which supports the special effect of Ca^{2+} on AgNP-Cit due to the complexation of Ca^{2+} and citrate. Based on the tested concentrations, agglomeration over 1 week changed precisely between 3.3 mM and 2.5 mM. NO_3^- and SO_4^{2-} lead to slight agglomeration over 1 week, while Cl^- and CO_3^{2-} have stabilizing

effects when they are countered with monovalent cations. Natural organic matter and surfactants disperse TiO_2 over 1 week. (Zhang et al., 2008) found similar results for sodium dodecyl sulphate media and showed that TiO_2 did not disperse anymore, which was in agreement with our study.

Fulvic acid and alkyl ethoxylate seem to be the most effective stabilizing agents for TiO_2. However, both humic and fulvic acid are very effective in keeping TiO_2 in suspension. High humic acid concentrations lead to a slight increase in mean particle size. Although sodium dodecyl sulphate did not increase mean size significantly, there was a minor increase in time. Humic acid (dissolved organic carbon (DOC)) could be the second major parameter for TiO_2 (Chapter 4). More sensitive toxicity studies are needed for TiO_2 as its size might change to around 200-800 nm under conditions very similar to those in natural surface water. Since pH fluctuations could be expected more than fluctuation of divalent cation concentration, TiO_2 has higher uncertainty in terms of pH effects on agglomeration and ERA methodologies should take this into consideration.

8.1.2 Effects of the combination of water chemistry parameters on ENP agglomeration

According to the results of the ion mixture experiments for AgNP-Cit, the type of ion rather than the ionic strength of the medium were the distinctive factor and state of agglomeration was determined by Ca^{2+} and Mg^{2+} at environmentally relevant concentrations. The agglomeration level of AgNP-Cit did not change for Mg^{2+} concentrations in the range of 2.5-3.3 mM. Z-average size changed significantly with Ca^{2+} concentrations ranging between 1.7 and 3.3 mM due to both its screening of surface charge and complexation with citrate. Humic acid, fulvic acid (5 mg/L) and sodium dodecyl sulphate (1 mg/L) provided stabilization in ionic media especially after 1 day, which is in agreement with the results of experiments on individual factors. Alkyl ethoxylate slightly promoted agglomeration especially over 1 day, which could be due to the fact that it is a nonionic surfactant and its adsorption to ENPs may lead to bridging effects. Kvitek et al. (2008) also reported a low stabilization effect for non-ionic surfactants (Brij group) due to steric effects. Agglomeration or stabilization of ENPs might be promoted depending on the type of organic matter. Agglomeration of ENPs in relation to the structure of DOC should be investigated by applying fractionation in terms of molecular weight or hydrophobicity (Chapter 4).

For AgNP-PVP, combination of ionic medium with DOC and/or surfactants resulted in very slight agglomeration around 80 nm, which can be attributed the effect of divalent cations

slightly compressing the EDL. AgNP-PVP was stable during 1 week both in simulated and in natural surface water media where Mg^{2+} concentration was only 0.8 mM and no agglomeration effect was expected based on the synthetic media experiments. Therefore, for the purpose of risk assessment studies, AgNP-PVP can be evaluated as least suspicious for agglomeration.

While environmentally relevant concentrations of monovalent cations have no effect on ENP agglomeration (1 week), divalent cations promote agglomeration significantly in a very short time. The presence of DOC or surfactants can have a stabilizing effect and keep the size constant for 1 day up to over 1 week. Chowdhury et al., (2012) stated that humic acid is only effective for dispersing ENPs in the presence of monovalent cations. In agreement with this study, a combination of factors had no dispersing effect on ENPs after 1 day till up to over 1 week. TiO_2 agglomerated more in the co-presence of organic matter and divalent cations. Domingos (2010) also reported similar effects of Ca^{2+} in the presence of fulvic acid. However, they also reported a dispersing effect of fulvic acid when present at high concentrations (more than 5 mg/L) by adsorbing on the ENP surface and leading to thick steric barriers.

Ionic medium (2 mM $CaCl_2$, 0.8 mM Na_2CO_3, 0.5 mM KCl and 0.4 mM $MgCl_2$) was combined with DOC (5 mg/L of humic and fulvic acid) and synthetic organic materials (1 mg/L of alkyl ethoxylate and sodium dodecyl sulfate) to simulate natural surface water. Its performance was quite well in terms of agglomeration of ENPs over 1 week, as was shown from validation with a natural surface water sample. Agglomeration occurred as expected from Ca^{2+} and DOC concentrations in the medium. AgNP-Cit was more vulnerable to agglomeration as expected since citrate is a low molecular weight compound and provides electrostatic stabilization resulting in a more negative charge (Chinnapongse et al., 2011; Hyunh and Chen, 2011; Thio et al., 2012; El-Badavy et al., 2010; Cuberland and Lead, 2009; Mukherjee and Weaver, 2010).

To conclude, the agglomeration of AgNP-Cit and TiO_2 was significantly affected by Ca^{2+} concentration, and the presence of organic matter (DOC) provided stabilization after 1 day, which could be correlated with the agglomeration level of AgNP-Cit (Chapter 4). Although pH is highly critical for the agglomeration of TiO_2, the concentration of Ca^{2+} seems to determine the initial agglomeration levels. The effect of pH may be evaluated by expert judgement for risk assessment studies as proposed in Chapter 7. These water chemistry parameters may enable the prediction of the behavior and toxicity of AgNP-Cit and TiO_2 for risk assessment studies (Chapter 7). ENP agglomeration may change significantly between 1

hour and 1 week. Therefore, agglomeration behavior of ENPs can be predicted from Ca^{2+}, Mg^{2+} and DOC concentrations not only in aquatic systems, but also in the pore water of soil **(second part of second research question)**, which is supposed to be the main exposure phase for soil organisms (Chapters 5&6). Since these are the dominant factors that predict the long-term behavior of ENPs in the aquatic systems (Chapter 4), a significant number of natural water samples, having different levels of Ca^{2+}, Mg^{2+}, DOC and alkalinity, should be studied.

8.2 Correlation of Water Chemistry Parameters with ENP Agglomeration in Natural Water

Although there are several studies on the agglomeration of ENPs in natural water samples (Chinnapongse et al., 2011; Ottofuelling et al., 2011; Hammes et al., 2013), they are lacking different aspects limiting their use for the prediction of agglomeration behavior. Based on the results of agglomeration experiments in synthetic media, which showed that Ca^{2+}/ Mg^{2+} and DOC concentration were most relevant water chemistry parameters for the prediction of agglomeration (Chapter 3), classification scales based on the concentration of these parameters were suggested for aquatic media from low to very high in terms of agglomeration. Surface water and wastewater sample sites were selected as to represent each class in the classification scale. Agglomeration experiments were conducted in unfiltered samples to obtain environmentally relevant conditions. Samples were also filtered and passed through 10 kDa and 1 kDa cut-off membrane filters to fractionate the dissolved organic matter.

Correlations between the agglomeration of the model ENPs and selected water quality parameters **(the first part of the Research Question 3)** were explained in detail for each ENP:

AgNP-PVP

AgNP-PVP was stable in all fractions of all water samples for over 1 week. Therefore, for risk assessment predictions AgNP-PVP can be evaluated as stable in most natural water environments, only by considering its heteroaggregation potential and Mg^{2+} concentration (Chapter 7). The fraction of AgNP-PVP that is not adsorbed to soil may be easily mobilized in the pore water and increase the exposure potential for soil organisms (Chapters 5&6).

AgNP-Cit

The change in Z-average size of AgNP-Cit in 1 hour was correlated (R^2>0.7) with Ca^{2+} concentration in the water, with samples with DOC< 1 mg/L or DOC> 2 mg/L slightly

deviating from the correlation due to higher agglomeration and stabilization, respectively. The effect of DOC concentration was more pronounced after 1 day and correlation (R^2>0.5) was less good than after 1 hour. Agglomeration of AgNP-Cit was more sensitive to the concentration range of Ca^{2+} (1-2 mM) which leads to Z-average sizes of 200-400 nm. Agglomeration over time was more significant for the samples with high concentrations of Ca^{2+}. As is clear from Figure 4S.18, agglomeration of ENPs in surface water and wastewater in 1 day and 1 week might be predicted depending on the concentration of Ca^{2+} and DOC. Evaluation of AgNP-Cit in terms of agglomeration for environmental risk assessment studies (Chapter 7) can be provided with the classification scale suggested in Chapter 4. The bioavailability of AgNP-Cit could be variable with time and with different water chemistry conditions in pore water, which could be taken into account for soil ecotoxicity studies (Chapters 5&6). When AgNP-Cit and AgNP-PVP were compared in terms of agglomeration, a significant effect of surface coating material on their behavior was demonstrated in Chapter 4, which should be considered in studies on their toxicity (Chapters 5&6).

TiO_2

TiO_2 nanoparticles were usually agglomerated up to 400 nm in 1 hour when the concentration of Ca^{2+} changed between 1 and 3 mM. The correlation of particle sizes with Ca^{2+} concentration is not as good as the correlation of AgNP-Cit, however, the correlation is more pronounced for the samples with DOC< 2 mg/L. DOC is more effective for the stabilization of TiO_2 than that of AgNP-Cit and the correlation becomes worse after 1 day. Results of tests in natural water samples showed that aquatic media can be classified based on Ca^{2+} concentration and DOC for the agglomeration in 1 hour and 1 day (Figure 4.7).

Classification of aquatic sources based on the agglomeration

Surface water simulated media prepared with nano pure water (Chapter 3), in which ions and natural organic matter are dissolved at concentrations close to those in natural surface water, is precise enough to predict ENP agglomeration level in surface water and wastewater based on concentrations of Ca^{2+} and DOC. This helped preparing a classification scheme of aquatic media for the agglomeration of ENPs over time, which is shown in Figure 4.7 **(the second part of Research Question 3)**. Therefore, usage of this simulated media is highly recommended for experimental studies on ENP behavior in the future. For AgNP-Cit, the agglomeration studies conducted with filtered natural water should be considered carefully since the agglomeration was much higher in filtered samples than in unfiltered ones. The uncertainty of the data of the studies with filtered natural water should be taken into account

for ERA studies (Chapter 7). Another point to consider for ERA studies is the presence of high molecular weight organic compounds (> 10 kDa) which are supposed to be an effective fraction of organic matter for stabilizing ENPs over time (Chapter 4). Especially, biopolymers could be the major compounds responsible for the stabilization of ENPs in the presence of organic matter. Fractions below 10 kDa led to significantly higher agglomeration in 1 day than other fractions. In the literature, there is a lack of studies on the effect of specific components of natural organic matter on the agglomeration behavior of ENPs. Recently, Louie et al. (2013) studied the effect of organic fractions on the agglomeration of gold nanoparticles and found that the residue remaining on the filter (Molecular weight 691,000 g/mol) provided significantly higher stabilization than the filtrate (Molecular weight of 12,800 g/mol). Further detailed studies are needed with more natural water samples for supporting the effect of biopolymers on the stabilization of ENPs. The classification scheme for the agglomeration behavior of AgNP-Cit, AgNP-PVP and TiO_2 over time based on the experimental results with natural water samples and the determination of the effect of the molecular weight of organic carbon on the stabilization of these ENPs (Chapter 4) are the first in the literature to the best of our knowledge.

8.3 Uptake of AgNPs in model soil organism, *Enchytraeus crypticus*

The studies about uptake of ENPs in soil organisms are limited to arthropods (Waalewijn-Kool et al., 2014) and earthworms (Heckmann et al., 2011; Shoults-Wilson et al., 2011; Hu et al., 2012; Schlich et al., 2013; Van der Ploeg et al., 2014). The integration of toxicokinetic and toxicodynamic studies to understand the link between internal and external concentrations of ENPs and their toxic effects (Chapter 5) may help predicting ENP exposure and effects for ERA (Chapter 7). No such studies have been done on ENPs before. The model soil organism, the potworm *Enchytraeus crypticus***,** was exposed to different concentrations of AgNP-Cit and AgNP-PVP for 2, 3, 5, 7 and 10 days in an inert sand medium embedded with solution phase containing essential elements. TiO_2 was not included in the soil toxicity studies because it is only toxic at very high concentrations. The use of an inert sand medium with solution phase avoided the complexity of natural soils and did provide clear insight into the toxicokinetics and toxicodynamics of the AgNPs. To our best knowledge, this is the first study in the literature reporting the toxicity of AgNPs to *E. crypticus* and applying a toxicokinetic/ toxicodynamic approach.

For all Ag compounds (ionic Ag, AgNP-Cit, AgNP-PVP), most of the Ag was adsorbed to the sand fraction. Ag concentration did not change significantly in both the solution and the sand phases from day 2 to day 7 of the tests. The adsorption of AgNP-PVP was higher than that of AgNP-Cit, which could be due to the lipophilic nature of PVP. Considering the limited dissolution of AgNP-Cit (3%, Odzak et al., 2014), most of the Ag in the solution phase for AgNP-Cit was supposed to be in nano form. The amount of Ag in the solution phase of the AgNP-PVP treatments was very close to that for $AgNO_3$, and was comparable with the known dissolution of AgNP-PVP (10%, Odzak et al., 2014). Therefore, the fraction bioavailable for the enchytraeids would be expected to be ionic Ag for both $AgNO_3$ and AgNP-PVP and mainly in nano form AgNP-Cit (Chapter 6). The form in which Ag is present should be considered for determining exposure potential and toxic effects for ERA studies (Chapter 7).

Ag measurements in *E. crypticus* showed that Ag can be taken up **(first part of Research Question 4)** and accumulate over time for all Ag compounds tested **(second part of Research Question 4)**. Ultimate LC50 for effects on enchytraeid survival toxicity was ten times lower for $AgNO_3$ (0.081 mg Ag/L) and AgNP-PVP (0.047 mg Ag/L) than for AgNP-Cit (0.322 mg Ag/L). LC50 values decreased from day 2 to day 10 and reached steady state **(first part of Research Question 4)**. The change of LC50 over time should be considered when using results from standard toxicity tests with a fixed exposure time (Chapters 6&7). Moreover, LC50 values and the form of Ag in the solution fraction of the test medium showed that toxicity is mainly due to ionic Ag for all Ag compounds. LC50 values based on internal Ag concentrations in the animals also supported the role of the Ag ion in toxicity since LC50internal was higher for AgNP-Cit than for the other Ag forms. LC50 internal did not change over time, indicating that this parameter may be a more suitable measure of toxicity **(third part of Research Question 4)**, being less dependent on exposure time (Chapter 6). LC50internal values therefore could be useful for ERA studies. Ag uptake and elimination rates were similar for AgNP-PVP and $AgNO_3$ exposures, supporting the conclusion that the form of Ag in the test organisms was the same. Toxicodynamics provided important information especially for AgNP-Cit because its internal concentration and its toxicity were not the same. Although AgNP-Cit was taken up, probably mostly in nano-form, they did not affect the target sites which could be due to internal agglomeration of AgNP-Cit in biological fluids (Kwak et al., 2013). The agglomeration potential of AgNP-Cit (Chapters 3&4) also supports this speculation. Hence, the evaluation of exposure potential and toxic effects of AgNP-Cit should be considered carefully for ERA studies (Chapter 7).

8.4 Toxicity of AgNPs to *Enchytraeus crypticus* and the correlation of toxicity with soil organic matter content

After understanding the linkage between internal and external concentrations and the toxicity and bioavailability for *E. crypticus* (Chapter 5), a reproduction toxicity test with *E. crypticus* (ISO (2004) and OECD (2004)) was performed with AgNPs in three different soils with different organic matter contents and different pH (Chapter 6). This enabled the use of possible correlation of toxicity with organic matter content or soil pH for ERA studies (Chapter 7).

