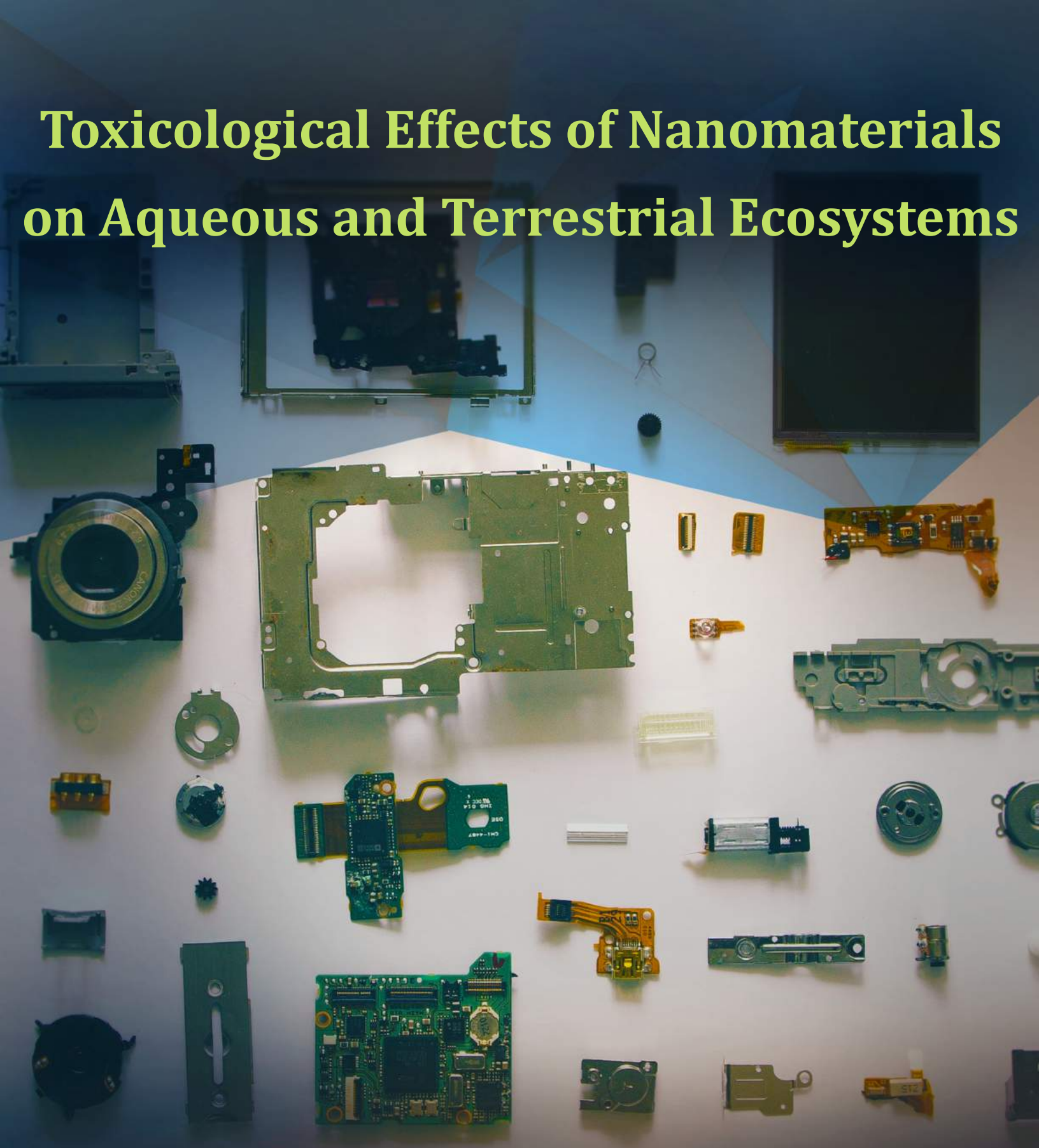
Toxicological Effects of Nanomaterials on Aqueous and Terrestrial Ecosystems



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Abstract

This paper provides a holistic overview of the effects of engineered and natural nanomaterials (ENMs and NNMs) in the environment. Through increasing production and technology, NMs are contaminating the air, soil, and water with large toxicological implications. The proposed pathways of nanomaterials in the environment suggest that they accumulate in terrestrial and aquatic environment due to emissions, surface runoff and effluent from wastewater treatment facilities. Nanomaterial toxicological studies have also shown large bactericidal and mutagenic effects due to the metal ion properties, stabilizing agents and the size and shape of the NMs. (Suresh et al., 2013). These adverse effects that were seen on bacteria and microbes were also observed in plants and fish. Plants were seen to have inhibited growth, and even death when coming into contact with specific nanoparticles. (Burke et al., 2015) However, some conflicting data exist where plants have shown improvement in growth under specific exposure. (Lahiani et al., 2008) Fish and aquatic organisms were also studied in order to determine the health impacts on these species as well as the potential for biomagnification. Organ failure was observed in zebrafish and carp due to acute exposure to metal NPs (Griffit et al., 2011). Other harmful effects have also been observed in aquatic ecosystems, with many engineered nanoparticles settling in the sediment of freshwater and marine environments causing risk to benthic species (Handy et al., 2011). The impact on human health was also reviewed, revealing that the exposure to nanoparticles has lead to nausea, vomiting and even death. Human organs can also be severely affected by inhaling, ingesting or coming into contact with large amounts of nanomaterials. (Tang et al., 2015).

Key Words: Nanomaterials, Nanotechnology, Nanotoxicology, Aqueous Ecosystems, Terrestrial Ecosystems, Biomagnification, Engineered Nanoparticles

1.0 Introduction

Nanotechnology and nanomaterial (NM) containing products are part of a multi billion-dollar industry, with a wide variety of uses ranging from electronics to biomedical applications. Due to growing industry, it is inevitable that engineered and natural NMs will be released into the atmosphere, affecting both the water and soil. This could be from intentional or unintentional release, which means that it is vital to determine the potential impacts this growing industry can have on environmental, human, animal and plant health.

Nanoparticles are naturally occurring in the environment, however through technological breakthroughs the abundance of new and engineered nanoparticles not found naturally are becoming prevalent in the atmosphere. Due to lack of knowledge there is no regulation on the emissions and many scientists are concerned that this could pose a threat to as a new class of environmental hazards. Nanomaterials are "structures with at least one dimension less than 100nm" which, due to their small size and large surface area NMs are widely used in various products, increasing the routes in which they can come in contact with organisms and plants. (Van Aken, 2015) (Hou. et al., 2013) Release of nanomaterials in the environment through emissions, industrial and commercial products can have a large effect causing them to end up in wastewater treatment plants and furthermore to surface water. (Hou. et al., 2013) The nanoparticles that are not filtered in wastewater treatment plants are most likely to "accumulate in benthic sediments" which can possibly cause problems for many species in lakes and rivers. (Batley. et al., 2011) Aquatic organisms are "particularly susceptible to pollutants due to their large, fragile, respiratory epithelium". (MacCormack. et al., 2008) Changes in pH, water temperature, oxygen levels can also increase the risks associated with nanomaterials in aqueous environments and must be considered when addressing risk. Plants are also susceptible to high exposure of nanomaterials

through soil contamination or accidental release. Nanomaterials have been shown to enter living organisms and "exert toxic effects at the cellular level, including membrane disruption, protein inactivation, DNA damage, and disruption of energy transfer and release of toxic substances". (Van Aken, 2015) Due to the importance of plants and bacteria in the food chain it is crucial to understand the impact that this large industry could have in the future of environmental and human health. This paper will focus on the toxicological effects of waste engineered nanomaterials in terrestrial and aquatic environments and their implications on human health and environmental safety.

