Nanoparticles: Case studies of their synthesis, properties and biological interaction

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Nanoparticles: Synthesis, properties and their interactions with plants and plant interfaces

Abstract

Similarly, as the rapid development of genetics during the end of the 20th century, did nano research, also change the way we see and make things. Perhaps it was not a coincidence, genetics, is indeed, a nano phenomenon. As our understanding of the physics and chemistry at the nanoscale has changed, our understanding of biology has improved and fields such as quantum biology has emerged. Because, it is within this certain size range, where giant molecules and tiny materials meet at the edge of quantum physics, that properties radically change. It has become evident, how little we know about what is happening at this scale and how important it is that we learn. This thesis, is but a small scratch on the surface of the investigation of nanomaterial interaction with biological organisms and systems. A small selection of some of the most common inorganic nanoparticles have been investigated, for a small set of properties and interactions.

First, investigations of titanium dioxide nanoparticles and their possible interactions with microbes in the rhizosphere have been described. How the chemistry of titanium dioxide affects their nanoparticle interaction with microbes and plants through phosphonate affinity.

Second, iron oxide nanoparticles and their enzymatic behaviour have been studied. How these enzymatic properties might have beneficial effect in plants during abiotic stress, due to large build-up of hydrogen peroxide during such conditions.

Third, calcium carbonate nanoparticle synthesis has been developed. Substantially changing production of possible sizes of calcite nanoparticles. These particles have then been studied for their interaction with organic molecules and formation of nanocomposites for their delivery mechanisms. Further, these particles have been tested for their effect on aphid infestations on plants and how it might be possible to unleash plants innate defence mechanisms that aphids otherwise can overcome.

Keywords: Nanoparticles, nanoscience, nanoagriculture, agriculture, titania, iron oxide, silica, calcium carbonate, plant protection, fertilizer, nanozyme, plant biology, agronomy, sol gel, inorganic chemistry

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Dedication

To my father (probably the only one, except supervisors and evaluators, who will make an effort to go through this heap of cellulose, calcium carbonate and ink)

“Nobody ever figures out what life is about, and it doesn´t matter. Explore the world. Nearly everything is really interesting if you go into it deeply enough. Work as hard and as much as you want on the things you like to do the best. Don´t think about what you want to be, but what you want to do. Keep up some sort of minimum on other things so that society doesn´t keep you from doing anything at all.”

“The first principal is that you must not fool yourself and you are the easiest person to fool.”

Richard P. Feynman

“If it is not right do not do it; if it is not true do not say it.”

Marcus Aurelius

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I N. G. Martin Palmqvist, Sarosh Bejai, Johan Meijer, Gulaim A. Seisenbaeva, Vadim G. Kessler (2015). Nano titania aided clustering and adhesion of beneficial bacteria to plant roots to enhance crop growth and stress management. *Scientific reports* vol.5 (10146)

II N. G. Martin Palmqvist, Gulaim A. Seisenbaeva, Peter Svedlindh, Vadim G. Kessler (2017). Maghemite nanoparticles acts as nanozyme, improving growth and abiotic stress tolerance in Brassica napus. *Nanoscale Research Letters.* (Under review)

III N.G. Martin Palmqvist, Jean-Marie Nedelec, Gulaim A. Seisenbaeva, Vadim G. Kessler (2017). Controlling nucleation and growth of nano-CaCO3 via CO2 sequestration by a calcium alkoxide solution to produce nanocomposites for drug delivery applications. *Acta Biomaterialia* vol. 57, 426-434.

IV N.G. Martin Palmqvist, Dimitrije Markovic, Robert Glinwood, Karin Knapp Norrfors, Geert Cornelis, Gulaim Seisenbaeva, Velemir Ninkovic, Vadim Kessler (2017). Effects of calcium carbonate nanoparticles on plants and plant tolerance to aphids. (manuscript).

Papers I and III are reproduced with the permission of the publishers.

The contribution of Nils Gustav Martin Palmqvist to the papers included in this thesis was as follows:

I Took equal part in development of the idea and design of the study. Performed the majority of all of the experiments and wrote most of the manuscript.

II Developed the idea, designed and performed all experiments, except running the magnetometer. Wrote the manuscript with assistance from co-authors.

III Developed the idea and design of the study together with Vadim and Gulaim. Performed all experiments except nitrogen adsorption. Wrote the manuscript with help from co-authors.