The speciation of $AgNO_3$ and AgNPs showed the same trend in soil as in the inert sand medium. According to the Freundlich isotherm, adsorption of AgNP-PVP was much more pronounced than for the other Ag compounds. The highest fraction of Ag in pore water was observed for AgNP-Cit which could be due to the complexation of the citrate coating with soil organic matter leading to reduced binding of Ag to the soil solid phase. Organic matter content and pH also influenced Ag speciation. For the soil with pH>7, lowest Ag concentrations were measured in the pore water. When soil pH was similar, a higher soil organic matter content led to lower Ag concentrations in the pore water, which could be due to the binding of Ag with dissolved organic matter. Therefore, assessing the exposure potential of soil organisms should consider the pH and organic matter content of the soil (Chapter 7).

To the best of knowledge, Chapter 6 was the first to report LC50 (111 and 335 mg Ag/kg dry soil for $AgNO_3$ and AgNP-PVP, respectively) and EC50 (75.2 and 92.3 mg Ag/kg dry soil, respectively) values for effects of AgNPs on the reproduction of *E. crypticus* which was expected to be more sensitive than arthropods and earthworms **(first part of Research Question 5)**. The results of this study agreed with this expectation and lower LC/EC50 values were achieved for AgNPs than in other studies. Reproduction was found to be more sensitive to AgNPs than survival. Species sensitivity distribution and endpoint sensitivity need great care for ERA studies (Chapter 7). The extremely high pH of the AgNP-Cit stock solution led to dramatic increase of soil pH to values >8.5, which appeared lethal to the enchytraeids. Therefore no LC/EC50 values could be calculated for AgNP-Cit. Nevertheless, the importance of the nature of the capping agent for ENP behavior and toxicity was shown again.

$AgNO_3$ and AgNP-PVP did not differ in reproduction toxicity, while the effect on enchytraeid survival was higher for $AgNO_3$ than for AgNP-PVP which could be due to the slow release of

Ag ions from AgNP-PVP. The NOECs for the effect of $AgNO_3$ (<9.90 mg Ag/kg dry soil) and AgNP-Cit (<20.3 mg Ag/kg dry soil) were relatively low and fairly close to predicted environmental concentrations reported for sewage sludge by Lazareva and Keller (2014) (0.18-2.01 mg Ag/kg sludge) Gotschalk et al. (2009) (1.29-6.24 mg nano-Ag/kg sludge).

Enchytraeid survival was influenced by neither pH nor organic matter content of the soil. EC50 for effects on reproduction negatively correlated with soil organic matter content, probably because of the high affinity of Ag for binding to organic matter. There was no significant effect of pH on enchytraeid survival and reproduction **(second part of Research Question 5)**. Therefore, organic matter content of the soil could be used for the prediction of reproduction toxicity for ERA studies (Chapter 7). EC50 values based on pore water concentrations may lead to conflicting results, which may be due to the complex interactions of dissolved organic carbon, pH and cations with metal uptake and toxicity, as described in the so-called Biotic Ligand Models (Ardestani et al., 2014; Di Toro et al., 2001). Thus, the uncertainty about the toxic effect of AgNPs to soil organisms should be considered for ERA studies (Chapter 7) until toxicity evaluations based on pore water concentrations are studied in more detail. The uptake of Ag from AgNPs in *E. crypticus*, both in soil and in the sand-solution medium, pointed at possible effects due to the bioaccumulation and food web transfer of Ag, which should also be taken into consideration for ERA studies (Chapter 7).

8.5 Environmental Risk Assessment with Multiple Criteria Decision Making Tools and Fuzzy Inference Models

The short history of ENP usage in products (Nanotechproject, 2013), their approved release from products to the environment (Gottschalk and Nowack, 2011) and their potential to pose harm to ecosystems (Chapter 5&6; Bondarenko et al., 2013; Kwak et al., 2014 and Heckman et al., 2011) have triggered concerns about their occurrence in the environment and required ERA for the usage of ENPs in consumer products. Difficulties in measuring ENPs in the environment (Gondikas et al., 2014) and the highly variable and limited data about their usage in products (Holden et al., 2014), their release from the products, behavior (Chapter 4) and toxic effects (Chapter 6) in the environment highlight the need for predictive risk assessment studies (Quik et al., 2015). The unique properties of ENPs, such as their size leading high reactivity or coating materials resulting in totally different characteristics compared to their pristine versions (Farre et al., 2011), raised the need of specific ERA approaches for ENPs instead of using conventional approaches (Schauman et al., 2014). Among the alternative

tools for the prediction oriented prognostic ERA, multiple criteria decision making (MCDM) tools (Miseljic and Olsen, 2014) and fuzzy inference models are considered useful (Chapter 7) and they have not been applied yet in existing studies.

One of the most widely used Multiple Criteria Decision Making Tools (MCDM) tools, the analytical hierarchy process (AHP), allows defining the main components of risk for ENP usage and the most relevant sub-factors composing the main components in a hierarchic manner which enable the prediction of the risk considering all possible conditions. Therefore, the user does not need exact data or all information and can predict the risk by focusing on the sub-factors in the hierarchy (Zeng et al., 2007). Not only the contribution of the factors to the risk, but also the priority of the factors in determining the risk are included in the evaluations which makes fuzzy inferences more accurate and precise, since fuzzy inference use detailed expert opinions instead of formulations.

As shown in the flow chart of the proposed ERA approach in Figure 7.1, the most elaborate task is the preliminary step including the definition of the conditions and the gathering of as much as possible data and information. This step however, also provides an accurate evaluation of risks. The most critical aspect of the definition is to determine the possible transformations during the release of ENPs from the product (Mitrano et al., 2015) and their fate in transition phases, such as wastewater treatment plants (WWTPs), before they enter the environment. Users can define the form of ENPs that enter environmental compartments and gather data accordingly.

A systematic evaluation procedure with MCDM tools for ERA of ENPs is applied by constructing a hierarchy which consists of risk factors (**first part of Research Question 1**). Environmental risk is the result of the occurrence of ENPs in the environment, exposure potential for ecosystem components and their possible toxic effects; these are the main factors in the hierarchy of ERA. The sub-factors related with the release of ENPs from products and their transformation/transportation before they enter the environment provide an estimate of the likelihood for occurrence in the environment. Most ENPs are released in different conditions, such as via washing of textiles (Geranio et al., 2009), cosmetic make ups (Keller et al., 2014), and run off from rain washing the outside of buildings (Al-Kattan et al., 2013). They end up in WWTPs, recycling centers or landfills and pass through engineered processes which certainly affect the amount and form of ENPs reaching the environment (Barton et al., 2015; Lazareva and Keller, 2014; Mueller et al., 2013). Knowledge of the characteristics of the ENP and the receiving environment enables the evaluation of fate processes to assess the

exposure potential (Chapters 3&4). The key processes for the fate of ENPs, namely agglomeration and dissolution (Garner et al., 2014), have been understood well in terms of exposure potential from the combination of ENP and environmental characteristics. Agglomeration studies helped predicting agglomeration behavior based on these characteristics (Chapters 3&4). Toxicity studies coupled with the determination of ENP speciation in soil media (Chapters 5&6) were highly useful for scoring the factors related to their contribution to the risk and for prioritization of these factors. Similar studies may apply for estimating the contribution of dissolution processes. Toxic effects are evaluated to reveal the strength of the risk, which was categorized at the single species and the community level. This categorization provides a precise evaluation since the acute and chronic endpoints for single species and structural and functional parameters at the community level are more representative of toxic effects.

Uncertainty regarding the data and information on the release (Quik et al., 2015), fate and effects of ENPs were successfully reduced with incorporating of expert judgements into the approach by using fuzzy scale scores and fuzzy inference rules **(first part of Research Question 1)**. Expert opinions on different items, such as high contribution to risk, were beneficial to compensate for missing data or uncertainty stemming from the limited number of studies available. Fuzzy inference rules were instrumental to combine main components of the risk, including occurrence likelihood, exposure potential and toxic effects with expert opinions to achieve an ultimate risk magnitude instead of using complicated formulations based on several assumptions. Therefore, there is no need for the formulation to quantify the risk and the risk is reported with its class (such as major or minor) which points out the necessary risk management action. Since the risk classification is also applied with fuzzy scales, reported risk class (membership degree), for instance Major (0.7), can provide comparison or ranking of risks, e.g. for different scenarios of ENP use or under different environmental conditions.

The proposed ERA approach was successfully applied to the model ENPs, AgNP-Cit, AgNP-PVP and TiO_2 ***(second part of Research Question 1)***. The proposed hierarchy was good enough to include all the factors that are needed to be evaluated in the context of the case study. Risk characterization with the fuzzy inference model provided the risk classes for each ENP coupled with membership degrees. Risk was quantified separately for aquatic and soil media owing to the flexible structure of the approach. In this way, more precise and informative results were obtained for the risk management step.

8.6 Risk Management, Communication and Legislative aspects

ERA is the basis for risk management and risk communication. ERA results therefore should provide an input for management activities (Wessberg et al., 2007). Since the history of ENP usage is relatively short and prediction of its risks are more important rather than evaluating the risk of existing situations, AHP provided priority weights were useful to address which aspects should be considered to reduce risk or take precautions. These results are helpful for both decision makers at legislative levels and manufacturing industries. Integration of this ERA approach with the research development stage of nanoparticle production or the usage of ENPs for consumer product manufacturing could provide valuable information for the nanomaterial or nano-based product development. It will be easier to identify which ENP characteristic needs to be improved or modified to reduce the risk. It is also possible to use the suggested approach as quantification of how risk reduction measures can be efficient (Topuz et al., 2011).

Priority weights could help identifying which environmental characteristics may be included in the legislation in order to limit exposure potential for ENPs or which parameters could be used to regulate the manufacturing of nano-based products. Based on the results of risk class and occurrence likelihood due to the release of ENPs, regulations can be defined in which nanomaterial production or nano-based product manufacturing activities could be included. It may also help deciding whether there is a need for new regulations specific to new (and existing) nanomaterials such as REACH.

Risk communication among stakeholders, like scientists, industries, decision makers of legislative issues and the public, should be stimulated (Kuhnel et al., 2014). Since the usage of ENP is relatively new and the communication for its safety for the environment is highly necessary to convince the usage of nano-based products without any doubt in terms of its risk to the environment, quantified ERA results will make the communication with the public easier. For decision makers, quantified risk and its class and the priority weights of the factors contributing to the risk may be much more informative. As such, the ERA approach proposed in this thesis may offer several advantages and tools also for risk communication between stakeholders.

8.7 Conclusion and Recommendation for Further Studies

This PhD study showed that a new approach for the ERA of ENPs is able to provide informative and meaningful results. Since the usage of ENPs in products is relatively new and there are few data with high uncertainty on its emission, fate and effects, predictive ERA approaches might help to predict possible harms that could be posed to the environment and to identify the critical steps and data gaps. The proposed approach allowed evaluating all the factors including environmental and ENP characteristics related to the risk by using analytical hierarchy process (AHP) to define the components of risk. To reduce major uncertainties, it is recommended to support ERA studies with critically designed experimental studies. Therefore, studies oriented to understanding the mechanisms of the fate and effects of ENPs should be expanded to provide data for improving the predictions in ERA. The ENP agglomeration and soil ecotoxicity studies described in this thesis provided useful data for the predictions and significantly supported the application of the proposed ERA approach.

Aquatic media can be classified based on the Ca^{2+}, Mg^{2+} and DOC concentrations in terms of their role in ENP agglomeration. AgNPs are taken up by *E. crypticus*, which showed to be one of the most sensitive species for their toxicity. Reproduction toxicity of AgNPs was correlated with soil organic matter content while soil pH did not affect their toxicity.

Further studies are needed to find correlations between water/soil chemistry parameters or ENP characteristics and their agglomeration/dissolution behavior to support ERA studies. The speciation of ENPs in soil pore water should be studied in more detail to better understand agglomeration/dissolution in the soil compartment. Toxicity studies should be designed in such a way that they enable predicting ENP toxicity based on both environmental and ENP characteristics. The proposed hierarchy for ERA could be improved based on the results of such studies.

Decision makers at the legislative level and research and development departments of nano-based industries are recommended to integrate the proposed AHP approach in their studies. This will help obtaining informative data for risk assessment and to include precautions to reduce environmental risk at the product development stage, which is the first level and the most desired step of waste management. Finally, it is suggested to apply the proposed ERA approach also in the Life Cycle Analysis (LCA) of ENPs. Since LCA considers all the stages of the usage and discarding of ENPs , the modular and hierarchic structure of the proposed ERA approach has the flexibility to be adapted for all LCA stages

REFERENCES

Abdullah, M., Low, G.K.C., Matthews, R.W. (1990). Effects of common inorganic anions on rates of photocatalytic oxidation of organic carbon over illuminated titanium dioxide. *Journal of Physical Chemistry, 94,* 6820-6825.

Achour, M. H., Haroun, A. E., Schult, C. J., Gase, K. A. M. (2005). A new method to assess the environmental risk of a chemical process. *Chemical Engineering and Processing*. *44*, 901-909.

Adams, L. K., Lyon, D. Y. and Alvarez, P. J. J. (2006). Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions, *Water Research, 40,* 3527-3532.

Adams, N. W. H. and Kramer, J. R. (1999). Silver speciation in wastewater effluent, surface waters, and pore waters, *Environmental Toxicology and Chemistry, 18,* 2667-2673.

Ahn, J.M, Eoma, H.J., Yang, X., Meyer, J.N., Choi, J. (2014). Comparative toxicity of silver nanoparticles on oxidative stress and DNA damage in the nematode, Caenorhabditis elegans. *Chemosphere, 108,* 343-352.

Aiken, G.R. Dissolved Organic Matter in Aquatic Systems, in: Ahuja, S. (Ed.), (2014). Comprehensive Water Quality and Purification. Elsevier,. Waltham, pp. 205-220.

Aitken, R. J., Chaudhry, M. Q., Boxall, A. B. A., and Hull, M. (2006). Manufacture and use of nanomaterials: current status in the UK and global trends. Occupational *Medicine-Oxford, 56(5),* 300-306. doi: 10.1093/occmed/kq1051

Akaighe, N., MacCuspie, R. I., Navarro, D. A., Aga, D. S., Banerjee, S., Sohn, M., Sharma, V. K. (2011). Humic Acid-Induced Silver Nanoparticle Formation Under Environmentally Relevant Conditions. *Environ. Sci. Technol., 45 (9),* 3895-3901.

Al-Kattan, A., Wichser, A., Vonbank, R., Brunner, S., Ulrich, A., Zuin, S., and Nowack, B. (2013). Release of TiO2 from paints containing pigment-TiO2 or nano-TiO2 by weathering. Environmental Science. Processes & Impacts, 15(12), 2186–2193 doi:10.1039/c3em00331k

Al-Kattan, A., Wichser, A., Zuin, S., Arroyo, Y., Golanski, L., Ulrich, A., Nowack, B. (2014). Behavior of TiO2 released from nano-TiO2-containing paint and comparison to pristine nano-TiO2. *Environ. Sci. Technol., 48,* 6710–6718. doi:10.1021/es5006219

Andersson, A.S., Tysklind, M. and Fangmark, I., (2007). A method to relate chemical accident properties and expert judgements in order to derive useful information for the development of environment-accident index, *J. Hazard. Mater., 147,* 524-533.