2.0 Background

2.1 Definition of Nanomaterials

Nanoparticles are naturally occurring in both aquatic and terrestrial environments in form of colloids, "mineral precipitates (Al, Fe, MgO, OH)" and dissolved organic matter". (Batley et al., 2011) The International Organization for Standardization (ISO) has classified three groups of nanomaterials. First, nanoparticles, which include all "three dimensions between 1 and 100 nm", are the most commonly referred nanomaterials. However, dimensionality plays a large role in determination of NM's showing that nanoplates (two dimensions between 1 and 100nm) and nanofibers (one dimension between 1 and 100nm) are also classified as NM's by ISO. (Batley et al., 2011) NNM's (natural nanomaterials) have always existed in the environment with little known toxicological effects. However, these definitions based only on size may not be sufficient in addressing toxicological risk, due to the fact that the nanomaterial version of a material exhibits different properties than it's non-nano counterpart. (Boverhof et al., 2015) With increasing nanotechnology and new products, engineered nanomaterials (ENM's) are now emitted into the air, water and soil. These ENM's can be categorized in "seven main classes: carbonaceous nanomaterials (carbon nanotubes (CNT's));

semiconductors (quantum dots); metal oxides (zinc oxide); nanopolymers (dendrimers); nanoclays; emulsions (acrylic latex); and metals (silver, gold)." (Buckley et al., 2011)

2.2 Properties of Engineered Nanomaterials and Microbial Toxicity

Suresh et al., (2013) have written a comprehensive review of the properties of nanomaterials and their relationship to microbial toxicity. Due to the fact that "microbial consortia underlie environmental processes" it is crucial to study the toxic effects of nanoparticles and the impacts this can have on "trophic balances". Nanomaterials are used in medicine because of their bactericidal and fungicidal properties, the most well known being Ag and CuO nanoparticles. (Suresh et al., 2013) However, when these engineered NM's are released into the environment such properties might have serious long-term impacts on both terrestrial and aquatic species. Some studies have tried to correlate the parent metal toxicity with that of its nano-form, however the size, shape, coating and the way the nanoparticle is synthesized determine that its toxicity is different than its parent metal. Furthermore, manufacturing processes add detergents, additives and other chemicals, which are not fully removed from the final product, increasing the toxicity. (Suresh et al., 2013) Metal nanoparticles are studied because they dissolve into ions in aquatic environments, which is "often a primary step and common cause for nanoparticle toxicity". (Suresh et al., 2013) The most widely studied and used ion for its microbial toxicity is silver, yet there are many studies showing that the "correlation between nanoparticle toxicity and that of its dissolved ion" is seen with other materials as well. (Suresh et al., 2013) Secondly, the coating of a nanomaterial determines the toxicity and effect it will have on bacteria. Usually, engineered nanoparticles are "surrounded by a shell or cap to act as a stabilizing, biocompatibility and or reacting agent". (Suresh et al., 2013) This shell can affect the charge of the nanoparticle and its interaction with the environment creating a completely different toxic effect than the nanoparticles parent material. Baumann et al., studied the acute effects on

Daphnia Magna of four different types of coatings on iron oxide nanoparticles which stabilized the NPs yet had toxic effects on the organisms, including death. Lastly, the size and shape of a nanoparticle can have a large effect on bacteria "as the particle size decreases, the ratio of surface area to mass increases" resulting in changes to the "physical-chemical properties" of the nanoparticle. (Suresh et al., 2013) This creates novel applications due to "surface atom reactivity, electronic and optical properties" which can influence binding characteristics in bacteria and increased reactivity. (Suresh et al., 2013) A general trend of increased toxicity with a decrease in size has been observed due to increased reactivity of smaller particles. (Suresh et al., 2013) Below is a table highlighting the known microbial nanotoxicity studies.

Table 1. Known effects of nanoparticles on bacterial strains. (Source: Suresh et al., 2013)

Nanomaterial	Size (nm)	Surface coating	Dosage[a] mg L^{-1}	Bacterial strains	Mode of action[b]	Ref.
Ag	10	Myrasmistin	2.5–5	*B. subtilis*, *S. aureus*, *L. mesenteroides*	Bactericidal	74
Ag	6.7 or 7.2	Mercaptopropionic acid or polylysine	7	*E. coli*	Growth inhibition and inactivation	104
Ag	25	-	4.8 ± 2.7	*S. mutans*	Cell membrane damage	63
Ag	1	-	10	*P. putida*	Bactericidal	105
Ag	9–21	-	>1	Nitrifying bacteria	ROS	106
Ag	20 80	Phosphate	0.25–1 ppm	*N. europaea*	Decreased ammonia monooxygenase activity and outer membrane destabilization	46
Ag-oleate	4 ± 1	Protein	5–7.5	*E. coli*, *B. subtilis*, *S. oneidensis*	Non-inhibitory	3
Ag-colloidal	9 ± 2	-	2–12	*E. coli*, *B. subtilis*, *S. oneidensis*	Cell membrane damage	3
Ag-biogenic	4 ± 1.5	Protein				
Ag–TiO_2	10–80	-	-	*B. subtilis*, *P. putida*	Bactericidal	80
Ag_2S	2–20	Protein	50–150	*E. coli*, *B. subtilis*, *S. oneidensis*	Non-inhibitory	41
Ag@MESs	100	MES	-	*E. coli*, *B. anthracis*	Growth inhibition	107
Ag–MgO	ND	-	4	*E. coli*, *B. subtilis*	Cell-membrane damage	48
Al_2O_3	179	-	0.1–1	*E. coli*	Minor growth inhibition	108
Al_2O_3, SiO_2, TiO_2, ZnO	1–100	-	10–200	*E. coli*, *B. subtilis*, *P. fluorescens*	Bactericidal	38
CeO_2	3–50	-	50–150	*E. coli*, *B. subtilis*	ROS	4
CeO_2	3–50	-	50–150	*S. oneidensis*	Non-inhibitory	4
CeO_2	10 25 50 60 5000	-	2.4–29.6	*P. subcapitata*	Cell wall and membrane disruption	109
SiO_2, TiO_2, ZnO	10–1000	-	205–480	*E. coli*	ROS, cellular internalization, membrane disorganization	15
CuO	10	-	70–300	*P. putida*	Bactericidal	105
CuO, spiked multiarms	500–1000	Uncoated	500	*E. coli*, *S. typhi*, *S. aureus*, *B. subtilis*	Bactericidal activity	70
CdSe–CdS	2–10	-	-	*P. aeruginosa*	Non-toxic	57
CdSe	8	Citrate	50	*P. aeruginosa*	Membrane damage, impaired growth and ROS	40