Angel, B.M., Batley, G.E., Jarolimek, C. V., Rogers, N.J. (2013). The impact of size on the fate and toxicity of nanoparticulate silver in aquatic systems. *Chemosphere , 93,* 359–365. doi:10.1016/j.chemosphere.2013.04.096

Angelstorf, J.S., Ahlf, W., Kammer, F. Von Der, Heise, S. (2014). Impact of particle size and light exposure on the effects of TiO2 nanoparticles on Caenorhabditis elegans. *Environ. Toxicol. Chem., 33*, 2288–2296. doi:10.1002/etc.2674

Ardestani, M. M., Van Straalen, N. M., and Van Gestel, C. a M. (2014). Uptake and elimination kinetics of metals in soil invertebrates: A review. *Environmental Pollution, 193*, 277–295 doi:10.1016/j.envpol.2014.06.026

Arunraj, R.S. and Maititi, J. (2008)**.** Development of Environmental consequence index (ECI) using fuzzy composite programming, J. Hazard. Mater., 162, 29-43.

Aruoja, V., Kahru, A. and Dubourguier, H.-C. (2008). Toxicity of ZnO, TiO2 and CuO nanoparticles to microalgae pseudokirchneriella subcapitata, *Toxicology Letters, 180*, S220-S220.

AshaRani, P. V., Mun, G. L. K., Hande, M. P. and Valiyaveettil, S. (2009). Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells, *Acs Nano, 3*, 279-290.

Asharani, P. V., Wu, Y. L., Gong, Z. and Valiyaveettil, S. (2008). Toxicity of silver nanoparticles in zebrafish models. *Nanotechnology, 19*.

Badawy, a. M. E., Luxton, T. P., Silva, R. G., Scheckel, K. G., Suidan, M. T., and Tolaymat, T. M. (2010). Impact of environmental conditions (pH, ionic strength,and electrolyte type) on the surface charge and aggregation of silver nanoparticle suspensions. *Environmental Science & Technology, 44(4)*, 1260–1266. Retrieved from http://dx.doi.org/10.1021/es902240k

Barton, L.E., Auffan, M., Durenkamp, M., McGrath, S., Bottero, J.-Y., Wiesner, M.R. (2015). Monte Carlo simulations of the transformation and removal of Ag, TiO2, and ZnO nanoparticles in wastewater treatment and land application of biosolids. *Sci. Total Environ., 511*, 535–543. doi:10.1016/j.scitotenv.2014.12.056

Behra, R., Sigg, L., Clift, M.J.D., Herzog, F., Minghetti, M., Johnston, B., Alke, P.F., Rothen-Rutishauser, B. (2013). Bioavailability of silver nanoparticles and ions: from a chemical and biochemical perspective. *J. R. Soc. Interface, 10*, 1-17.

Bell, R. A. and Kramer, J. R. (1999), Structural chemistry and geochemistry of silver-sulfur compounds: Critical review, *Environmental Toxicology and Chemistry, 18*, 9-22.

Benn, T., Cavanagh, B., Hristovski, K., Posner, J.D., Westerhoff, P. (2010). The release of nanosilver from consumer products used in the home. *J. Environ. Qual., 39*, 1875–1882. doi:10.2134/jeq2009.0363

Benn, T.M., Westerhoff, P. (2008). Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol. 42*, 4133–4139. doi:10.1021/es7032718

Bhatt, I. and Tripathi, B. N. (2011). Interaction of engineered nanoparticles with various components of the environment and possible strategies for their risk assessment. *Chemosphere, 82 (3)*, 308-317.

Bleeker, E. a J., de Jong, W.H., Geertsma, R.E., Groenewold, M., Heugens, E.H.W., Koers-Jacquemijns, M., van de Meent, D., Popma, J.R., Rietveld, A.G., Wijnhoven, S.W.P., Cassee, F.R., Oomen, A.G. (2013). Considerations on the EU definition of a nanomaterial: Science to support policy making. *Regul. Toxicol. Pharmacol., 65*, 119–125 doi:10.1016/j.yrtph.2012.11.007

Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbuhler, K.. (2008). Estimation of cumulative aquatic exposure and risk due to silver; contribution of nano-functionalized plastics and textiles. *Sci Total Environ, 390*:396–409.

Boldrin, A., Hansen, S.F., Baun, A., Hartmann, N.I.B., Astrup, T.F. (2014). Environmental exposure assessment framework for nanoparticles in solid waste. *J. Nanoparticle Res., 16*. doi:10.1007/s11051-014-2394-2

Bolyard, S.C., Reinhart, D.R., Santra, S., States, U. (2013). Behavior of Engineered Nanoparticles in Land fi ll Leachate.

Borm, P., Klaessig, F.C., Landry, T.D.,Moudgil, B., Pauluhn, J., Thomas, K., et al. (2006). Research strategies for safety evaluation of nanomaterials, part V: role of dissolution in biological fate and effects of nanoscale particles. *Toxicol Sci, 90*, 23–32.

Bondarenko, O., Juganson, K., Ivask, A., Kasemets, K., Mortimer, M., and Kahru, A. (2013). Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: A critical review. *Archives of Toxicology, 87(7)*, 1181–1200. doi:10.1007/s00204-013-1079-4

Bottero, J.-Y., Auffan, M., Rose, J., Mouneyrac, C., Botta, C., Labille, J., Masion, A., Thill, A. and Chaneac, C. (2011). Manufactured metal and metal-oxide nanoparticles: Properties and perturbing mechanisms of their biological activity in ecosystems, *Comptes Rendus Geoscience ,343*, 168-176.

Brar, S. K. and Verma, M. (2011). Measurement of nanoparticles by light-scattering techniques. *Trac-Trends in Analytical Chemistry, 30*, 4-17.

Brown, D.F. and Dunn, W.E., (2007). Application of a quantitative risk assessment method to emergency response planning, *Comput. Oper. Res.*, 34, 1243-1265.

Brown, G.E., Henrich, V.E., Casey, W.H., Clark, D.L., Eggleston, C., Felmy, A., Goodman, D.W., Grätzel, M., Maciel, G., McCarthy, M.I., Nealson, K.H., Sverjensky, D.A., Toney, M.F., Zachara, J.M. (1999). Metal oxide surfaces and their interactions with aqueous solutions and microbial organisms. *Chem. Rev., 99*, 77-174.

Brunelli, A., Pojana, G., Callegaro, S., Marcomini, A. (2013). Agglomeration and sedimentation of titanium dioxide nanoparticles (n-TiO2) in synthetic and real waters. *J. Nanoparticle Res., 15*. doi:10.1007/s11051-013-1684-4

Cañas, J.E., Qi, B., Li, S., Maul, J.D., Cox, S.B., Das, S., Green, M.J. (2011). Acute and reproductive toxicity of nano-sized metal oxides (ZnO and TiO2) to earthworms (Eisenia fetida). *J. Environ. Monit., 13*, 3351. doi:10.1039/c1em10497g

Castro-Ferreira, M,P., Roelofs, D., van Gestel, C.A.M., Verweij, R.A., Soares, A.M.V.M., Amorim, M.J.B. (2012). Enchytraeus crypticus as model species in soil ecotoxicology. *Chemosphere, 87*,122-1227.

Chambers, B. a., Afrooz, a. R.M.N., Bae, S., Aich, N., Katz, L., Saleh, N.B., Kirisits, M.J. (2014). Effects of chloride and ionic strength on physical morphology, dissolution, and bacterial toxicity of silver nanoparticles. *Environ. Sci. Technol., 48*, 761–769. doi:10.1021/es403969x

Chen, K. L. and Elimelech, M. (2006). Aggregation and deposition kinetics of fullerene (C-60) nanoparticles. *Langmuir, 22*, 10994-11001.

Chen, K. L. and Elimelech, M. (2007), Influence of humic acid on the aggregation kinetics of fullerene (C-60) nanoparticles in monovalent and divalent electrolyte solutions. *Journal of Colloid and Interface Science, 309*, 126-134.

Chinnapongse, S. L., MacCuspie, R. I., Hackley, V. A. (2011). Persistence of singly dispersed silver nanoparticles in natural freshwaters, synthetic seawater, and simulated estuarine waters. *Sci. Total Environ., 409 (12)*, 2443-2450.

Choi, O. and Hu, Z. (2008), Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria, *Environmental Science & Technology, 42*, 4583-4588.

Chowdhury, I., Cwiertny, D. M., Walker, S. L. (2012). Combined Factors Influencing the Aggregation and Deposition of nano-TiO2 in the Presence of Humic Acid and Bacteria. *Environ. Sci. Technol., 46 (13*), 6968-6976.

Ciglenecki, I., Cosovic, B., Vojvodic, V., Plavsic, M., Furic, K., Minacci, A. and Baldi, F. (2000).The role of reduced sulfur species in the coalescence of polysaccharides in the Adriatic Sea, Marine Chemistry 71, 233-249.

Clark, R.M. and Besterfield-Sacre, M.E., (2009). A New Approach to Hazardous Materials Transportation Risk Analysis: Decision Modeling to Identify Critical Variables, *Risk Anal., 29*, 344-354.

Cleveland, D., Long, S.E., Pennington, P.L., Cooper, E., Fulton, M.H., Scott. G.I., Brewer. T., Davis. J., Petersen. E.J., Wood, L. (2012). Pilot estuarine mesocosm study on the environmental fate of silver nanomaterials leached from consumer products. *Sci Total Environ, 421-422*, 267-272.

Colman, B.P., Arnaout, C.L., Anciaux, S., Gunsch, C.K., Hochella, M.F., Kim, B., Lowry, G.V., McGill, B.M., Reinsch, B.C., Richardson, C.J., Unrine, J.M, Wright, J.P., Yin, L.Y., Bernhardt, E.S. (2013). Low concentrations of silver nanoparticles in biosolids cause adverse ecosystem responses under realistic field scenario. *Plos One 8.*

Cornelis, G., Kirby, J.K., Beak, D., Chittleborough, D., McLaughlin, M.J. (2010). A Method for determination of retention of silver and cerium oxide manufactured nanoparticles in soils. *Environ Chem, 7*, 298–308.

Cornelis, G., Thomas, D.C.M., McLaughlin, M.J., Kirby, J.K, Beak, D.G, Chittleborough, D. (2012). Retention and dissolution of engineered silver nanoparticles in natural soils. *Soil Sci Soc Am J, 76*, 891–902.

Cumberland, S. A., Lead, J. R. (2009). Particle size distributions of silver nanoparticles at environmentally relevant conditions. *J Chromatogr A, 1216 (52),* 9099-9105.

Darbra, R.M., Eljarrat, E., Barcelo, D. (2008). How to measure uncertainties in environmental risk assessment. *Trac-Trends in Analytical Chemistry, 27(4),* 377-385.

Dale, A.L., Lowry, G.V., Casman, E. a, (2013). Modeling Nanosilver Transformations in Freshwater Sediments Modeling Nanosilver Transformations in Freshwater Sediments Center for Environmental Implications of Nanotechnology. *Environ. Sci. Technol. Just Accep.*

Das, P., Metcalfe, C.D., Xenopoulos, M. A. (2014). Interactive E ff ects of Silver Nanoparticles and Phosphorus on Phytoplankton Growth in Natural Waters.

De Juan Luna, A., Fernandez Diego, I., Perez Hernando, M.A., Arroyabe Hernaez, P.F., Ruiz Puente, C.M., (2005). Analysis and development of factors to locate sustainable

industrial areas, *WSEAS Transactions on Environment and Development, 2(1),* 258-264.

Delay, M., Dolt, T., Woellhaf, A., Sembritzki, R., Frimmel, F.H. (2011). Interactions and stability of silver nanoparticles in the aqueous phase: Influence of natural organic matter (NOM) and ionic strength. *J. Chromatogr. A, 1218,* 4206–4212. doi:10.1016/j.chroma.2011.02.074

Dick, W., Belluz, D. and Current, C. L., (1999). Directions in Risk Assesment and Management, Accessed: 20 November 2008, http://www.neram.ca

Didden, W., Römbke, J. (2001). Enchytraeids as indicator organisms for chemical stress in terrestrial ecosystems. *Ecotox Environ Safe, 50,* 25-43.

Di Marzioa, W.D., Saenz, M., Alberdia, J., Tortorellia M. and Silvana, G., (2005). Risk assessment of domestic and industrial effluents unloaded into a freshwater environment, *Ecotoxicol. Environ. Saf., 61,* 380-391.

Domingos, R. F., Peyrot, C., Wilkinson, K. J. (2010). Aggregation of titanium dioxide nanoparticles: role of calcium and phosphate. *Environ. Chem., 7 (1),* 61-66.

Domingos, R. F., Tufenkji, N., Wilkinson, K. J. (2009). Aggregation of Titanium Dioxide Nanoparticles: Role of a Fulvic Acid. *Environ. Sci. Technol., 43 (5*), 1282-1286.

Dror-Ehre, A., Mamane, H., Belenkova, T., Markovich, G. and Adin, A. (2009). Silver nanoparticle-E. coli colloidal interaction in water and effect on E-coli survival, *Journal of Colloid and Interface Science, 339,* 521-526.

Duffus, J.H. and Worth, H.G.J., (1996). Fundamental Toxicology for Chemists, The Royal Society of Chemistry, Cambridge, UK.

EC, (2006). Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), in E.C. (EC) (Editor), Vol. Regulation (EC) No 1907/2006, Official Journal of the European Union**.**

El Badawy, A. M., Luxton, T. P., Silva, R. G., Scheckel, K. G., Suidan, M. T., Tolaymat, T. M. (2010). Impact of Environmental Conditions (pH, Ionic Strength, and Electrolyte Type) on the Surface Charge and Aggregation of Silver Nanoparticles Suspensions. *Environ. Sci. Technol., 44 (4),* 1260-1266.

Eom, H.J., Ahn, J.M., Kim, Y., Choi, J. (2013). Hypoxia inducible factor-1 (HIF-1)-flavin containing monooxygenase-2 (FMO-2) signaling acts in silver nanoparticles and silver ion toxicity in the nematode Caenorhabditis elegans. *Toxicol. Appl. Pharmacol, 270,*106–113.

EPA (2007). Nanotechnology White Paper, Science Policy Counci U.S. Environmental Protection Agency Washington, DC

EPA (2009). Nanomaterial Research Strategy, Office of Research and Development

U.S. Environmental Protection Agency Washington, D.C.

EU (2011) Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). O. J. L 275:38–40

Fabiano, B., Curro, F., Reverberi, A.P. and Partorino, R., (2005). Dangerous good transportation by road: from risk analysis to emergency planning, *J. Loss Prevent. Proc., 18,* 403-413.

Fabrega, J., Luoma, S.N., Tyler, C.R., Galloway, T.S., Lead, J.R. (2011). Silver nanoparticles: Behaviour and effects in the aquatic environment. *Environ. Int. 37*, 517–531 doi:10.1016/j.envint.2010.10.012

Fabrega, J., Renshaw, J.C., Lead, J.R. (2009). Interactions of Silver Nanoparticles with. *Environ. Sci. Technol., 43*, 9004–9009.

Farré, M., Sanchís, J., and Barceló, D. (2011). Analysis and assessment of the occurrence, the fate and the behavior of nanomaterials in the environment. TrAC - Trends in Analytical *Chemistry, 30(3)*, 517–527. doi:10.1016/j.trac.2010.11.014

Fortin, C. and Campbell, P. G. C. (2000). Silver uptake by the green alga Chlamydomonas reinhardtii in relation to chemical speciation: Influence of chloride, *Environmental Toxicology and Chemistry, 19*, 2769-2778.