CdTe	3.6	Alkanethiols	21.2 mM 11.6 mM 17.4 mM 11.1 mM	*E. coli, S. aureus, P. aeruginosa, B. subtilis*	Toxic, charge transfer	77
CdTe nanowires	40–60	Mercaptosuccinic acid	100 nM	*E. coli*	Oxidative damage	110
Fe_3O_4@TiO_2	>100	-	2.57	*S. pyogenes, S. saprophyticus*	Photokilling	111
FePt	9	-	2.5 per plate	*E. coli, S. typhimurium*	Mutagenicity, DNA damage	112
Iron doped ZnO	0.3–43	-	-	*E. coli, B. subtilis*	Bactericidal	51
Si	50	AAPTS and TES	0.8	*P. aeruginosa*	NO release	66
Si	100 200	AAPTS and TES	1.5	*P. aeruginosa*	NO release	67
TiO_2/Fe_3O_4	22–33	Oleic acid	-	*S. iniae, E. tarda*	Photokilling	113
TiO_2	Varying	-	-	*C. metalliruans, E. coli*	ROS	71
TiO_2	-	-	0.2 mM	*E. coli*	Bacterial inactivation	114
TiO_2	15–20	-	0.05–0.5	Soil bacterial communities	Substrate induced respiration and DNA damage	83
ZnO	20–30	-				
ZnO, CuO	1.9–79		-	*V. fischeri*	Bactericidal	37
ZnO	260 ± 40 6.8 ± 2	Uncoated DMF	0.1–10	*E. coli, S. aureus*	Cell division arrest and oxidative stress	34
ZnO	20–40	2-Mercaptoethanol	4	*E. coli*	Bactericidal	64
ZnO	13	Diethylene glycol	3.4 mM 1 mM	*E. coli* *S. aureus*	Bactericidal	115

Nanomaterial	Size (nm)	Surface coating	Dosage[a] mg L^{-1}	Bacterial strains	Mode of action[b]	Ref.
ZnO	27–71	PEG and PVP	-	*E. coli*	Non-toxic and increased stability of the particles	62
ZnO rods	250	Hexamethylene tetramine	7.5 mM 1 mM	*E. coli, B. atrophaeus*	ROS mediated membrane damage	75

[a] Concentration is mg L^{-1} or as noted. [b] ROS: reactive oxygen species, ND: not determined; - unknown/commercial.

As previously mentioned; the coating, size, dosage and type of nanomaterial have different effects on different bacterial strains. Some surface coatings such as PEG and PVP are used as stabilizing agents for ZnO nanoparticles and display no known toxic effects on *E. coli* whereas an MES stabilized Ag^+ ion displays inhibition in *E. coli.* PVP was also found to be the most promising stabilizer for medical applications due to its polymer coating, which reduces the release of iron ions through high colloidal stability. (Baumann et al., 2014) Most of the studied nanoparticles however, present possibilities of being bactericidal, mutagenic, cause membrane damage, act as inhibitors and destabilizers. Very few of the studied nanoparticles have no side effects in bacteria.

2.3 Potential Mechanism of Biological Uptake and Toxicity

Klein et al. have described some possible mechanisms of nanomaterial toxicity to bacteria, as shown below in **Figure 1.**

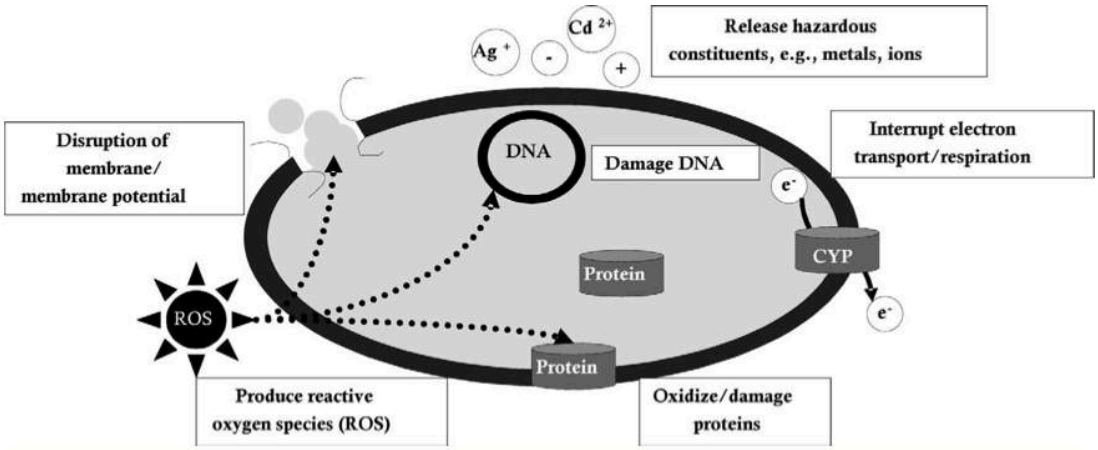

Figure 1. A proposed schematic diagram by Klein et al., of the possible mechanisms of nanomaterial toxicity to bacteria. (Image retrieved from Klein et al., 2008)

Nanoparticles are shown to enter the cell by diffusion through the membranes as well as through endocytosis and adhesion. (Klein et al., 2008) Quantum dots and carbon nanotubes (CNTs) are designed to interact with "proteins, nucleic acids or cell membranes for drug delivery purposes" which makes the unintentional interactions with the environment potentially hazardous. (Klein et al., 2008) As mentioned previously, CNTs are also some of the most mass produced nanoparticles for many applications, which increases the risk of environmental exposure through product use and industry leaks. The diagram depicts possible mechanisms, which include "disruption of membranes or membrane potential, oxidation of proteins, genotoxicity, interruption of energy transduction, formation of reactive oxygen species and release of toxic constituents." (Klein et al., 2008) These effects are all seen in the data provided in **Table 1.** of the known effects of nanomaterials on different bacterial strains.

3.0 Discussion and Analysis

3.1 Pathways of Nanomaterials in the Environment

NMs find their way into the environment intentionally and unintentionally, through consumer products such as medicinal devices, paints, electronics, cosmetics, car catalysts, plastics, ceramics and more. Other pathways could include industrial runoff, spills and waste. Most research is focused on ENMs that have a high potential for industrial spills or waste such as carbon nanotubes and metal oxides due to their mass production and possibly toxic effect on the environment. (Lowry et al., 2010) Some large risks associated with the manufacturing of these NMs are the unintentional leakage that can occur during the transportation of the NP's to secondary or tertiary sites.

Kalavrouziotis et al., 2008) have studied the impact of the platinum group elements (Pt, Pd, Rh) emitted into the atmosphere through automobile catalyst converters. The study suggests that even though catalytic converters minimize the pollution emitted by the car exhaust fumes, the platinum group elements are being emitted in forms of particulate matter and accumulating in soil, plants and air. (Kalavrouziotis et al., 2009) Due to the nature of these emissions, the particulate matter is being transported over long distances and have "increased significantly during the last ten to fifteen years, especially along the road side of high ways". (Kalavrouziotis et al., 2009) Intentional releases of nanomaterials also occur when remediating contaminated soils, and the "use of iron NPs to remediate groundwater". (Klaine et al., 2008) Other pathways of intended release are through consumer goods, such as sunscreen, health care products, fabrics and paints, which enter the environment "proportional to their use". (Klaine et al., 2008) In order to effectively filter these NM's requires "a new class of nanostructured sorbents" which is not widely available due to unregulated emissions.