French, A.J., Jacobson, A.R., Kim, B., Isley, S.L., Penn, R.L., Baveye, P.C. (2009). Influence of Ionic Strength, pH, and Cation Valence on Aggregation Kinetics of Titanium Dioxide Nanoparticles. *Environ. Sci. Technol., 43*, 1354-1359.

Gajewicz, A., Rasulev, B., Dinadayalane, T.C., Urbaszek, P., Puzyn, T., Leszczynska, D., Leszczynski, J. (2012). Advancing risk assessment of engineered nanomaterials: Application of computational approaches. *Adv. Drug Deliv. Rev., 64*, 1663–1693. doi:10.1016/j.addr.2012.05.014

Gao, J., S, Y., a, H., Vl, L., Y, W., G, B., Jc, B. (2009). Dispersion and toxicity of selected manufactured nanomaterials in natural river water samples : effects of water chemical composition . Episodic discharge of lead , copper and antimony from a Norwegian small arm shooting The effects of Anabaena spiroides . *J. Environ. Sci., 43*, 1–153.

Gao, J., Powers, K., Wang, Y., Zhou, H., Roberts, S.M., Moudgil, B.M., Koopman, B., Barber, D.S. (2012). Influence of Suwannee River humic acid on particle properties and toxicity of silver nanoparticles. *Chemosphere ,89*, 96–101.

Garner, K.L., Keller, A. a., (2014). Emerging patterns for engineered nanomaterials in the environment: A review of fate and toxicity studies. *J. Nanoparticle Res., 16*. doi:10.1007/s11051-014-2503-2

Gentile, M., Rogers, W.J. and Mannan, M.S., (2003). Development of a Fuzzy Logic-Based Inherent Safety Index, Process Saf. Environ. Prot., 444-456.

Geranio, L., Heuberger, M., and Nowack, B. (2009). The behavior of silver nanotextiles during washing. *Environmental Science and Technology, 43(21),* 8113–8118 doi:10.1021/es9018332

Gomes, S.I.L., Amadeu, A.M.V.M., Soares, A.M.V.M., Scott-Fordsmand, J.J., Amorim. M.J.B. (2013). Mechanisms of response to silver nanoparticles on Enchytraeus albidus (Oligochaeta): Survival, reproduction and gene expression profile. *J Haz Mat, 254-255*, 336-344.

Gondikas, A. P., Jang, E. K. & Hsu-Kim, H. (2010). Influence of amino acids cysteine and serine on aggregation kinetics of zinc and mercury sulfide colloids, *Journal of Colloid and Interface Science, 347*, 167-171.

Gondikas, A. P., Morris, A., Reinsch, B. C., Marinakos, S. M., Lowry, G. V., and Hsu-Kim, H. (2012). Cysteine-induced modifications of zero-valent silver nanomaterials: Implications for particle surface chemistry, aggregation, dissolution, and silver speciation. Environmental Science and Technology, 46(13), 7037–7045 doi:10.1021/es3001757

Gondikas, A. P., Von Der Kammer, F., Reed, R. B., Wagner, S., Ranville, J. F., and Hofmann, T. (2014). Release of TiO2 nanoparticles from sunscreens into surface waters: A one-year survey at the old danube recreational lake. *Environmental Science and Technology, 48(10),* 5415–5422 doi:10.1021/es405596y

Gottschalk, F., Sondere, T., Schols, R., and Nowack, B. (2009). Modeled Environmental Concentrations of Engineered Nanomaterials for Different Regions. *Environmental Science & Technology, 43(24),* 9216–9222.

Gottschalk, F., Sonderer, T., Scholz, R.W., Nowack, B. (2010). Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis. *Environ Toxicol Chem, 29,* 1036–1048.

Gottschalk, F., Nowack, B. (2011). The release of engineered nanomaterials to the environment. *J Environ. Monitor., 13 (5),* 1145-1155.

Gottschalk, F., Sun, T.Y., Nowack, B. (2013). Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. *Environ. Poll., 181,* 287-300.

Grieger, K.D., Hansen, S.F., Sørensen, P.B., Baun, A. (2011). Conceptual modeling for identification of worst case conditions in environmental risk assessment of nanomaterials using nzvi and C 60 as case studies. *Sci. Total Environ., 409,* 4109–4124 doi:10.1016/j.scitotenv.2011.06.021

Gubbins, E. J., Batty, L. C. & Lead, J. R. (2011). Phytotoxicity of silver nanoparticles to Lemna minor. *Environmental Pollution 159,* 1551-1559.

Gunasekera, M.Y. and Edwards, D.W., (2003). Estimating the Environmental Impact of Catastrophic Chemical Releases to the Atmosphere: An Index Method for Ranking Alternative Chemical Process Routes, *Process Saf. Environ. Prot., 81,* 463-474.

Gunasekera, M.Y. and Edwards, D.W., (2006). Assessing the inherent atmospheric environmental friendliness of chemical process routes: An unsteady state distribution approach for a catastrophic release, *Chemosphere, 30,* 744-757.

Haanstra, L., Doelman, P., Oude Voshaar, J.H. (1985). The use of sigmoidal dose response curves in soil ecotoxicological research. *Plant Soil ,84,* 293–297

Hamilton, M.A., Russo, R.C., Thurston, R.V. (1977). Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ Sci Technol, 11,*714–719.

Hammes, J., Gallego-Urrea, J.A., Hasselöv, M. (2013). Geographically distributed classification of surface water chemical parameters influencing fate and behavior of nanoparticles and colloid faciliated contaminant transport. *Water Res.*

Handy, R. D., von der Kammer, F., Lead, J. R., Hassellov, M., Owen, R. and Crane, M. (2008). The ecotoxicology and chemistry of manufactured nanoparticles, *Ecotoxicology, 17,* 287-314.

Hansen, P.D., (2007). Risk assessment of emerging contaminants in aquatic systems, *Trends Anal. Chem.,* 26, 1095-1099.

Hassim, M. H. and Edwards, D. W., (2006). Development of a methodology for assessıng ınherent occupatıonal health hazards, *Process Saf Environ Protect, 84(B5),* 378-390.

Holt, M.S., Fox, K., Grieubach, E., Johnsen, S., Kinnunen, J., Lecloux, A., Murray-Smith, R., Peterson, D.R., Schroder, R., Silvani, M., tenBerge, W.F.J., Toy R.J.

and Feijtel, T.C.M., (2000). Monitoring, modelling and environmental exposure assessment of industrial chemicals in the aquatic environment, *Chemosphere, 41,* 1799-1808.

He, E., Van Gestel, C.A.M. (2013). Toxicokinetics and toxicodynamics of nickel in Enchytraeus crypticus. *Environ Toxicol Chem, 32*,1835-1841.

Heckmann, L.H., Hovgaard, M.B., Sutherland, D.S., Autrup, H., Besenbacher, F., Scott-Fordsmand, J.J. (2011). Limit-test toxicity screening of selected inorganic nanoparticles to the earthworm Eisenia fetida. *Ecotoxicology 20,* 226-233.

Hedberg, J., Skoglund, S., Karlsson, M.E., Wold, S., Wallinder, I.O., Hedberg, Y. (2014). Sequential studies of silver released from silver nanoparticles in aqueous media simulating sweat, laundry detergent solutions and surface water. *Environ Sci Technol, 48*,7314-7322.

Hennebert, P., Avellan, A., Yan, J., Aguerre-Chariol, O. (2013). Experimental evidence of colloids and nanoparticles presence from 25 waste leachates. *Waste Manag ,33*, 1870–1881 doi:10.1016/j.wasman.2013.04.014

Hiriart-Baer, V. P., Fortin, C., Lee, D.-Y. & Campbell, P. G. C. (2006). Toxicity of silver to two freshwater algae, Chlamydomonas reinhardtii and Pseudokirchneriella subcapitata, grown under continuous culture conditions: Influence of thiosulphate, *Aquatic Toxicology, 78,* 136-148.

Holden, P. A, Klaessig, F., Turco, R. F., Priester, J. H., Rico, C. M., Avila-arias, H., … Gardea-torresdey, J. L. (2014). Evaluation of Exposure Concentrations Used in Assessing Manufactured Nanomaterial Environmental Hazards: Are They Relevant? *Environmental Science & Technology, 48*, 10541–10551.

Hsiao, I.L., Hsieh, Y.K., Wang, C.F., Chen, I.C., Huang, Y.J. (2015). Trojan-Horse mechanism in the cellular uptake of silver. *Environ Sci Technol, 49*, 3813-3821

Hu, C., Mei, L., Wang, W., Cui, Y., Chen, J., Yang, L. (2012). Ecotoxicity of silver nanoparticles on earthworm Eisenia fetida: responses of the antioxidant system, acid phosphatase and ATPase. *Toxicol Environ Safe, 94*, 732-741.

Hunt, G., Lynch, I., Cassee, F., Handy, R.D., Fernandes, T.F., Berges, M., Kuhlbusch, T. a J., Dusinska, M., Riediker, M. (2013). Towards a consensus view on understanding nanomaterials hazards and managing exposure: Knowledge gaps and recommendations. *Materials (Basel). 6*, 1090–1117. doi:10.3390/ma6031090

Huynh, K. A., Chen, K. L. (2011). Aggregation Kinetics of Citrate and Polyvinylpyrrolidone Coated Silver Nanoparticles in Monovalent and Divalent Electrolyte Solutions. *Environ. Sci. Technol., 45 (13)*, 5564-5571.

Hwang, E. T., Lee, J. H., Chae, Y. J., Kim, Y. S., Kim, B. C., Sang, B.-I. and Gu, M. B. (2008). Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria, *Small, 4*, 746-750.

ISO 1996. Water quality- Determination of the inhibition of the mobility of Daphnia magna. ISO 6341:1996. Straus (Cladocera, Crustacea) – Acute toxicity test. International Organization for Standardization. Geneva, Switzerland.

ISO 2004. Soil quality-Effects of pollutants on Enchytraeidae (Enchytraeus sp.) - Determination of effects on reproduction and survival. ISO 16387: International Organization for Standardization. Geneva, Switzerland.

Jin, X., Li, M., Wang, J., Marambio-Jones, C., Peng, F., Huang, X., Damoiseaux, R., Hoek, E. M. V. (2010). High-Throughput Screening of Silver Nanoparticle Stability and Bacterial Inactivation in Aquatic Media: Influence of Specific Ions. *Environ. Sci. Technol, 44 (19),* 7321-7328.

Johnson, A.C., Jürgens, M.D., Lawlor, A.J., Cisowska, I., Williams, R.J. (2014). Particulate and colloidal silver in sewage effluent and sludge discharged from British wastewater treatment plants. *Chemosphere, 112*, 49-55.

Jung, S.-K., Qu, X., Aleman-Meza, B., Wang, T., Riepe, C., Liu, Z., Li, Q., Zhong, W. (2015). Multi-endpoint, High-Throughput Study of Nanomaterial Toxicity in Caenorhabditis elegans. *Environ. Sci. Technol*. doi:10.1021/es5056462

Kaegi, R., Wagner, T., Hetzer, B., Sinnet, B., Tzuetkov, G. and Boller, M. (2008). Size, number and chemical composition of nanosized particles in drinking water determined by analytical microscopy and LIBD, *Water Research, 42*, 2778-2786.

Kaegi, R., Voegelin, A., Sinnet, B., Zuleeg, S., Hagendorfer, H., Burkhardt, M. and Siegrist, H. (2011). Behavior of Metallic Silver Nanoparticles in a Pilot Wastewater Treatment Plant. *Environmental Science & Technology, 45,* 3902-3908.

Kaegi, R., Voegelin, A., Ort, C., Sinnet, B., Thalmann, B., Krismer, J., Hagendorfer, H., Elumelu, M., Mueller, E. (2013). Fate and transformation of silver nanoparticles in urban wastewater systems. *Water Res, 47*, 3866-3877.

Keller, A. a., Vosti, W., Wang, H., & Lazareva, A. (2014). Release of engineered nanomaterials from personal care products throughout their life cycle. *Journal of Nanoparticle Research, 16(7)* Doi:10.1007/s11051-014-2489-9

Keller, A. a., Wang, H., Zhou, D., Lenihan, H. S., Cherr, G., Cardinale, B. J., … Zhaoxia, J. I. (2010). Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environmental Science and Technology, 44(6),* 1962–1967. http://doi.org/10.1021/es902987d

Kennedy, A.J., Hull, M.S., Bednar, A.J., Goss, J.D., Gunter, J.C., Bouldin, J.L., Vikesland, P.J., Steevens, J. A. (2010). Fractionating nanosilver: Importance for determining toxicity to aquatic test organisms. *Environ. Sci. Technol., 44,* 9571–9577 doi:10.1021/es1025382

Khan, F.I. and Abbasi, S.A., (2001). Risk analysis of a typical chemical industry using ORA procedure, J. Loss Prevent. Proc., 14, 43-59.

Khan, F. I., Sadiq, R. and Husain, T., (2002). *GreenPro-I*: a risk-based life cycle assessment and decisionmaking methodology for process plant design, *Environ. Modell. Softw., 17*, 669-692.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J.-H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., Hwang, C.-Y., Kim, Y.-K., Lee, Y.-S., Jeong, D. H. and Cho, M.-H. (2007). Antimicrobial effects of silver nanoparticles, *Nanomedicine-Nanotechnology Biology and Medicine, 3*, 95-101.

Kirkegaard, P., Hansen, S.F., Rygaard, M. (2015). Potential exposure and treatment efficiency of nanoparticles in water supplies based on wastewater reclamation. *Environ. Sci. Nano, 2*, 191–202 doi:10.1039/C4EN00192C

Kittler, S., Greulich, C., Diendorf, J., Koeller, M. & Epple, M. (2010). Toxicity of Silver Nanoparticles Increases during Storage Because of Slow Dissolution under Release of Silver Ions. *Chemistry of Materials, 22*, 4548-4554.

Klaine, S. J., Alvarez, P. J. J., Batley, G. E., Fernandes, T. F., Handy, R. D., Lyon, D. Y., . . . Lead, J. R. (2008). Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. Environmental Toxicology and Chemistry, 27(9), 1825-1851. doi: 10.1897/08-090.1

Klitzke, S., Metreveli, G., Peters, A., Schaumann, G.E., Lang, F. (2015). The fate of silver nanoparticles in soil solution -Sorption of solutes and aggregation. *Sci Total Environ, In Press*, doi:10.1016/j.scitotenv.2014.10.108

Krueger, T., Page, T., Hubacek, K., Smith, L., Hiscock, K. (2012). The role of expert opinion in environmental modelling. *Environmental Modeling and Software, 36*, 4-18.

Kühnel, D., Marquardt, C., Nau, K., Krug, H. F., Mathes, B., and Steinbach, C. (2014). Environmental impacts of nanomaterials : providing comprehensive information on exposure , transport and ecotoxicity - the project DaNa2 . 0.

Kvtek, L., Panc, A., Soukupov, J., Kol, M., Prucek, R., Holecov, M., … Nps, N. (2008). Effect of Surfactants and Polymers on Stability and Antibacterial Activity of Silver Nanoparticles (NPs) Effect of Surfactants and Polymers on Stability and Antibacterial Activity of Silver. *Society*, 5825–5834. doi:10.1021/jp711616v

Kwak, J., Lee, W. M., Kim, S. W. and An, Y. J. (2014). Interaction of citrate-coated silver nanoparticles with earthworm coelomic fluid and related cytotoxicity in *Eisenia andrei*. *Journal of Applied Toxicology*, doi:10.1002/jat.2993

Lazareva, A., and Keller, A. A. (2014). Estimating potential life cycle releases of engineered nanomaterials from waste water treatment plants. *ACS Sustainable Chemistry & Engineering* doi:10.1021/sc500121w

Le Faucheur, S. V., Behra, R. & Sigg, L. (2005). Thiol and metal contents in periphyton exposed to elevated copper and zinc concentrations: A field and microcosm study. *Environmental Science & Technology, 39*, 8099-8107.

Lee, D. Y., Fortin, C. and Campbell, P. G. C. (2004). Influence of chloride on silver uptake by two green algae, Pseudokirchneriella subcapitata and Chlorella pyrenoidosa, *Environmental Toxicology and Chemistry, 23*, 1012-1018.

Leeuwen, C. J. and Vermeire, T. G. (2007). Risk Assesment of Chemicals: An Introduction, Springer, The Netherlands.

Levard, C., Reinsch, B. C., Michel, F. M., Oumahi, C., Lowry, G. V. and Brown, G. E., Jr. (2011). Sulfidation Processes of PVP-Coated Silver Nanoparticles in Aqueous Solution: Impact on Dissolution Rate. *Environmental Science & Technology, 45*, 5260-5266.

Levard, C., Hotze, E.M., Colman, B.P., Dale, A.L., Truong, L., Yang, X.Y., Bone, A.J., Brown, G.E., Tanguay, R.L., Di Giulio, R.T., Bernhardt, E.S., Meyer, J.N., Wiesner, M.R., Lowry, G. V. (2013). Sulfidation of silver nanoparticles: Natural antidote to their toxicity. *Environ. Sci. Technol., 47*, 13440–13448. doi:10.1021/es403527n

Lewinski, R., Graf, C., Langer, B., Flesch, R., Bresch, H., Wassermann, B. and Ruehl, E. (2009). Size-effects in clusters and free nanoparticles probed by soft X-rays, *European Physical Journal-Special Topics, 169*, 67-72.

Li, J.B., Huang, G.H., Zeng, G.M., Maqsood, I. and Huang, Y.F., (2007). An integrated fuzzy-stochastic modeling approach for risk assessment of groundwater contamination, *J. Environ. Manage., 82*, 173-188.

Li, P., Wang, X., Allinson, G., Li, X. and Xiong, X., (2008). Risk assessment of heavy metals in soil previously irrigated with industrial wastewater in Shenyang, China, *J. Hazard. Mater., 161*, 516-521.

Li, X., Lenhart, J. J., Walker, H. W. (2010). Dissolution-Accompanied Aggregation Kinetics of Silver Nanoparticles. *Langmuir, 26 (22)*, 16690-16698.

Liau, S. Y., Read, D. C., Pugh, W. J., Furr, J. R. and Russell, A. D. (1997). Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions, *Letters in Applied Microbiology, 25*, 279-283.

Linkov, I., Steevens, J., Adlakha-Hutcheon, G., Bennett, E., Chappell, M., Colvin, V., Davis, J.M., Davis, T., Elder, A., Foss Hansen, S., Hakkinen, P.B., Hussain, S.M., Karkan, D., Korenstein, R., Lynch, I., Metcalfe, C., Ramadan, A.B., Satterstrom, F.K. (2009). Emerging methods and tools for environmental risk assessment, decision-making, and policy for nanomaterials: Summary of NATO Advanced Research Workshop. *J. Nanoparticle Res., 11*, 513–527. doi:10.1007/s11051-008-9514-9

Liu, J. and Hurt, R.H. (2010). Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ Sci Technol, 44*, 2169–75.

Liu, J., Sonshine, D. A., Shervani, S. and Hurt, R. H. (2010). Controlled Release of Biologically Active Silver from Nanosilver Surfaces, *Acs Nano, 4*, 6903-6913.

Liu, W., Zhou, Q.F., Liu, J.Y., Fu, J.J., Liu, S.J., Jiang, G. Bin. (2011). Environmental and biological influences on the stability of silver nanoparticles. *Chinese Sci. Bull. 56*, 2009–2015 doi:10.1007/s11434-010-4332-8

Lock, K., Janssen, C.R. (2001). Zinc and cadmium body burdens in terrestrial oligochaetes: Use and significance in environmental risk assessment. *Environ Toxicol Chem, 20*, 2067–2072.

Lombi, E., Donner, E., Scheckel, K.G., Sekine, R., Lorenz, C., Von Goetz, N., Nowack, B. (2014). Silver speciation and release in commercial antimicrobial textiles as influenced by washing. *Chemosphere, 111*, 352-358.

Long, T. C., Saleh, N., Tilton, R. D., Lowry, G. V. & Veronesi, B. (2006). Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): Implications for nanoparticle neurotoxicity, *Environmental Science & Technology 4*0, 4346-4352.

Loosli, F., Le Coustumer, P., Stoll, S. (2015). Impact of alginate concentration on the stability of agglomerates made of TiO2 engineered nanoparticles: Water hardness and pH effects. *J. Nanoparticle Res. 17*, 44 doi:10.1007/s11051-015-2863-2

Lowry, G. V., Hotze, E. M., Bernhardt, E. S., Dionysiou, D. D., Pedersen, J. A., Wiesner, M. R. and Xing, B. (2010). Environmental Occurrences, Behavior, Fate, and Ecological Effects of Nanomaterials: An Introduction to the Special Series. *Journal of Environmental Quality, 39*, 1867-1874.

Louie, S., Tilton, R., & Lowry, G. (2013). Effects of molecular weight distribution and chemical properties of natural organic matter on gold nanoparticle aggregation. *Environmental Science & …. Retrieved from* http://pubs.acs.org/doi/abs/10.1021/es400137x

Lu, W., Senapati, D., Wang, S., Tovmachenko, O., Singh, A. K., Yu, H. and Ray, P. C. (2010). Effect of surface coating on the toxicity of silver nanomaterials on human skin keratinocytes, *Chemical Physics Letters, 487,* 92-96.

Ma, R., Levard, C., Marinakos, S. M., Cheng, Y., Liu, J., Michel, F. M., Brown, G. E., Jr. & Lowry, G. V. (2012). Size-Controlled Dissolution of Organic-Coated Silver Nanoparticles, *Environmental Science & Technology, 46,* 752-759.

Ma, R., Levard, C., Judy, J.D., Unrine, J.M., Martin, B., Jefferson, B., Lowry, G.V., Durenkamp, M., Lowry, G. V. (2013). Fate of zinc oxide and silver nanoparticles in a pilot waste water treatment plant and in processed biosolids Fate of zinc oxide and silver nanoparticles in a pilot waste water treatment plant and in processed biosolids. *Environ. Sci. Technol. Just Accep.*

MacCuspie, R. I., Rogers, K., Patra, M., Suo, Z. Y., Allen, A. J., Martin, M. N., Hackley, V. A. (2011). Challenges for physical characterization of silver nanoparticles under pristine and environmentally relevant conditions. *J Environ. Monitor., 13 (5),* 1212-1226.

Marcoux, M. A., Matias, M., Olivier, F., Keck, G. (2013). Review and prospect of emerging contaminants in waste - Key issues and challenges linked to their presence in waste treatment schemes: General aspects and focus on nanoparticles. *Waste Manag., 33,* 2147–2156. doi:10.1016/j.wasman.2013.06.022

McShane, H., Sarrazin, M., Whalen, J.K., Hendershot, W.H., Sunahara, G.I. (2012). Reproductive and behavioral responses of earthworms exposed to nano-sized titanium dioxide in soil. *Environ. Toxicol. Chem., 31,* 184–193. doi:10.1002/etc.714

Meyer, J. N., Lord, C. A., Yang, X. Y., Turner, E. A., Badireddy, A. R., Marinakos, S. M., Chilkoti, A., Wiesner, M. R. and Auffan, M. (2010). Intracellular uptake and associated toxicity of silver nanoparticles in Caenorhabditis elegans. *Aquatic Toxicology, 100,* 140-150.

Miseljic, M., and Olsen, S. I. (2014). Life-cycle assessment of engineered nanomaterials: A literature review of assessment status. *Journal of Nanoparticle Research, 16(6)* doi:10.1007/s11051-014-2427-x

Mitrano, D. M., Motellier, S., Clavaguera, S., & Nowack, B. (2015). Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products. *Environment International, 77,* 132–147 doi:10.1016/j.envint.2015.01.013

Money, E.S., Reckhow, K.H., Wiesner, M.R. (2012). The use of Bayesian networks for nanoparticle risk forecasting: Model formulation and baseline evaluation. *Sci. Total Environ., 426,* 436–445 doi:10.1016/j.scitotenv.2012.03.064

Moore, G.S., (2007). Living with the Earth: Concepts in Environmental Health Science, CRC Pres, Boca Raton.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramirez, J. T. and Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles, *Nanotechnology, 16,* 2346-2353.

Mueller, N. C., Buha, J., Wang, J., Ulrich, A., and Nowack, B. (2013). Modeling the flows of engineered nanomaterials during waste handling. *Environmental Science: Processes & Impacts, 15(1),* 251 doi:10.1039/c2em30761h

Mukherjee, B., and Weaver, J. W. (2010). Aggregation and charge behavior of metallic and nonmetallic nanoparticles in the presence of competing similarly-charged inorganic

ions. *Environmental Science and Technology, 44(9),* 3332–3338 doi:10.1021/es903456e

Mylon, S. E., Chen, K. L. & Elimelech, M. (2004). Influence of natural organic matter and ionic composition on the kinetics and structure of hematite colloid aggregation: Implications to iron depletion in estuaries. *Langmuir, 20,* 9000-9006.

Navarro, E., Piccapietra, F., Wagner , B., Marconi, F., Kaegi , R., Odzak , N., Sigg , L., Behra, R. (2008). Toxicity of silver nanoparticles to Chlamydomonas reinhardtii. *Environ Sci Technol, 42,* 8959-8964.

Navarro, D,A,, Kirby, J.K., McLaughlin, M.J., Waddington, L., Kookana, R.S. (2014). Remobilisation of silver and silver sulphide nanoparticles in soils. *Environ Pollut, 193,*102-110.

Nel, A. E., Maedler, L., Velegol, D., Xia, T., Hoek, E. M. V., Somasundaran, P., Klaessig, F., Castranova, V. and Thompson, M. (2009). Understanding biophysicochemical interactions at the nano-bio interface. *Nature Materials, 8,* 543-557.

Nowack, B., Ranville, J.F., Diamond., S., Gallego-Urrea, J.A., Metcalfe, C., Rose, J., Horne, N., Koelmans, A.A., Klaine, S.J. (2012). Potential scenarios for nanomaterial release and subsequent alteration in the environment. *Environ Toxicol Chem, 31,*50-59.

OECD. (2004). Guideline for the testing of chemical 220-Enchytraeid reproduction test. Organisation for Economic Co-operation and Development. Paris, France.

O'Brien, N., Cummins, E. (2010). Nano-Scale Pollutants: Fate in Irish Surface and Drinking Water Regulatory Systems. *Hum. Ecol. Risk Assess. An Int. J., 16,* 847–872 doi:10.1080/10807039.2010.501270

Odzak, N., Kistler, D., Behra, R., and Sigg, L. (2014). Dissolution of metal and metal oxide nanoparticles in aqueous media. *Environmental Pollution, 191,* 132–138. doi:10.1016/j.envpol.2014.04.010

Ottofuelling, S., Von Der Kammer, F., & Hofmann, T. (2011). Commercial titanium dioxide nanoparticles in both natural and synthetic water: Comprehensive multidimensional testing and prediction of aggregation behavior. *Environmental Science and Technology, 45(23),* 10045–10052. doi:10.1021/es2023225

Panacek, A., Kvitek, L., Prucek, R., Kolar, M., Vecerova, R., Pizurova, N., Sharma, V. K., Nevecna, T. j. and Zboril, R. (2006). Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. *Journal of Physical Chemistry B, 110,* 16248-16253.

Papadakis, G. A. and Chalkidou, A. A., (2008). The exposure–damage approach in the quantification of occupational risk in workplaces involving dangerous substances, *Saf. Sci., 46,* 972-991.

Pavagadhi, S., Sathishkumar, M., Balasubramanian, R. (2014). Uptake of Ag and TiO2 nanoparticles by zebrafish embryos in the presence of other contaminants in the aquatic environment. *Water Res., 55,* 280–291 doi:10.1016/j.watres.2014.02.036

Piccinno, F., Gottschalk, F., Seeger, S., Nowack, B. (2012). Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. *J. Nanoparticle Res., 14* doi:10.1007/s11051-012-1109-9

Praetorius, A., Tufenkji, N. (2014). The road to nowhere: equilibrium partition coefficients for nanoparticles. *Environ. … 317–323* doi:10.1039/c4en00043a

Ratte, H. T. (1999). Bioaccumulation and toxicity of silver compounds: A review. *Environmental Toxicology and Chemistry, 18*, 89-108.

Ribeiro, F., Gallego-Urrea, J.A., Jurkschat, K., Crossley, A., Hassellöv, M., Taylor, C., Soares, A.M.V.M., Loureiro, S. (2014). Silver nanoparticles and silver nitrate induce high toxicity to Pseudokirchneriella subcapitata, Daphnia magna and Danio rerio. *Sci. Total Environ., 466-467*, 232–241. doi:10.1016/j.scitotenv.2013.06.101

Rick VandeVoort, A., Tappero, R., Arai, Y. (2014). Residence time effects on phase transformation of nanosilver in reduced soils. *Environ. Sci. Pollut. Res., 21*, 7828–7837. doi:10.1007/s11356-014-2743-9

Roh, J.Y., Sim, S. J., Yi, J., Park, K., Chung, K. H., Ryu, D.Y. and Choi, J. (2009). Ecotoxicity of Silver Nanoparticles on the Soil Nematode Caenorhabditis elegans Using Functional Ecotoxicogenomics. *Environmental Science & Technology, 43*, 3933-3940.

Royce, S.G., Mukherjee, D., Cai, T., Xu, S.S., Alexander, J. A., Mi, Z., Calderon, L., Mainelis, G., Lee, K., Lioy, P.J., Tetley, T.D., Chung, K.F., Zhang, J., Georgopoulos, P.G. (2014). Modeling population exposures to silver nanoparticles present in consumer products. *J. Nanoparticle Res., 16*, doi:10.1007/s11051-014-2724-4

Ruiz Puente, C.M., Fernandez Diego, I., Santa Maria, J.J.O., Perez Hernando, M.A., Arroyabe Hernaez, P.F., (2007). The development of a new methodology based on GIS and fuzzy logic to locate sustainable industrial areas, 10th AGILE International Conference on GIS Science. Aalborg University, Denmark.

Saaty, T.L., 2001. Decision making for leaders: the analytic hierarchy process for decisions in a complex world, RWS Publications, Pittsburgh

Saaty, T.L. (1994). Fundemantels of Decision Making and Priority Theory with the AHP, RWS Publications, Pittsburgh.

Saaty, T.L. (1990). The analytic hierarchy process: planning, priority setting, resource allocation. RWS Publications, Pittsburgh.

Sadiq, R., Khan, F. I. and Veitch, B., (2005). Evaluating offshore technologies for produced water management using *GreenPro-I*—a risk-based life cycle analysis for green and clean process selection and design, *Comput. Chem. Eng.*, *29*, 1023-1039.

Santorufo L., Van Gestel C.A.M., Maisto, G. (2013). Ecotoxicological assessment of metal-polluted urban soils using bioassays with three soil invertebrates. *Chemosphere, 88*, 418-425.

Saouter, E., Pittinger, C., and Feijtel, T. (2001). Aquatic Environmental Impact of Detergents: From Simple to More Sophisticated Models. *Ecotoxicology and Environmental Safety, 50*, 153-159.