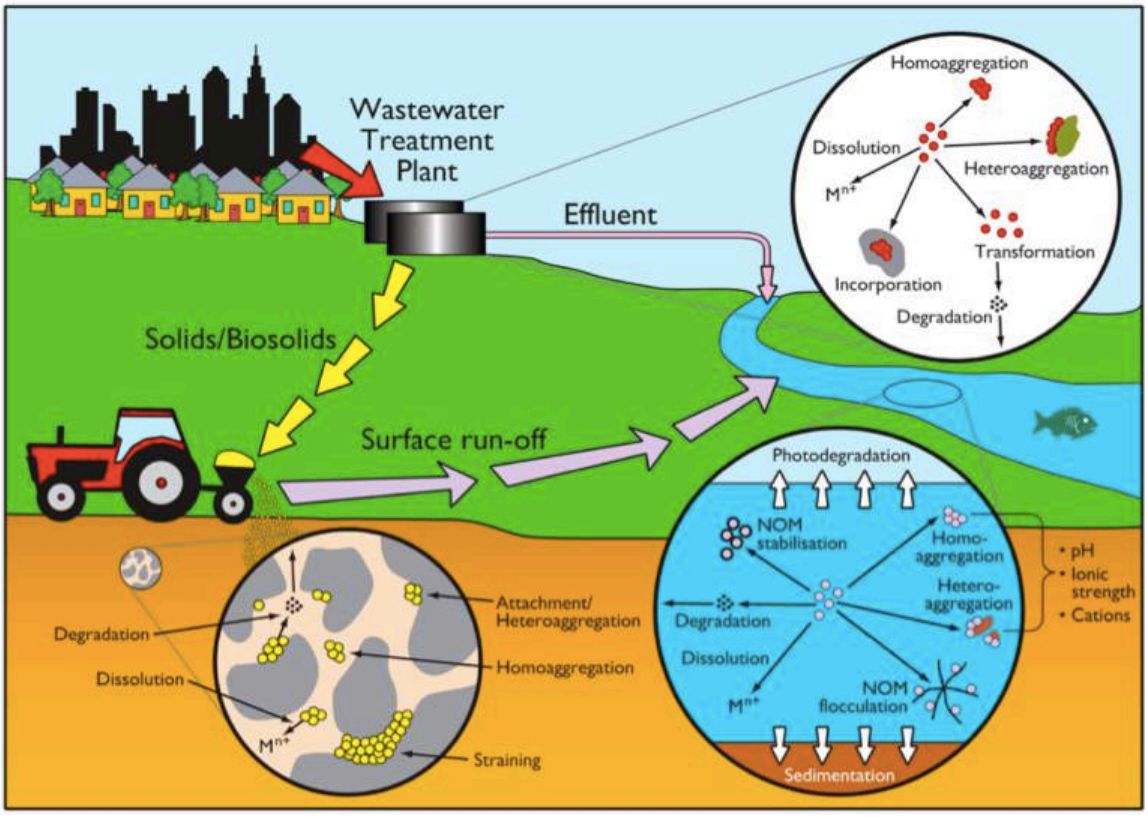

Figure 2. Proposed schematic diagram of pathways of NPs into the environment. (Image retrieved from Batley et al., 2011)

Despite knowledge of the pathways into the environment, trying to analyze the life cycle of the nanoparticles and the exact mechanisms of entry is quite challenging. Most studies have focused on *in situ* experiments, which can be vague and unlikely to show the correct processes. (Nam et al., 2014) However, "researchers have turned to microcosm and mesocosm systems as miniaturized ecosystems to simplify experimental conditions", which helps to understand "the uptake and bioaccumulation" of NPs. (Nam et al., 2014)

3.2 Uptake of Nanoparticles in Aquatic Ecosystems

Since nanoparticles are designed to "persist as particulate matter in aqueous media", they are able to pass through biological membranes due to their size. (Velzeboer et al., 2008) Nanoparticles in aqueous solutions form colloidal suspensions, which can potentially interact with aggregates, other colloidal suspensions and agglomerates. (Velzeboer et al., 2008) This occurs in marine ecosystems because they are generally more alkaline, have higher ionic strength and already have a "wide variety of colloids and natural organic matter". (Klaine et al., 2008) Coastal zones are very likely to have a high concentration of colloids, organic matter and nanoparticles, being closer to discharges or spills from plants or industries than deep ocean water.

In freshwater, the nanoparticle aggregates have a high chance of sinking slowly to the bottom and accumulating in the sediment; this can have a negative effect on the benthic species. In marine ecosystems, it is possible that "nanoparticles will accumulate at the interface between cold and warm currents" which is not likely with freshwater. (Klaine et al., 2008) Another possibility for the mechanism of NPs in marine ecosystems could be a recycling through biota. This can increase the risk of the species, which feed within these cold and warm zones such as tuna. Lastly, Klaine et al, 2008 introduced another possibility, namely accumulation in the "surface microlayers of the oceans" where nanoparticles are trapped due to surface tension and viscous properties. This presents a risk to marine birds and mammals as well as the organisms living in the surface microlayer. (Klaine et al., 2008) However, there is no research that studies the different effects of accumulated nanoparticles in the surface microlayer of oceans. **Figure 3.** represents a schematic diagram of the proposed behavior of nanoparticles in marine ecosystems. It shows the nanoparticles and organic matter discharged from coastal runoff and suggests that the NPs are diluted and transported in the open ocean, and can also sink to the bottom and become

aggregates. These aggregates are further transported by microbes or settle in sediment causing toxic effects to benthic species. Another mechanism shows that the free nanoparticles that are transported can have a toxic effect on pelagic species through direct contact, as well as become a risk to seabirds and mammals through aerosol formation. (Klaine et al., 2008)