Schaumann, G. E., Philippe, A., Bundschuh, M., Metreveli, G., Klitzke, S., Rakcheev, D., … Vogel, H.J. (2014). Understanding the fate and biological effects of Ag- and TiO2-nanoparticles in the environment: The quest for advanced analytics and interdisciplinary concepts. *Science of The Total Environment*, doi:10.1016/j.scitotenv.2014.10.035

Schlich, K., Klawonn, T., Terytze, K., and Hund-Rinke, K. (2013). Effects of silver nanoparticles and silver nitrate in the earthworm reproduction test. *Environmental Toxicology and Chemistry, 32(1)*, 181–188 doi:10.1002/etc.2030

Scown, T. M., van Aerle, R. and Tyler, C. R. (2010). Review: Do engineered nanoparticles pose a significant threat to the aquatic environment? *Critical Reviews in Toxicology, 40,* 653-670.

Shah, V., Jones, J., Dickman, J., Greenman, S. (2014). Response of soil bacterial community to metal nanoparticles in biosolids. *J. Hazard. Mater., 274,* 399–403 doi:10.1016/j.jhazmat.2014.04.003

Shahverdi, A. R., Fakhimi, A., Shahverdi, H. R. and Minaian, S. (2007). Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli, *Nanomedicine-Nanotechnology Biology and Medicine, 3*, 168-171.

Shih, Y. H., Liu, W. S., and Su, Y. F. (2012a). Aggregation of stabilized TiO_2 nanoparticle suspensions in the presence of inorganic ions. *Environmental Toxicology and Chemistry, 31(8),* 1693–1698 doi:10.1002/etc.1898

Shih, Y.H., Zhuang, C.M., Tso, C.P., Lin, C.H. (2012b). The effect of electrolytes on the aggregation kinetics of titanium dioxide nanoparticle aggregates. *J Nanopart. Res.*, 14 (8).

Shoults-Wilson, W.A., Reinsch, B.C., Tsyusko, O.V., Bertsch, P.M., Lowry, G.V., Unrine, J.M. (2011a). Effect of silver nanoparticle surface coating on bioaccumulation and reproductive toxicity in earthworms (Eisenia fetida). *Nanotoxicology, 5*, 432–444.

Shoults-Wilson, W. A., Zhurbich, O. I., McNear, D. H., Tsyusko, O. V., Bertsch, P. M., and Unrine, J. M. (2011b). Evidence for avoidance of Ag nanoparticles by earthworms (Eisenia fetida). *Ecotoxicology, 20(2),* 385–396. doi:10.1007/s10646-010-0590-0

Sigg, L. and Stumm, W. (2011). Aquatische Chemie, 5. ed. vdf / UTB, Zürich.

Sokal, R.R. and Rohlf, F.J. (1995). Biometry. third ed., San Francisco, CA, USA: W.H. Freeman

Som, C., Wick, P., Krug, H., Nowack, B. (2011). Environmental and health effects of nanomaterials in nanotextiles and fa??ade coatings. *Environ. Int., 37,* 1131–1142. doi:10.1016/j.envint.2011.02.013

Sondi, I. and Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on E-coli as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science, 275*, 177-182.

Sonnemann, S. G., Castells, F. and Schuhmacher, M., (2004). Integrated Life-Cycle and Risk Assessment for Industrial Processes Environmental Risk Assessment, CRC Press.

Sørensen, P.B., Thomsen, M., Assmuth, T., Grieger, K.D., Baun, A. (2010). Conscious worst case definition for risk assessment, part I. A knowledge mapping approach for defining most critical risk factors in integrative risk management of chemicals and nanomaterials. *Sci. Total Environ., 408,* 3852–3859. doi:10.1016/j.scitotenv.2009.11.010

Spurgeon, D.J. and Hopkin, S.P. (1996). Effects of variations of the organic matter content and pH of the soils on the availability and toxicity of zinc to the earthworm Eisenia fetida. *Pedobiologia, 40,* 80-96.

Stam, G.J., Bottelberghs, P.H., Post, J.G. and Bos, H.G., (2000). PROTEUS, a technical and management model for aquatic risk assessment of industrial spills, *J. Hazard. Mater., 17*, 439-448.

Stapleton, P.J., Glover, M.A. and Davis, S.P., (2001). Environmental Management Systems: An Implementation Guide for Small and Medium Sized Organizations, NSF International, Ann Arbor.

Piccapietra, F., Sigg, L., Behra, R. (2012). Colloidal Stability of Carbonate-Coated Silver Nanoparticles in Synthetic and Natural Freshwater. *Environ. Sci.Technol., 46 (2)*, 818-825.

Powers, C.M., Yen, J., Linney, E.A., Seidler, F.J., Slotkin, T.A. (2010). Silver exposure in developing zebrafish (Danio rerio): persistent effects on larval behavior and survival. *Neurotoxicol Teratol, 32*,391–397.

Quik, J.T.K., Velzeboer, I., Wouterse, M., Koelmans, a. a., van de Meent, D. (2014). Heteroaggregation and sedimentation rates for nanomaterials in natural waters. *Water Res., 48*, 269–279 doi:10.1016/j.watres.2013.09.036

Quik, J.T.K., Vonk, J.A., Hansen, S.F., Baun, A., Van De Meent, D. (2011). How to assess exposure of aquatic organisms to manufactured nanoparticles?. *Environ. Int., 37*, 1068–1077 doi:10.1016/j.envint.2011.01.015

Quadros, M.E., Pierson, R., Tulve, N.S., Willis, R., Rogers, K., Thomas, T. A., Marr, L.C. (2013). Release of silver from nanotechnology-based consumer products for children. *Environ. Sci. Technol., 47*, 8894–8901 doi:10.1021/es4015844

Ribeiro, F., Gallego-Urrea, J.A., Jurkschat, K., Crossley, A., Hassellöv, M., Taylor, C., Soares, A.M.V.M., Loureiro, S. (2014). Silver nanoparticles and silver nitrate induce high toxicity to Pseudokirchneriella subcapitata, Daphnia magna and Danio rerio. *Sci Total Environ, 466-467*, 232-241.

Tejamaya, M., Römer, I., Merrifield, R.C., Lead, J.R. (2012). Stability of citrate, PVP, and PEG coated silver nanoparticles in ecotoxicology media. *Environ. Sci. Technol., 46*, 7011–7017 doi:10.1021/es2038596

The Project on Emerging Technologies. Available at: http://www.nanotechproject.org/cpi/ Accessed on 30 March 2015

Thio, B. J. R., Montes, M. O., Mahmoud, M. a., Lee, D. W., Zhou, D.,and Keller, A. (2012). Mobility of capped silver nanoparticles under environmentally relevant conditions. *Environmental Science and Technology, 46(13),* 6985–6991 doi:10.1021/es203596w

Thio, B. J. R., Zhou, D., and Keller, A. (2011). Influence of natural organic matter on the aggregation and deposition of titanium dioxide nanoparticles. *Journal of Hazardous Materials, 189(1-2)*, 556–563 doi:10.1016/j.jhazmat.2011.02.072

Tixier, J., Dusserre, G., Salvi, O. and Gaston, D., (2002). Review of 62 risk analysis methodologies of industrial plants, *J. Loss Prevent. Proc., 15*, 291-303.

Tixier, J., Dandrieux, A., Dusserre, G., Bubbico, R., Mazzarotta, B., Silvetti, B., Hubert, E., Rodrigues, N. and Salvi, O., (2006). Environmental vulnerability assessment in the vicinity of an industrial site in the frame of ARAMIS European project, *J. Hazard. Mater., 130*, 251-264.

Topuz, E., Talinli, I., Aydin, E. (2011). Integration of environmental and human health risk assessment for industries using hazardous materials: a quantitative multi criteria approach for environmental decision makers. *Env. Int, 37*, 393–403. doi:10.1016/j.envint.2010.10.013

Topuz, E., Sigg, L., Talinli, I. (2014). A systematic evaluation of agglomeration of Ag and TiO2 nanoparticles under freshwater relevant conditions. *Environ Pollut, 193*, 37-44.

Topuz, E., Traber, J., Sigg, L., Talinli, I. (2015). Agglomeration of Ag and TiO2 nanoparticles in surface and waste water: role of calcium ions and of organic carbon fractions. *Environ Pollut, 204*, 313-323.

Topuz, E,, van Gestel, C.A.M. (2015). Toxicokinetics and toxicodynamics of differently coated silver nanoparticles and silver nitrate in Enchytraeus crypticus upon aqueous exposure in an inert sand medium. *Environ Toxicol Chem., accepted.*

Tourinho, P.S., Van Gestel, C.A.M., Lofts, S., Svendsen, C., Soares, A.M.V.M., Loureiro, S. (2012). Metal-Based nanoparticles in soil: Fate, behavior, and effects on soil invertebrates. *Environ Toxicol Chem, 31*,1679-1692.

UNEP. Rio Declaration on Environment and Development, United Nations Enviromental Programme; 1992.

USEPA. (2009). Targeted national sewage sludge report: Overview report. United States Environmental Protection Agency. Washington, DC.

Trouiller, B., Reliene, R., Westbrook, A., Solaimani, P. and Schiestl, R. H. (2009). Titanium Dioxide Nanoparticles Induce DNA Damage and Genetic Instability In vivo in Mice, *Cancer Research, 69*, 8784-8789.

Van Der Ploeg, M.J.C.Y., Handy, R.D., Waalewijn-Kool, P.L., Van Den Berg, J.H.C., Rivera, Z.E.H., Bovenschen, J., Molleman, B., Baveco, J.M., Tromp, P., Peters, R.J.B., Koopmans, G.F., Rietjens, I.M.C.M., Van Den Brink, N.W. (2014). Effects of silver nanoparticles (Nm-300k) on Lumbricus rubellus earthworms and particle characterization in relevant test matrices including soil. *Environ Chem, 33*,743-752.

Vassiliadis, C.G. and Pistikopoulos, E. N., (2000). Maintenance-based strategies for environmental risk minimization in the process industries, *J. Haz. Mat., 71*, 481-501.

Vevers, W. F. and Jha, A. N. (2008). Genotoxic and cytotoxic potential of titanium dioxide (TiO_2) nanoparticles on fish cells in vitro, *Ecotoxicology, 17*, 410-420.

Verma, M., (2009). A cost and expected consequence approach to planning and managing railroad transportation of hazardous materials, *Transport. Res. D- TR E, 14*, 300-308.

Von Goetz, N., Lorenz, C., Windler, L., Nowack, B., Heuberger, M., Hungerbühler, K. (2013). Migration of Ag- and TiO2-(nano)particles from textiles into artificial sweat under physical stress: Experiments and exposure modeling. *Environ. Sci. Technol., 47*, 9979–9987 doi:10.1021/es304329w

Waalewijn-Kool, P.L., Klein, K., Fornies, R.M., van Gestel C.A.M. (2014). Bioaccumulation and toxicity of silver nanoparticles and silver nitrate to the soil arthropod Folsomia candida. *Ecotoxicology, 23*,1629-1637.

Wang, Y., Westerhoff, P. and Hristovski, K. D. (2012). Fate and biological effects of silver, titanium dioxide, and C-60 (fullerene) nanomaterials during simulated wastewater treatment processes. *Journal of Hazardous Materials, 201*, 16-22.

Weinberg, H., Galyean, A. and Leopold, M. (2011). Evaluating engineered nanoparticles in natural waters, *Trac-Trends in Analytical Chemistry, 30*, 72-83.

Wessberg, N., Molarius, R., Seppala, J., Koskela, S., Pennanen, J. (2007). Environmental risk analysis for accidental emissions. *Journal of Chemical Health & Safety*, 24-31.

Westerhoff, P., Song, G., Hristovski, K. and Kiser, M. A. (2011). Occurrence and removal of titanium at full scale wastewater treatment plants: implications for TiO2 nanomaterial, *Journal of Environmental Monitoring, 13*, 1195-1203.

Whitley, A. R., Levard, C.M., Oostveen, E., Bertsch, P.M., Matocha, C.J., Kammer, F.V., Unrine, J.M. (2013). Behavior of Ag nanoparticles in soil: Effects of particle surface coating, aging, and sewage sludge amendment. *Environ Pollut, 182*, 141−149.

Wiesner, M.R., Bottero, J.Y. (2011). A risk forecasting process for nanostructured materials, and nanomanufacturing. *Comptes Rendus Phys., 12*, 659–668. doi:10.1016/j.crhy.2011.06.008

Windler, L., Lorenz, C., Von Goetz, N., Hungerbühler, K., Amberg, M., Heuberger, M., Nowack, B. (2012). Release of titanium dioxide from textiles during washing. *Environ. Sci. Technol., 46*, 8181–8188 doi:10.1021/es301633b

Wolf, W. and Feijtel, T., (1998). Terrestrial Risk Assessment For Linear Alkyl Benzene Sulfonate (LAS) in Sludge Amended Soils, *Chemosphere, 36*, 1319-1343.

Wood, C. M., Playle, R. C. and Hogstrand, C. (1999). Physiology and modeling of mechanisms of silver uptake and toxicity in fish, *Environmental Toxicology and Chemistry, 18*, 71-83.

Yang, K., Lin, D. and Xing, B. (2009). Interactions of Humic Acid with Nanosized Inorganic Oxides. *Langmuir, 25*, 3571-3576.

Yang, X., Jiang, C., Hsu-Kim, H., Badireddy, A.R., Dykstra, M., Wiesner, M., Hinton, D.E., Meyer, J.N. (2014). Silver nanoparticle behavior, uptake, and toxicity in Caenorhabditis elegans: effects of natural organic matter. *Environ Sci Technol, 48*, 3486−3495.

Yin, L., Cheng, Y., Espinasse, B., Colman, B. P., Auffan, M., Wiesner, M., Rose, J., Liu, J., Bernhardt, E. S. (2011). More than the Ions: The Effects of Silver Nanoparticles on Lolium multiflorum. *Environmental Science & Technology, 45*, 2360-2367.

Zadeh, L.A. (1965). Fuzzy Sets. *Information and Control, 8*, 338-353.

Zeng, J., An, M., and Smith, N. G. (2007). Application of a fuzzy based decision making methodology to construction project risk assessment. *Project Management, 25*, 589-600

Zhang, R., Zhang, H., Tu, C., Hu, X., Li, L., Luo, Y., Christie, P. (2015). Facilitated transport of titanium dioxide nanoparticles by humic substances in saturated porous media under acidic conditions. *J. Nanoparticle Res., 17*, doi:10.1007/s11051-015-2972-y

Zhang, H., Smith, J. A., and Oyanedel-Craver, V. (2012). The effect of natural water conditions on the anti-bacterial performance and stability of silver nanoparticles capped with different polymers. *Water Research, 46(3)*, 691–699 doi:10.1016/j.watres.2011.11.037

Zhang, Y., Chen, Y., Westerhoff, P., and Crittenden, J. (2009). Impact of natural organic matter and divalent cations on the stability of aqueous nanoparticles. *Water Research, 43(17)*, 4249-4257 doi: 10.1016/j.watres.2009.06.005

Zhang, Y., Chen, Y., Westerhoff, P., Hristovski, K., and Crittenden, J. C. (2008). Stability of commercial metal oxide nanoparticles in water. *Water Research, 42(8-9)*, 2204–2212 doi:10.1016/j.watres.2007.11.036

Zhao, C.M., Wang, W.X. (2011). Comparison of acute and chronic toxicity of silver nanoparticles and silver nitrate to Daphnia magna. *Environ Toxicol Chem, 30*, 885–92.