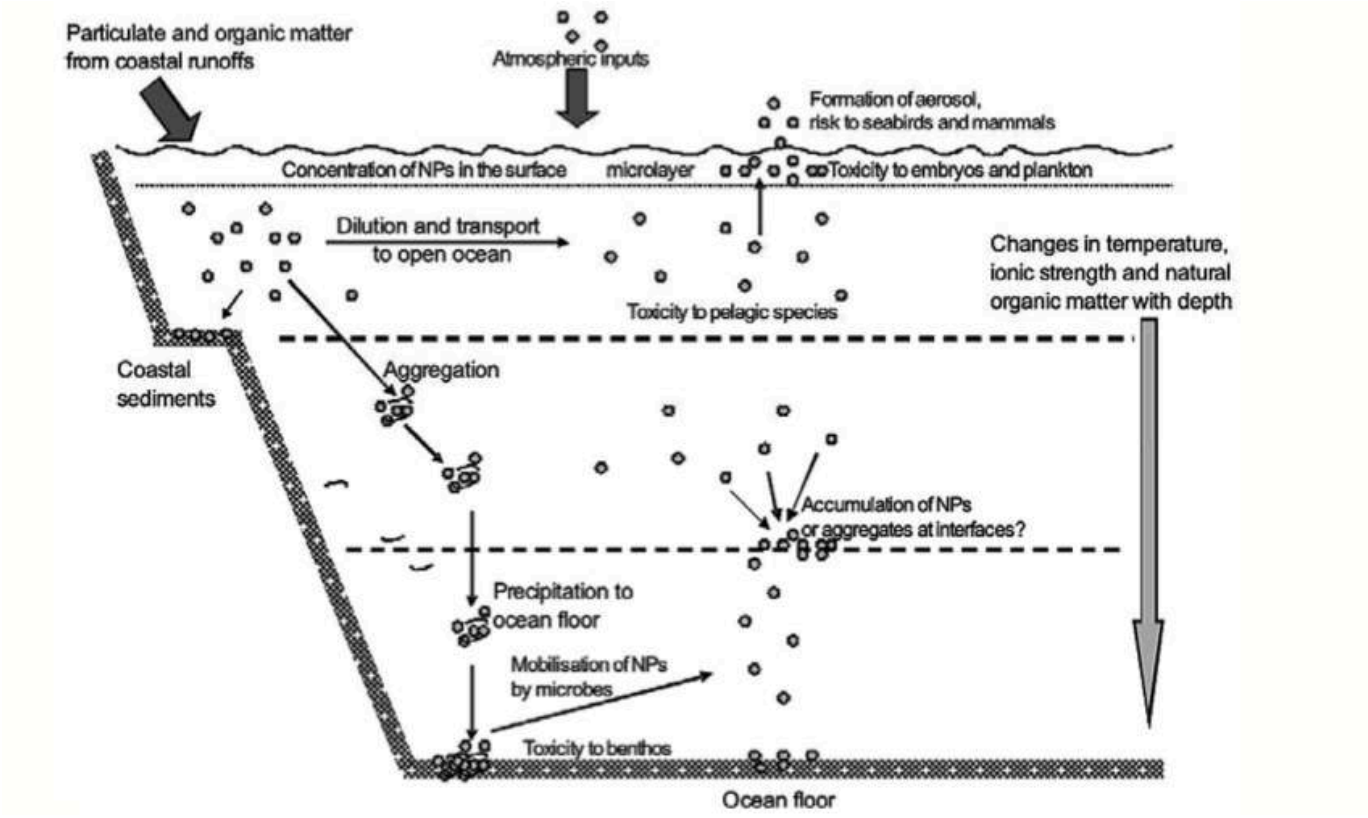

Figure 3. Schematic diagram showing the behavior and effects of NPs on marine environments as well as the organisms at risk of exposure. (Image retrieved from Klaine et al., 2008)

3.3 Bioaccumulation of NMs in Aquatic Ecosystems

Nam et al., have written a comprehensive review, which focused on the bioaccumulation of NPs in aquatic environments. Due to the complexity of nanoparticles and the effects on the environment depending on the size, shape, coating and functionality, they decided to focus specifically on TiO_2 NPs and Ag NPs. A simplified microcosm system was designed to "assess the bioaccumulation of TiO_2 NPs in

multiple model species". (Nam et al., 2014) They discovered that a high level of TiO_2 NPs were present in the sediment layer due to settling of NPs as previously mentioned in the proposed schematic by Klaine et al. 2008. However, there was also experimental evidence, which showed a movement of the TiO_2 from the sediment to "water dropwort roots and nematodes living on these plants". (Nam et al., 2014) Nanoparticles were also transferred through different trophic levels as shown by Nam et al., through transfer from "dropwort roots, to nematodes and snails feeding on these roots as well as from biofilm-consuming plankton to ricefish feeding on the plankton". (Nam et al., 2014) This shows that the engineered nanoparticles can travel through feeding patterns of aquatic organisms. There was also direct evidence of trophic transfer of TiO_2 NPs showing NPs "transferred from water fleas to zebrafish, indicating potential biomagnification of TiO_2 via food chain transfer". (Nam et al., 2014) Ag nanoparticles were also studied in order to measure the bioavailability of these nanoparticles to higher trophic organisms. (Nam et al., 2014) Nam et al., show that algal cells concentrate nanoparticles due to adhesion of NPs to the cell wall. They also provide a table summarizing the different uptake endpoints of the Ag NPs in different species. In the earthworm they found that the bioaccumulation depended on the concentration and could be possibly distributed throughout the body. In rainbow trout they propose that the possible adsorption occurs through the gill, which was similar to the Japanese medaka. (Nam et al., 2014) The two tables are presented below of both the bioaccumulation of TiO_2 NPs as well as the Ag NPs.

Table 2. Uptake and bioaccumulation of TiO_2 nanoparticles in aquatic organisms (Table retrieved from Nam et al., 2014)

Species tested		NP size (nm)	Type	NP characterization	Concentration range	Duration	Uptake endpoints
Water dropwort Biofilm Ricefish Mud snail Nematodes Quillworts Algae	*Oenanthe javanica* *Oryzias sinensis* *Cipangopaludina chinensis* *Meloidogyne* sp. *Isoetes japonica* *Spirogyra* spp.	9		TEM	2 mg/L	17 d	Bioaccumulation and trophic transfer
Biofilm, plankton		20	P25	TEM	5.3 mg/L	24 d	Deposition and adsorption to biofilm
nematode	*Caenorhabditis elegans*	50	Anatase	TEM	24-239.6 mg/L	24 h	Possible to adsorption
Earthworm	*Lumbricus terrestris*			XRF	1-100 mg/L	7 d	Unpredictable
Water flea	*Daphnia magna*	< 20	Anatase	Microscope	0.5-500 mg/L	48 h	Uptake and distribution in body
		21	Anatase, Rutile	SEM	0.1-100 mg/L	72 h	Bioaccumulation in body
Water flea Zebrafish	*D. magna* *Danio rerio*	21	P25	SEM	0.1-1.0 mg/L	24 h	Trophic transfer and biomagnification
Carp	*Cyprinus carpio*	21	P25	TEM	10 mg/L	25 d	Bioaccumulation in intestine, stomach, and gills
		21	P25	TEM	10 mg/L	25 d	Bioaccumulation in viscera and gill
Rainbow trout	*Oncorhynchus mykiss*	21	P25	TEM	0-1.0 mg/L	0-14 d	Bioaccumulaton in tissues
Zebrafish, trout							Adsorption and translocation in gill and skin
Duckweed	*Lemna minor*	21	P25	TEM	0.01-10 mg/L	72 h	Attachment onto cell wall, but no cellular uptake

TEM: Transmission electron microscopy; SEM: Scanning electron microscopy; XRF: X-ray fluorescence

Table 3. Uptake and bioaccumulation of Ag nanoparticles in aquatic organisms (Table retrieved from Nam et al., 2014)