FATE AND EFFECTS OF ENGINEERED NANOPARTICLES IN THE ENVIRONMENT AND NEW APPROACHES FOR THEIR RISK

SUMMARY

Considering the needs for the Environmental Risk Assessment (ERA) of Engineered Nanoparticles (ENPs), the main aim of this PhD study is to propose an ERA approach for ENPs that enables evaluating all representative factors for the risk, provides tools for reducing uncertainties and points out further research needs and risk management strategies with quantitative risk communication. Silver nanoparticles (AgNPs) and TiO_2 NP were chosen as model ENPs due to their common usage in consumer products. Two different AgNPs, with citrate and PVP coating, were used to compare the effects of coating material. In the context of this main aim, the following objectives were studied to generate data or knowledge for the proposed ERA: 1) to determine in a systematic manner the most relevant water chemistry parameters for the agglomeration of model ENPs, which is the key fate process for the fate of ENPs 2) to classify the aquatic environment based on the relevant water chemistry parameters for agglomeration by considering the possible temporal changes according to the results of ENP agglomeration behavior in natural water samples 3) to evaluate the toxicokinetics and toxicodynamics of AgNPs in *E. crypticus* by investigating the relation between uptake and effects and 4) to determine the survival and reproductive toxicity of AgNPs to *E. crypticus* and the relation between toxicity and soil organic matter content and pH of the soil.

In the context of Chapter 2, selected anions, cations, natural organic matter (humic acid and fulvic acid) and synthetic organic compounds (sodium dodecyl sulphate and ethoxylates) that were commonly observed in surface water were tested for their effects on ENP agglomeration under environmentally realistic conditions. ENP agglomeration was characterized using the change in size as measured using dynamic light scattering and Nanosight Nanotrack. Then, the combinations of ions and natural organic matters were evaluated in terms of agglomeration behavior to compare with the specific effects and most related parameters with the agglomeration of selected compounds were determined as Ca^{2+}/Mg^{2+} and dissolved organic matter concentrations in the aquatic medium. All of the agglomeration studies were

conducted for 1 hour, 1 day and 1 week to assess ENP agglomeration changes over time depending on the type of ENP. A surface water simulated media in terms of agglomeration was proposed which was validated by comparison with natural surface water samples.

Based on the results of Chapter 2, six surface water and three wastewater treatment plants were selected with different Ca^{2+}/Mg^{2+} and dissolved organic matter concentrations to determine its effect on the change in ENP size. ENP agglomeration experiments were conducted in unfiltered and filtered samples to have environmentally realistic conditions. Agglomeration characterization was performed using the same methods as in previous chapter. Agglomeration of citrate-coated AgNPs correlated well with Ca^{2+} concentration. However, dissolved organic carbon led to deviations at a certain concentration range. PVP-coated AgNPs were stable at their original size regardless of water chemistry. TiO_2 NPs agglomerated up to micrometer scale in most of the water samples after 1 week. Correlation of their agglomeration behavior with Ca^{2+} concentration was weaker than that of citrate-coated AgNPs. However, the correlation improved when dissolved organic carbon content was higher than 2 mg/L. The effect of dissolved organic carbon on the stabilization of TiO_2 NPs was more pronounced after 1 day. Fractionation of the samples based on molecular weight of the organic matter using ultrafiltration showed that agglomeration was much more pronounced for the fraction below 10 kDa than for unfiltered samples. Based on the correlations found, a classification scheme for the agglomeration of model ENPs in water over time was proposed.

The uptake of AgNPs and $AgNO_3$ in *E. crypticus* was followed for 10 days. A background solution with essential elements was spiked to inert quartz sand to prepare the exposure medium. *E. crypticus* were exposed to AgNPs at different dose levels for different times (2,3,5,7,10 days). Survival of *E. crypticus* was determined, and sand, filtered sand solution and *E. crypticus* were analyzed for total Ag. Ag mostly adsorbed to the sand, with strongest sorption found for ionic Ag and PVP-coated AgNPs. Citrate-coated AgNPs gave much higher Ag concentrations in the solution than other two Ag compounds. However, the LC50 was also higher for citrate-coated AgNPs, so it was less toxic. The other Ag compounds had similar toxicity. Accumulation of Ag was observed depending on time and external concentration. For all compounds, the LC50 decreased with time and reached steady state after 7 days of exposure. However, LC50 calculated based on internal Ag concentrations in the enchytraeids were constant over time and could be considered more representative of toxicity regardless of time.

Survival and reproduction toxicity of AgNPs and $AgNO_3$ to *E. crypticus* were determined in three different standard soils, namely Lufa 2.2, Lufa 2.3 and Lufa 5M. The standard ISO (2004) guideline 16387 was used for the toxicity tests. Effects on enchytraeid survival were observed at concentrations higher than 500 mg Ag/kg dry soil for the AgNPs, while $AgNO_3$ was more toxic. Reproduction was more sensitive than survival and there was no significant difference in toxicity between AgNPs and $AgNO_3$. Toxicity decreased with increasing soil organic matter content, but not by soil pH.

An ERA approach for ENPs was proposed with using analytical hierarchy process (AHP) and fuzzy inference tools. Risk of ENPs were based on the occurrence likelihood (OL), exposure potential (EP) and toxic effects (TE). According to the principles of AHP, sub-factors that are related with OL, EP and TE were determined systematically and a hierarchical structure was developed considering the placing of comparable factors at the same level. A fuzzy scale was proposed to score the factors in the hierarchy using expert judgement. Sub factors were scored based on their contribution to risk and compared in terms of their importance for the risk to obtain priority weights for the factors. Then overall scores were calculated with a weight-average method and converted to standard trapezoidal numbers. Fuzzy sets corresponding to the overall scores were determined using the proposed scoring scale. OL, EP and TE were combined with a fuzzy inference rule base using expert judgement to obtain the risk magnitude and the risk class based on the scale proposed. Three case studies were analyzed to demonstrate the applicability of the method. The case studies showed that this approach can provide more informative results since it gives the risk class which helps identifying the required risk management strategy. Moreover, the priority weights of the factors may give a clue about research needs or may help identifying which factor should be focused on to reduce the risk.

NANOPARTİKÜLLERİN ÇEVREDEKİ DAVRANIŞ VE ETKİLERİ İLE RİSK DEĞERLENDİRMESİ İÇİN YENİ YAKLAŞIMLAR

ÖZET

Modern çağın gereklilikleri ile birlikte hızla değişen ve gelişen teknoloji, günlük hayatta kullanılan ürünlere ihtiyaç duyulan işlevlerin kazandırılması amacıyla farklı malzemelerin üretilmesine neden olmuştur. Bu malzemeler, kullanıldıkları ürünlerin hayat döngüleri içerisinde çeşitli yollarla çevresel ortamlara girmeye başlamış ve çevre için "öncelikli kirleticiler" adı altında, çevredeki davranışları ve çevreye olan etkileri tam olarak bilinmeyen, yeni bir kirletici gurubu oluşmasına neden olmuştur. Son on yıldır üretilmeye başlanan ve kullanımı oldukça hızlı artan nanopartiküller de, bu grupta yer almakla birlikte, gerek yüzey alanını oldukça büyüten nano boyutları ve gerekse çeşitli işlevler kazandırılmak amacıyla herbiri değişik özelliklerde üretilen oldukça geniş ürün yelpazesiyle çevreye etki bakımından diğer öncelikli kirleticilerden de ayrılmaktadır. Bu nedenle, nanopartiküllerin kullanımı ile ilgili çevresel risk değerlendirmesi ihtiyacı gündeme gelmiş olup farklı özelliklerinden dolayı alternatif yöntemlerin geliştirilmesi de gerekmektedir.

Bu doktora tezinin amacı nanopartiküller için, çevresel riske katkısı olabilecek tüm faktörlerin değerlendirilmesini sağlayan, belirsizlikleri en aza indirgeyen, sayısallaştırılmış risk sonuçları ile gelecekteki çalışmalar için araştırma konularını ve risk yönetimi stratejilerini ortaya koyabilen bir çevresel risk değerlendirme yaklaşımı önermek ve önerilen yaklaşım için gerekli olan veri ve bilgi birikiminin kısmen oluşturulmasını sağlamaktır. Bu çalışmanın uygulanması için, tüketici ürünlerinde oldukça yaygın olarak kullanılmasından dolayı, Gümüş (Ag) ve TiO_2 nanopartiküller, model nanopartiküller olarak seçilmiştir. Nanopartiküllerin ürünlerdeki stabilizasyonunu sağlamak amacıyla kaplama malzemeleri ile yüzeyleri kaplanmaktadır. Bu çalışma kapsamında, kaplama malzemelerinin çevresel risk bakımından etkilerini koymak üzere iki farklı Ag nanopartikül, sitrat ve polyvinyl pyrrolidone (PVP) kaplı, kullanılmıştır. Önerilecek olan çevresel risk değerlendirme yaklaşımı için gerekli olan, ancak literatürde eksik olan veri ve bilgilerin oluşturulabilmesi için, çalışmanın ana amacı dahilinde dört farklı amaç belirlenmiştir.

Bölüm 2 kapsamında, yüzeysel sularda yaygın olarak bulunan anyonlar, katyonlar, doğal organik madderler (humik asit ve fulvic asit) ve sentetik organik maddeler (sodyum dodesil sülfat ve etoksilat) seçilmiş ve bu maddelerin çevresel ortamları temsil edici konsantrasyonlarda hazırlanan sentetik çözeltilerine Ag ve TiO_2 nanopartiküller ilave edilerek, bu maddelerin aglomerasyon üzerindeki etkileri tespit edilmiştir. Aglomerasyon karakterizasyonu, partikül çaplarındaki değişim dikkate alınarak yapılmış olup ölçümler için Dinamik Işık Dağıtıcı (DLS) ve Nanosight NTA kullanılmıştır. Daha sonra, bu parametrelerin tüm farklı kombinasyonları ile çevresel koşullara uygun konsantrasyonlarda hazırlanan sentetik çözelilere Ag ve TiO_2 nanopartiküller ilave edilmiş ve elde edilen sonuçlar, bu parametrelerin tekil olarak bulunduğu çözeltilerde elde edilen sonuçlarla karşılaştırılarak, aglomerasyon prosesine en çok etki eden parametreler, Ca^{2+}/Mg^{2+} ve çözünmüş organik karbon konsantrasyonları, olarak belirlenmiştir. Tüm aglomerasyon çalışmaları 1 saat, 1 gün ve 1 haftalık zaman dilimleri için gerçekleştirilmiş olup, özellikle sitrat kaplı Ag ve $Ti0_2$ nanopartiküllerin aglomerasyonunun zamanla artabileceği gözlenmiştir. Elde edilen tüm sonuçların analizi ile nanopartiküllerin çevredeki akibeti ve çevreye olan etkileri kapsamındaki çeşitli çalışmalarda, yüzeysel suları temsil edici olarak kullanılabicek "sentetik yüzeysel su ortamı" önerilmiştir.

Bölüm 3 kapsamında, bir önceki bölümde tespit edilen parametrelerin nanopartiküllerin boyutlarındaki değişimi ile olan korelasyonlarını tespit etmek üzere, farklı aralıklarda Ca^{2+}/Mg^{2+} ve çözünmüş organik karbon konsantrasyonları içerecek şekilde 6 yüzeysel su ve 3 atıksu arıtma tesisi seçilmiştir. Çevresel koşulları temsil edici olabilmesi için, süzülmüş numunelerde yapılan deneylere ek olarak, süzülmemiş numunelerde de aglomerasyon deneyleri yapılmıştır. Aglomerasyon karakterizasyonu bir önceki bölümde açıklandığı şekilde yapılmıştır. Sitrat kaplı Ag nanopartiküllerin aglomerasyonu ile, Ca^{2+} konsantrasyonu arasında oldukça güçlü bir ilişki tespit edilmiştir. Ancak, çözünmüş organik karbon içeriği, belirli konsantrasyon aralıklarında, bu korelasyondan küçük sapmalar yaşanmasına neden olmuştur. PVP kaplı Ag nanopartiküller ise, numunenin kimyasal içeriğinden bağımsız olarak, tüm numunelerde başlangıçtaki orijinal boyutlarını korumuştur. TiO_2 nanopartiküller ise, numunelerin çoğunda 1 hafta sonra, mikrometre seviyelerine kadar aglomere olmuştur. TiO_2 nanopartiküllerinin aglomerasyonunun Ca^{2+} konsantrasyonu ile olan korelasyonu, sitrat kaplı Ag nanopartiküllerinkine göre, daha zayıf olmuştur. Ancak, yanlızca çözünmüş organik karbon içeriği 2 mg/L değerinin üzerinde olan numuneler dikkate alındığında, korelasyon oldukça artmıştır. Hatta, çözünmüş organik karbonun TiO_2 nanopartiküllerinin stabilizasyonu

üzerindeki etkisi 1 gün içerisinde daha da belirgin hale gelmiştir. Tüm numuneler ultrafiltrasyon ile moleküler ağırlık bazında fraksiyonlarına (10 kDa and 1 kDa) ayrılmış ve her fraksiyonda aglomerasyon çalışmaları yürütülmüştür. 10 kDa altındaki organik maddeleri içeren numunelerdeki aglomerasyon, süzülmemiş numunelerdeki aglomerasyondan oldukça fazla gerçekleşmiştir. Bulunan korelasyonlar dikkate alınarak, model nanopartiküllerin aglomerasyonu için yüzeysel sulara ait bir sınıflandırma şeması önerilmiştir.

Bölüm 5 kapsamında, *Enchytraeus crypticus* tarafından gerçekleştirilen Ag nanopartikül ve $AgNO_3$ tüketimi 10 gün boyunca takip edilmiştir. Elzem elementleri içeren bir çözelti, inert bir kum ortamına ilave edilmiş ve bu ortamda, *E. crypticus* farklı konsantrasyon seviyelerindeki Ag nanopartiküllere farklı sürelerde (2,3,5,7,10 gün) maruz bırakılmıştır. Her zaman dilimi sonunda yaşan *E. crypticus* sayılmış, kum, çözelti fazı ve yaşayan organizmalar içinde Ag ölçümü yapılmıştır. Tüm nanopartiküller için LC50 zamanla azalmış ve 7 gün sonunda durağan koşullara gelmiştir. Ancak, organizmalaın bünyesinde ölçülen Ag konsantrasyonları dikkate alınarak yapılan hesaplamalarda elde edilen LC50 değerleri zamanla sabit kalmış ve zamandan bağımsız olması nedeniyle toksisite bakımından daha temsil edici olabileceği görülmüştür.

E. crypticus , üç farklı standart toprak içerisinde Ag nanopartikül ve Ag iyonlarına maruz bırakılmıştır. Yaşamsal ve üretimsel toksisite, "ISO (2004) 16387 Guideline" kullanılarak test edilmiştir. Ag nanopartiküllerin yaşam üzerindeki toksik etkileri 500 mg Ag/kg kuru toprak kadar yüksek kontrasyonlarda gözlenirken, Ag iyonları daha çok toksik etki göstermiştir. Üretimsel toksisitenin, yaşamsal toksisiteden daha hassas olduğu tespit edilmiş ve Ag nanopartiküller ile Ag iyonları arasında toksik etki bakımından farklılık gözlenmemiştir. Toksisite, organik madde içeriği yüksek olan topraklarda düşük iken, farklı pH değerlerinden dolayı toksisiteler arasında anlamlı bir fark gözlenmemiştir.