Species tested		NP size (nm)	Type	NP characterization	Concentration range	Duration	Uptake endpoints
Diatom	*Thalassiosira weissflogii*	10	PVP	TEM, EDS		48 h	Cellular distribution
Aquatic bacterium	*Pseudomonas fluorescens*	30-50		TEM, EDS	2-2000 ppb	24 h	Aggregation by nanoscale film formation
Eastern mud snails, Juvenile hard clams, Grass shrimp, Cordgrass, Biofilms		20-80		ICP-MS		60 d	Bioaccumulation and trophic transfer
Nematode	*Caenorhabditis elegans*	< 100		TEM	up to 0.5 mg/L	24 h	Uptake/adsorption to body
		7-25	PVP, Citrate	TEM	5-50 ppm	24 h	Uptake/transgenerational transfer to body
Earthworm	*Eisenia fetida*	30-50	PVP, OA	TEM, XAS		28 d	Bioaccumulation in a concentration-dependent manner
		10-50	PVP, OA	TEM	< 100 mg/kg	48 h	Unpredictable
			PVP	TEM, SEM	1000 mg/kg	28 d	Possible body distribution
Water flea	*Daphnia magna*	40-50	Carbonated	TEM	up to 5000 µg/L	8 h	Uptake and bioaccumulation
Zebrafish embryos	*Danio rerio*	5-15		TEM	0.71 nM	120 h	Uptake in embryos through chorion pore canals
		11.3		TEM	0.2 nM	21 h	Adsorption to embryos
		20-30		TEM, SEM	10-20 ppt	24 h	Penetrated skin and blood tube as aggregated particles
		20-30		TEM, SEM	0-4 ppm	10 d	Bioaccumulation in muscle and intestine
		20-30	P25	ICP-MS	10 mg/L	48 h	Possible body uptake
Eurasian perch	*Perca fluviatilis*	30-40	PVP	TEM	63-300 ppb	25 h	Possible to adsorb into gill
Rainbow trout	*Oncorhynchus mykiss*		PVP, Citrate	TEM	10-20 mg/L	48 h	Cellular compartmentallization, transport over epithelial layers
Japanese medaka	*Oryzias Latipes*		Citrate	TEM	20 µg/L	7 d	Bioaccumulation in liver and gill
Zucchini	*Cucurbita pepo*	100		ICP-MS	1000 mg/L	12 d	Translocation through shoots
Thale cress	*Arabidopsis thaliana*	20-80		Microscopy			Uptake and accumulation to roots
Common grass	*Lolium multiflorum*	6-25	GA	TEM	0-40 mg/L	24 h	Uptake into roots and shoots

EDS: Energy dispersive X-ray spectroscopy; GA: Gum arabic; ICP-MS: Inductively-coupled plasma mass spectrometry; PVP: Poly vinyl pyrrolidone; OA: Oleic acid

3.4 Impacts on Algae, Fish and Aquatic Organisms

The toxic impact of nanomaterials on fish and aquatic organisms is important to study because most contaminants released in the environment are consumed by aquatic species. Griffitt et al., 2008 have conducted a study in order to assess the toxicity of metallic nanoparticles in aquatic organisms. They used zebrafish, daphnids and *Pseudokirchneriella subcapitata* as models of the varying trophic levels and different feeding strategies. These organisms were exposed to Cu NPs, Al NPs, Co NPs, Ag NPs, Ni NPs and TiO_2 since they are the most commonly engineered nanoparticles with known parent metal toxicity.

The results of the study showed that the nanometals were causing “acute toxicity in multiple aquatic species”, and “filter-feeding invertebrates” having the highest susceptibility to metallic nanoparticle exposure. (Griffit et al., 2008) All the organisms tested were acutely susceptible to Ag NPs and Cu NPs, with the daphnids and algae being more affected by toxicity than zebrafish. (Griffit et al., 2008) Since daphnids are particulate filter feeders, they are more likely to be exposed to larger numbers of nanoparticles compared to larger organisms. (Griffit et al., 2008) These results also concluded that there was no apparent relation between size and surface area, and that the nanoparticles were capable of “causing acute toxicity in multiple aquatic species” regardless of their shape. (Griffit et al., 2008) Ag NPs and Cu NPs were the most toxic in all species yet both the size and shape of these nanoparticles varies greatly, and other nanomaterials with the same sizes have no effect on organisms. Furthermore, silver and copper are the most toxic when presented in a soluble form and the toxicity was confirmed to be partly because of dissolution of particles. (Griffit et al., 2008) These toxic effects however are not only limited to daphnids and MacCormack et al., suggest that the physical dimension of nanoparticles may allow them to “interact with cellular receptors or transport proteins” in aquatic animals. “The respiratory and ion transport surface area can be greater than 60% of the total surface area” of fish posing large health threats when interacting with nanoparticles. (MacCormack et al., 2008) It was found that the interaction of gill ion transport with metal nanoparticles resulted in ionoregulatory failure. (MacCormack et al., 2008) Furthermore, studies have mostly focused on the effects of NPs under “steady-state physiological conditions”, which is not the case in aquatic environments. (MacCormack et al., 2008) Marine ecosystems have changes in pH, salinity, pressure and temperature, which is not taken into account when studying nanomaterial toxicity in animals living under these conditions. (MacCormack et al., 2008) Specifically, cell membrane structure is altered during temperature changes by the “variation of fatty acids and cholesterol” which can be affected by nanomaterials that are specifically engineered to insert

"into biological membranes". (MacCormack et al., 2008) Salinity of marine ecosystems also has to be considered, as "fish adapt to changes in salinity" through changes in their gill membrane which also effects organisms "exposure to nanomaterials in the environment". (MacCormack et al., 2008) Lastly, due to limited oxygen exposure in water, "fish exhibit hypoxia" increasing their vulnerability of nanoparticle exposure and toxicity. Hypoxia is exhibited in forms of increased "ventilatory effort" as well as "cardiovascular changes necessary to exploit adjustments". (MacCormack et al., 2008) This increases the risk of toxicity to the respiratory system since many species double their skin capillary surface area in order to improve the efficiency of oxygen uptake, allowing for nanoparticles to enter the system and cause acute toxic effects in aquatic species. (MacCormack et al., 2008) Chronic exposure of nanomaterials to aquatic species is important to study in order to know long-term effects this will have on organisms. Zhu et al., have studied the chronic exposure to sublethal fullerenes aggregates on carp showing that the most susceptible organs were found to be the gills, the brain and the liver. They found that "oxidative stress due to long-term exposure could be the main mechanism for toxicity" for these fish under freshwater conditions. (Zhu et al., 2008) These findings are important in creating regulations that will penalize companies for spills or emissions of nanomaterials in rivers, lakes and oceans.

3.5 Evidence of Engineered NMs in Terrestrial Ecosystems

Natural nanomaterials have long been recognized to exist in terrestrial ecosystems. Soil contains many materials that are less than 2 micrometers, namely "loosely called colloidal soil". (Videa et al., 2011) The components of colloidal soil contain iron oxide nanoparticles as well as humic acids and phyllosilicates. (Videa et al., 2011) However due to the increase in production of nanotechnology, various engineered nanoparticles are entering the terrestrial environment through direct modes, such as "zero-valent metal for remediation" and indirect modes such as spills and emissions. (Videa et al., 2011)

Emissions are caused mainly through catalytic converters that increase the occurrence of Pt, Pd and Rh nanoparticles in the environment. (Kalavrouziotis et al., 2008) The concentrations of the nanoparticles either natural or anthropogenic are unknown in soil or terrestrial ecosystems due to the complexity of separating and identifying the nanomaterials. (Videa et al., 2011) It is assumed that the fate of the nanoparticles when they are released into such environments depends on their specific physical and chemical characteristics. (Videa et al., 2011) In addition, ionic strength, pH and soil texture also affect the impact and transport of nanomaterials, causing multiple behaviors such as "aggregation, transport, sorption, desorption, stabilization and dissolution" into the soil. (Videa et al., 2011)

3.6 Uptake and Bioaccumulation of NMs in Edible Plants

Rico et al., have reviewed relevant literature on the uptake and bioaccumulation of NM's in edible plants and the impacts this could have on the food chain. As previously discussed in the report, a lot of studies have gone into researching the uptake of NMs and their effects on cells, however little is known about the effects of NMs in edible plants. (Rico et al., 2011) The only carbon-based nanomaterials "shown to readily accumulate in plants" were fullerols and the C_{70} fullerene, whereas most of the metal-based nanomaterials such as Au, Ag, Cu and Fe were readily accumulated, "although some conflicting data exists". (Rico et al., 2011) Even though many nanomaterials are different depending on their size, shape, coating and core, data suggests that NP's can enter the plant cells by "binding to carrier proteins, through aquaporins, ion channels, or endocytosis, by creating new pores (in the case of Carbon Nanotubes (CNTs)) or by binding to organic chemicals in the environmental media". (Rico et al., 2011) Carbon based nanomaterials such as CNTs are used as novel drug delivery systems and ongoing studies are being conducted to find the mechanisms which allow CNTs to penetrate plant cells. Rico et al. (2011) hypothesize that the CNTs interact with proteins and polysaccharides on "the cell walls and elicit

hypersensitive responses mimicking plant pathogens due to their small size", which ultimately leads to "cell mortality". () Metal-based (MB) nanoparticles are also studied, and very little is known on the mechanisms and uptake of MB NP's. Due to the fact that the cell wall pore sizes vary from 2-20 nm the larger nanoparticles have a harder time penetrating the cell walls, which means that only the small NP's, which were found to be more reactive, can accumulate in plants. It is also not clear if the NP's remain the same when they are inside the plant walls or if they form aggregates or colloids, which will change the function and behavior. Below is a schematic diagram of the different methods in which nanoparticles could accumulate in edible plants also showing possible mechanisms, however the data for some mechanisms is still inconclusive, as shown in the diagram.

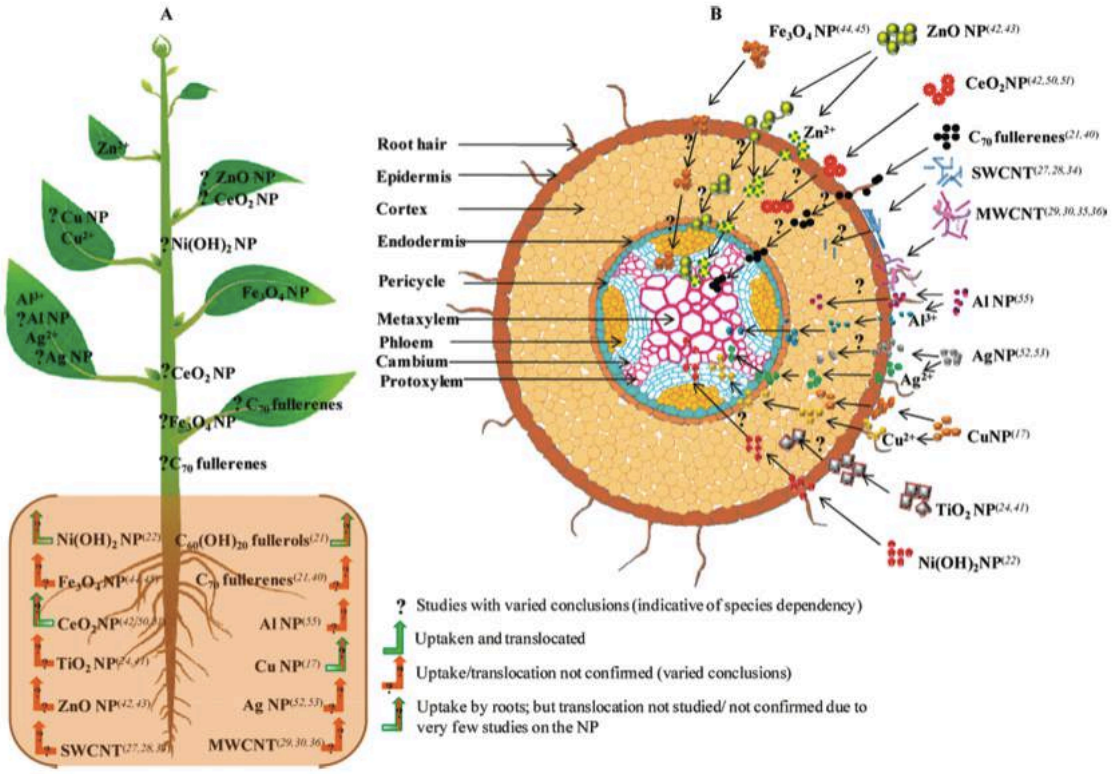

Figure 4. Diagram depicting the uptake, translocation, and biotransformation pathway of various nanoparticles in plants. Part A shows the uptake and the proposed location of the particles whereas part B shows the cross section of the absorption zone in the root, showing different nanoparticle interaction on exposure. (Rico et al., 2011)

Rico et al.(2011) also concluded that the medium in which the plants are grown is important, due to an observed zero intake of NMs in plants grown in soil. Furthermore, there are no studies that show how and where plants store nanoparticles that are accumulated.

3.7 Gene Expression Changes in Plants

The effects corresponding to NM's in edible plants are found to be both positive and negative. Rico et al.(2011) give a comprehensive review of the effects different nanoparticles have on growth and germination of many plants. Van Aken et al. (2015) have also studied the gene expression changes in plants and microorganisms exposed to nanomaterials with mostly negative results. These papers concluded that the TiO_2 nanoparticles on soil bacteria changed the "bacterial community structure", and showed a "reduction of nitrogen fixers and methane oxidizers" through direct toxicity to the soil. They have also concluded that many Ag nanoparticles varying in size from 45 nm to 85 nm show cell death, inhibition of cell growth, association with the cell wall, and antibacterial activity. (Van Aken, 2015) In plants, Kaveh et al., have concluded that Ag nanoparticles increase the growth of *A. Thaliana* at low doses and decrease it at high doses, positively increasing the "pathogen resistance and plant biomass" at low doses. (Van Aken, 2015) The studies suggest that the Ag nanoparticles are affecting the plants partly through the toxicity of the Ag metal and the size of the NP. (Van Aken, 2015) (Mendes et al., 2015) Some photosynthetic pathways have also been affected by NMs in plants, specifically Ma et al., have concluded that high levels of Cerium Oxide negatively "impacted plant growth and chlorophyll production". (Ma et al., 2011) (Van Aken, 2015) Positively, it was found that when *A. thaliana* is exposed to TiO_2 nanoparticles, a light harvesting complex gene is induced and results in increased efficiency of light absorption in the chloroplast. (Van Aken, 2015) Research has also shown that graphene oxide treated with PEG negatively impacts the development of *A. thaliana* seedlings due to the negative effect on genes