Nanopartiküller için, Analitik Hiyerarşi Proses (AHP) ve bulanık çıkarım modelleri kullanılarak bir çevresel risk değerlendirme yaklaşımı önerilmiştir. Nanopartiküllerin çevresel riskleri; çevrede bulunma durumlarına, organizmaların nanopartiküllere maruz kalma potensiyellerine ve organizmalar üzerinde gözlenen toksisk etkilerine bağlı olarak değerlendirilmiştir. AHP prensipleri gereği, bunlara etki eden alt faktörler sistematik olarak belirlenmiş ve karşılaştırılabilen faktörlerin aynı seviyeye yerleştirilmeleri dikkate alınarak hiyerarşik yapı oluşturulmuştur. Hiyerarşideki faktörlerin, riske olan katkılarını değerlendirmek üzere bir bulanık ölçek önerilmiştir. Bu faktörler, çevresel risk bakımından önemlerini belirlemek üzere matrislerle karşılatırılmış ve sayısal önem dereceleri elde

edilmiştir. Uzman görüşleri kullanılarak, bulanık çıkarım kural kümeleri sayesinde risk sayısallaştırılmış ve sınıflandırılmıştır. Uygulanan 3 farklı senaryo göstermiştir ki bu yöntem risk hakkında daha bilgilendirici sonuçlar sağlamaktadır, çünkü ele alınan durumun risk yönetimi için startejiler öneren risk sınıflarındaki yeri belirlenmiştir. Ayrıca, önem dereceleri gelecekteki nanopartikül çalışamalarının yönlendirilmesine ve riskin düşürülmesi için hangi faktörler üzerine eğilmek gerektiğine ilişkin ipuçları da sağlamaktadır.

FOREWORD

I am deeply thankful to my supervisors Prof. İlhan Talınlı, Prof Nico van Straalen, Dr. Cornelis A.M. van Gestel and Prof Dr. Laura Sigg. There are no words to express their contribution to my wisdom in science and life.

In addition to being perfect colleagues, many thanks to Dr. Elif Pehlivanoğlu Mantaş and Dr. Egemen Aydın for being kind and warm, sister and brother. The opportunity that they give me to work together and to share any significant momentus both happy and sad times in life is valuable for me.

Many other thanks to other faculty in the department that I gained valuable vision for my career and get supported everytime with many cheerful occasions. Prof. Emine Ubay Çokgör, Prof. Gülen İskender, Prof. Melike Gürel, Assoc Prof. Didem Okutman Taş, Assist Prof. Gülsüm Emel Zengin-Balcı, Assoc Prof Alpaslan Ekdal, Assoc Prof. Serdar Doğruel, Assoc Prof. Tuğba Ölmez-Hancı.

Both being my good friends and colleagues, I really enjoy sharing the every moment with you at anywhere, inside and the outside the university, many thanks to "K325+1": Ilker Akmırza, Zeynep Cetecioğlu Gürol, Çisem Ecer, Börte Köse and Fatih Yılmaz.

My deep thanks for my officemates, "girls party", İlke Pala Özkök, Tuğçe Katipoğlu Yazan, Gözde Özbayram, Asude Hanedar and our neighbor officemates: Ayşe Allar, Burçak Kaynak, Burçin Coşkun.

Not only officemates from Turkey, but aslo from Switzerland. I want to thank to Carmen Gill, Julita Stadnika, Anne Nyman, Carl Isaacson, Hannh Klug and İsabel Oliveria.

I really enjoyed my time in Amsterdam with a team, "Ecotoxic girls", I hope to meet you again: Jinxia Li, Nazaret Gonzalez, Lulu Zhang and Erkai (Cloris) He.

I want to thank for enjoyable time in Zurich and in Amsterdam for Soyoun Son, Eunyoung Young, Fatoş Hoşoğlu, Aslıhan Ural, Ceren Yüceçam, Özlem Tuna and Pınar Kuruüzüm.

And finally, it is time to admit that I am a member of two gangs. One is called "Maymuslar" with Gülten Yüksek and Handan Atalay. The oldest one is "Pomes" with Mutlu Çağlayan Köse, Pınar Karabacak, Şerife Ağacaoğlu Bitlis, Özlem Kaçar, Zeynep Karabacak and Junior Ela Köse. There are no words to tell how deep I can share everything and how I feel comfortable myself with them. I wish to have many many more years with you.

My special thanks to my family. My brother and his wife, Saadet-Emre Topuz, and my cute nephews "Zeynep-Elif", I really owe you too much for your great support to me at any time. It really feels me comfortable to know that you are beside me everytime and I always felt happy when I see you at home after an exhaustive working day. I appreciate the endless support that my parent, Emine-Hayrettin Topuz, gave me without any doubt. Nothing can express my thanks to you for the priority you gave us in the life. And there would be nothing else that I

would want so much, sharing this period with you, my dear father. Though I feel your spirit with me everytime at anywhere, I deeply miss your hug.

Thankfully my study was funded by TUBITAK 2211 and 2214 fellowship program for 4 years and for 1 year, respectively. I also get fund from COST ES 1205 Action for 3 months.

June 2015 Emel TOPUZ

Environmental Engineer

CURRICULUM VITAE

Name Surname:	Emel Topuz
Place and Date of Birth:	İstanbul / 17.04.1984
Address:	İTÜ İnşaat Fakültesi Çevre Müh. Bölümü Maslak-İstanbul
E-Mail:	topuze@itu.edu.tr
B.S. (Double Major):	Environmental Engineering
	Industrial Engineering
M.S. :	Environmental Sciences and Engineering

Professional Experience and Rewards:

2009 - : Research assistant at Istanbul Technical University, Environmental Engineering Department

September 2012 – September 2013: Guest researcher at Eawag, Switzerland

September 2014 – September 2015: PhD student at VU University Amsterdam

ITU Environmental Engineering Department First Ranked Graduate (B.S.)-2007

ITU Civil Engineering Faculty Third Ranked Graduate (B.S.) - 2007

TUBITAK National Master Scholarship 2007-2009

TUBITAK National Doctorate Scholarship 2009-2013

TUBITAK International Doctorate Research Scholarship 20012-2013

EU Cost ES 1205 Action Short Term Scientific Mission Grant (January-May) 2015

PUBLICATIONS/PRESENTATIONS ON THE THESIS

SCI Journal Articles:

Emel Topuz, Laura Sigg, Ilhan Talinli, A systematic evaluation of agglomeration of Ag and TiO_2 nanoparticles under freshwater relevant conditions, 2014, Environmental Pollution, 193, 37-44.

Emel Topuz, Jacqueline Traber, Laura Sigg, Ilhan Talinli, Agglomeration of Ag and TiO_2 nanoparticles in surface and waste water: role of calcium ions and of organic carbon fractions, 2015, Environmental Pollution, 204, 313-323.

Emel Topuz and Cornelis A.M. van Gestel, Toxicokinetics and toxicodynamics of differently coated silver nanoparticles and silver nitrate in *Enchytraeus crypticus* upon aqueous exposure in an inert sand medium, 2015, Environmental Toxicology and Chemistry, doi: 10.1002/etc.3123

International Conference Presentations:

Emel Topuz, Sevgi Sarı, Gamze Ozdemir, Egemen Aydin, Elif Pehlivanoğlu Mantaş, Didem Okutman Taş, Optimization of Diclofenac extraction from sludge samples, Micropol & Ecohazard 2013, 16-20 June 2013, Zurich, Switzerland.

Emel Topuz, Laura Sigg, Ilhan Talinli, Effect of freshwater relevant conditions on the agglomeration of titanium dioxide nanoparticles, International Conference on Environmental Science and Technology 2014, 09-13 June 2014, Houston, USA.

Laura Sigg, Niksa Odzak, **Emel Topuz**, Ursula Lindauer, Renata Behra, Agglomeration and dissolution of Ag and TiO_2 nanoparticles − Nanoparticle accumulation in sediments?, Contaminated Sediments: Environmental Chemistry, Ecotoxicology and Engineering, 08-13 March 2015, Ascona, Switzerland.

Emel Topuz, Cornelis A.M. van Gestel, Toxicokinetics and toxicodynamics of silver nanoparticles in *Enchytraeus crypticus*, SETAC Europe 25th Annual Meeting, 3-7 May 2015, Barcelona, Spain.

List of Publications:

SCI Journal Articles:

İlhan Talınlı, **Emel Topuz**, Mehmet Uygar Akbay, Comparative analysis for energy production processes (EPPs): Sustainable energy futures for Turkey, 2010, Energy Policy, 38 (8), 4479-4488.

Emel Topuz, İlhan Talınlı, Egmen Aydin, Integration of environmental and human health risk assessment for industries using hazardous materials: A quantitative multi criteria approach for environmental decision makers, 2011, Environment International, 37, 393-403.

Egemen Aydin, Fatma Büşra Yaman, Esra Ates Genceli, **Emel Topuz**, Esra Erdim, Melike Gurel, Murat Ipek, Elif Pehlivanoglu-Mantas, Occurrence of THM and NDMA precursors in a watershed: Effect of seasons and anthropogenic pollution, 2012, Journal of Hazardous Materials, 221–222, 86-91.

Emel Topuz, Egemen Aydin, Elif Pehlivanoglu-Mantas, A Practical LC-MS/MS Method for the detection of NDMA at nanogram per liter concentrations in multiple water matrices, 2012, Water, Air & Soil Pollution, 223, 5793-5802.

Emel Topuz, Sevgi Sari, Gamze Ozdemir, Egemen Aydin, Elif Pehlivanoglu-Mantas, Didem Okutman Tas, Optimization of ultrasonic assisted diclofenac extraction from wastewater treatment plant sludge, 2014, Journal of Chromatography B, 958, 48-54.

Sevgi Sari, Gamze Ozdemir, Çigdem Yangin Gömec, Gulsum Emel Zengin, **Emel Topuz**, Egemen Aydin, Elif Pehlivanoglu-Mantas, Didem Okutman Tas, Seasonal variation of diclofenac concentration and its relation with wastewater characteristics at two municipal wastewater treatment plants in Turkey, 2014, Journal of Hazardous Materials, 272, 155-164.

Gamze Ozdemir, Egemen Aydin, **Emel Topuz**, Cigdem Yangin-Gomec, Didem Okutman-Tas, Acute and chronic responses of denitrifying culture to diclofenac, 2015, Bioresource Technology, 167, 112-120.

International Book Chapter:

Talinli, I., Topuz, E., **Aydin, E**., Kabakci, S. (2011). A Holistic Approach for Wind Farm Site Selection by Using FAHP, Wind Farm, in Technical Regulations, Potential Estimation and Siting Assessment, Gastón O. Suvire (Ed.), InTech, ISBN: 978-953-307-483-2

International Conference Presentations:

Emel Topuz, İlhan Talınlı, An Environmental risk assessment approach for Industrial Hazardous Materials, 23rd Operational Research Conference, 5-8 July 2009, Bonn, Germany.

Emel Topuz, İlhan Talınlı, Atakan Öztekin, Environmental risk assessment for roadway transportaion of LNG, 24th Operational Research Conference, 11-14 July 2010, Lisbon, Portugal.

Emel Topuz, Esra Ateş Genceli, Nur Hanife Orak, Egemen Aydin, Melike Gurel and Elif Pehlivanoglu-Mantas, Effect of chlorinatıon and chloramination on THM and NDMA formation in a Drinking Water Treatment Plant, EuCheMS International Conference on Chemistry and the Environment, 11-15 September 2011, Zurich, Switzerland.

Emel Topuz, Egemen Aydin, Ilhan Talinli, An approach for environmental risk assessment of pharmaceuticals by Using Fuzzy AHP, SETAC World Congress 2012, 20-24 May 2012, Berlin, Germany.

Emel Topuz and Elif Pehlivanoglu-Mantas**,** Antibiotics and other pharmaceuticals in the environment: Occurrence and Measurement, Workshop on Antibiotics and Antibiotic-Resistant Bacteria in the Environment, Namık Kemal University, 8 June 2012, Tekirdağ, Turkey.

Tuğba Ozsenturk, Birge Didem Ozel, Esra Ates Genceli, Egemen Aydin, **Emel Topuz**, Melike Gurel, Elif Pehlivanoglu-Mantas, Fractionation of THM and NDMA precursors based on molecular weight and C-18 solid phase extraction, IWA World Water Congress & Exhibition, 16-21 September 2012, Busan, Korea.

Eren Gorenoglu, Egemen Aydin, **Emel Topuz**, Elif Pehlivanoglu-Mantas, Determination of toxicity of Triclosan on two different pure cultures (V. fischeri ve P. subcapitata) and activated sludge biomass, 6th International Perspective on Water Resources & the Environment Conference (IPWE 2013), 7-9 January 2013, Izmir, Turkey.

Emel Topuz, Sevgi Sarı, Gamze Ozdemir, Egemen Aydin, Elif Pehlivanoğlu Mantaş, Didem Okutman Taş, Optimization of Diclofenac extraction from sludge samples, Micropol & Ecohazard 2013, 16-20 June 2013, Zurich, Switzerland.

Gulsum Emel Zengin**, Emel Topuz**, Aysegul Unal, Nilay Ucar, Elif Banu Gencsoy, Emine Ubay Cokgor, Evaluation of ultrasonication and microwave disintegration of waste activated sludge, 17th International Symposium on Environmental Pollution and its Impact on Life in the Mediterranean Region, 28 September-1 October 2013, Istanbul, Turkey.

Elif Pehlivanoglu Mantas, Melike Gurel, Guclu Insel, **Emel Topuz,** Alpaslan Ekdal, Egemen Aydin, Nilay Sayi Ucar, Emine Cokgor, Didem Okutman Taş, Evaluation of enzyme addition in aerobic stabilization of biological sludge, 17th International Symposium on Environmental Pollution and its Impact on Life in the Mediterranean Region, 28 September-1 October 2013, Istanbul, Turkey.

Serdar Dogruel, Elif Pehlivanoglu Mantas, Tugba Olmez Hanci, Melike Gurel, **Emel Topuz,** Emine Cokgor, Fenton and ozone disintegration of excess sludge, 17th International Symposium on Environmental Pollution and its Impact on Life in the Mediterranean Region, 28 September-1 October 2013, Istanbul, Turkey.

Sevgi Sarı, Gamze Ozdemir, **Emel Topuz**, Egemen Aydin, Elif Pehlivanoğlu Mantaş, Didem Okutman Taş, Assessment of biodegradability of Diclofenac under methanogenic conditions, 13th Anaerobic Digestion World Congress, 25-28 June 2013, Santiago de Compostela, Spain.

Sevgi Sari, **Emel Topuz**, Egemen Aydin, Didem Okutman-Tas, Assessment of cometabolic biodegradation potential of Diclofenac in the presence of acetate under anaerobic conditions, International Conference on Environmental Science and Technology 2014, 09-13 June 2014, Houston, USA.

Sevgi Sari, **Emel Topuz**, Egemen Aydin, Didem Okutman-Tas, Effect of temperature and pH on anaerobic biodegradability of Diclofenac, 2nd IWA Specialist Conference on EcoTechnologies for Sewage Treatment, 23-27 June 2014, Verona, Italy.

Badel Gun, Melike Begum Tanis, **Emel Topuz**, Egemen Aydin, Elif Pehlivanoglu-Mantas, Determination of estrogeCnecity and BPA concentration in bottled drinking water, 5th EuCheMS Chemistry Congress, 31 August – 04 September 2014, Istanbul, Turkey.

Ilhan Talinli, **Emel Topuz,** Egemen Aydin, Treatment and disposal options for Hazardous Waste Management system: amendment proposal to lists, Wastenet Conference, 19-21 June 2015, Kavala, Greece.