involved in the development of the roots. Several studies, specifically Lahiani et al., have shown that multi-walled carbon nanotubes (MWCNTs) can have positive impacts on the seeds of barley, soybean and corn. Agglomerates of MWCNTs were found using Raman Spectroscopy and Transmission Electron Microscopy inside the seeds, and activated the expression of water channel genes (aquaporins) producing favorable results in growth and germination. (Lahiani et al., 2013) This study however, was created with the specific intent on increasing yield in these crops and not accounting for nanomaterials found in the environment due to unintentional release. Van Aken has also included that MWCNTs enhance the growth of tobacco over a wide range of concentrations. MWCNTs were however shown to reduce the root length of lettuce and cause cell death, plasma membrane detachment and cell shrinkage in rice. (Rico et al., 2013) Single walled carbon nanotubes (SWCNTs) have also raised concern because they inhibit growth of hair roots in maize plants. (Van Aken, 2015) Rico et al., have shown that Ag nanoparticles show reduced germination and shoot length on ryegrass, flax and barley as well as reduced transpiration and biomass on zucchini. Cu NPs were shown to have reduced seedling growth on mungbean and wheat as well as reduced root growth on zucchini. (Rico et al., 2013) CeO_2 NPs show negative effects on alfalfa, tomato, lettuce, cucumber, maize and soybean through reduced shoot growth, reduced germination and reduced root growth. (Rico et al., 2013) (Doolette et al., 2015) Lastly, it is observed that nanomaterials behave differently in plants than bacteria, affecting more than one transcription factor making it difficult to understand the pathway and mechanisms involved in activation.

3.8 Impacts on Humans Health

Tang et al., have studied the implications that engineered nanoparticles can have on the health of infants and children. They propose that humans have a risk of coming into contact with nanomaterials through consumer products, foods, sunscreens, toys, clothes, medical applications, drug delivery and

biomedical imaging. (Tang et al., 2015) Other possible mechanisms would be through bioaccumulation in the food chain through plants and crops, animals and fish as well as through direct contact with the air, water and soil. They propose that through these contact methods, nanoparticles can pose potentially threatening toxic effects for the skin when exposed to metallic nanoparticles such as iron, TiO_2 and quantum dots. (Tang et al., 2015) The nanoparticles were shown to penetrate the skin barrier and cause ROS mediated skin aging, and it can also lead to "systematic exposure and development of lesions". (Tang et al., 2015) A 17-year old patient "developed hepatotoxicity and argyria-like symptoms after treatment with an Ag – containing wound dressing". (Tang et al., 2015) The respiratory system is also very susceptible to airborne nanoparticles, which can be deposited in the alveoli increasing toxic symptoms. Due to the small size of the nanoparticles, they can penetrate the "thin blood-air barrier", and move to other organs increasing the damage. In 2006, 100 German consumers had symptoms including "coughing, sleep disruption, headache and vomiting" after using a bathroom cleaning aerosol product that contained ZnO nanoparticles. (Tang et al., 2015) The illness was later found not to be linked to the nanoparticles however, a 26-year old chemist handling nickel NPs had symptoms of throat irritation, nasal congestion and flushing whereas a 38-year old male died thirteen days after inhaling nickel nanoparticles. (Tang et al., 2015).

The same mechanisms that are shown in aquatic and terrestrial organisms can be expected in humans as well due to the size and shape of specific nanoparticles. Other case studies show adverse health effects to the gastrointestinal tract (GIT) and liver through exposure of engineered nanoparticles after ingesting food and pharmaceuticals. (Tang et al., 2015) Rats exposed to silver nanoparticles in the liver showed a reduced liver weight and "accumulation of granular material". (Tang et al., 2015) The brain, immune and circulation systems as well as the reproductive and developmental systems can also be

affected by engineered nanoparticles. Videa et al., also discuss the impacts of engineered nanoparticles in humans and mammals showing that CuO NPs are extremely toxic to lung epithelial cells due to induced DNA and oxidative lesions. CdSe quantum dots with a coating of polyethyleneglycol (PEG) lost their coating when tested in the intestinal cells due to the lowered pH, which increased their nanotoxicity. (Videa et al., 2011) The mechanisms for exposure are the same as previously seen, where the nanoparticle size and shape allows it to enter the cells of the organs and cause toxic effects. High instances of "cardiovascular disease" were seen in workers that are handling ENPs when compared to a control group. (Tang et al., 2015) Lastly, the reproductive and developmental systems are affected "causing early miscarriages and fetal malformations" in pregnant mice after "10 days of SWCNTs injection". (Tang et al., 2015) These observed health effects on humans have large implications for future studies, showing that engineered nanomaterials should be carefully assessed before being made widely available.

4.0 Limitations

There are many limitations when trying to assess the impact that nanomaterials have on the environment. Due to the many differently engineered nanoparticles and their different sizes and properties it is very difficult to make assumptions about all nanomaterials based on the findings of one. Some nanoparticles have shown that they are extremely toxic to bacteria, yet other studies show that nanoparticles can help in the growth of edible plants. (Lahiani et al., 2013) Other limitations include individual species and their interaction with the environment as well as their ability to store or expel small nanoparticles. Furthermore future studies should focus on the long-term impacts on the environment rather than acute toxicity tests on simple species. There are also limitations in understanding how the behavior of the nanoparticles differs in the environment, and creating replicable scientific studies on the mechanisms and the bioaccumulation of nanoparticles on both aquatic and terrestrial species. (Yao et al.,

2013) Very little is also discussed on the biomagnification of these nanoparticles within aquatic and terrestrial organisms and how this will affect food consumption. Moreover, fundamental studies between the structure and function relationships of nanoparticles are lacking which makes it more difficult to assess toxicity. (Rickerby et al., 2007) This study is also very wide and tries to provide a holistic view of the environmental impact of nanomaterials in many species, however in order to have a better understanding each of the aspects have to be extensively researched.

5.0 Conclusion

The increase in nanotechnology poses large toxicological implications because of the release of nanomaterials into the environment affecting bacteria, edible plants, fish and mammals. The size, shape, coating and functionality of nanoparticles are crucial in understanding their specific effects on aquatic and terrestrial ecosystems. Firstly, the small size of nanoparticles increases their surface area and their ability to enter cells causing cell mortality and increased reactivity in microbes. (Suresh et al., 2013) The coating and production process have large effects on the NP characteristics, increasing toxicity and environmental impact. NMs have been shown to enter cells through cell diffusion as well as through endocytosis and adhesion. (Klein et al., 2008) This poses a threat due to the mass production of these materials making them more widely available in the atmosphere increasing the risk for plants, animals and humans to come into contact with them. Moreover, nanoparticles are shown to settle in the sediment of waterbeds as well as soil, causing small organisms living in these systems to have high nanoparticle accumulation. Lastly, human health case studies have shown that humans can be affected through inhaling, eating and coming into contact with nanoparticles affecting all the organs due to their small size and ability to enter the circulatory system. (Tang et al., 2015) However, many of the studies available are only focused on the short-term acute effects of nanoparticles in the environment, yet long-term studies are also crucial due to

the fact that health impacts might not be relevant until decades later. (Tang et al., 2015) In order to study this, biomarkers can be used to examine nanoparticles in the environment and not only within the human body. (Tang et al., 2015) Finally, regulation needs to be discussed for companies that are producing nanomaterials and new technology in order to manage risk before seeing adverse health and environmental effects. Without governmental control nanomaterials are likely to accumulate in soil and water through extensive use and production of new technology, spills, runoff and emissions.

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