IV Performed all synthesis, material characterization and did some of the biological experiments. Wrote the manuscript with help from co-authors.

Abbreviations

DNA - Deoxy ribonucleic acid
EDX/EDS – Energy dispersive X-ray spectroscopy
TEM – Transmission electron microscopy
SEM – Scanning electron microscopy
ICP-MS – Inductively coupled plasma mass spectrometry
DLS – Dynamic light scattering
NTA - Nano tracking analysis
XPS – X-ray photoelectron spectroscopy
XAS – X-ray absorption spectroscopy
XRF – X-ray fluorescence spectroscopy
XES – X-ray emission spectroscopy
EELS – Electron energy loss spectroscopy
EGFP – Enhanced green fluorescent protein
MIQE – Minimum Information for Publication of Quantitative Real-Time PCR Experiments
TGA – Thermo gravimetric analysis
LB – Lysogeny broth

1 Introduction

> "If I were to take a very tiny thing in water and expand it to our dimensions. The analogous situation is that it is swimming in extremely thick honey. The ordinary ways of flapping, like a fish, of flapping the fins and so on. It doesn't work. It just goes eh eh and it is stuck. How do I know then, that I can make tiny machines and that it is possible according to the laws of physics? (Ironic voice) Because I am a physicist and I checked the laws of physics, and its ok, I tell you. But I got another way to tell you, living things have already done it. Bacteria swim. They swim through water, at the scale that corresponds to thick goo."
>
> Richard P. Feynman 1979, in the talk he called "tiny machines" the revision of his seminal talk in 1959, that was called "there is plenty of room at the bottom"

Richard Feynman was one of the earliest advocates of nanoscience. In the above quote, he vividly illustrates physical differences between things of different sizes. In terms of chemistry the changes are quite different. The changes, which can be observed in materials as you go from bulk to nano size, turns radical and exponential in properties such as surface tension, magnetism, electron conductivity and melting point (Roduner). Nanomaterials are much more reactive and vivid materials, as compared to their bulk counterparts. Atoms in bulk materials are compressed and share space to a higher degree. The space in a nanomaterial is much less occupied allowing for these changes, in physicochemical properties and reactivity, to appear. The small size also leads to more surface area and higher surface to volume ratio, i.e. more of and more reactive surfaces.

One of the most important fields of nanoscience is the development of new catalysers (Tao and Spivey, Xia et al., 2013). Particularly in the development of hydrogen fuel cells (Xia et al., 2013). Also, the improvement of chemical processing and production of organic compounds are greatly benefitted by nanomaterials (Sharma et al., 2015a). The development of medicines with nanomaterials has progressed far and there are already a multitude of products on the market, mostly in sensor applications and drug delivery (Marchesan and Prato, 2013, Bobo et al., 2016). Targeted delivery of drugs is a major discipline, where big advances have been made (Andresen et al., 2010). Targeted delivery has also become a major goal within agriculture. Targeted delivery of pesticides can help increase effectivity and reduce plant toxicity and none target impacts. There has been an incredible amount of praise and hope for development of nanotechnological advances that would solve the world's problem with hunger by revolutionizing agriculture. (Kristen Lyon; Pandey M.P. ;(Chen and Yada, 2011, Servin et al., 2015). This should be taken with a pinch of salt, but there is without a doubt a lot already happening and a lot more to be discovered in the realm of nanotechnology for agriculture, and this is the topic of this doctoral thesis.

A nanomaterial is defined as a material, with any geometric shape, that has at least one dimension within 1-100 nm in size. This means, that a hypothetical thin film, covering the whole of Kiruna municipality, is still a nanomaterial if the thickness would be below 100 nm. A nanoparticle has all three dimensions within 1-100 nm. Nanoscience denotes the field of investigation into these materials and the effects they have. Nanotechnology refers to, mostly, the development of devices and machines, but also materials, that utilizes these effects. The term nanotechnology was first coined by Norio Taniguchi at a conference in 1978 but was not widely recognized until after Eric Drexler´s book "Engines of Creation: The Coming Era of Nanotechnology" in 1986. His ideas would today, mostly, fall under the discipline of molecular nanotechnology, which is concerned with the creation of complex molecular machines that can perform certain tasks. The 2016 Nobel Prize in chemistry was awarded to three outstanding scientists in this discipline for ”*the design and synthesis of molecular machines”*, shortly after the first world championship nano car race was announced. One example of application of molecular machines could be a molecule that could perform specific gene editing. This is still science fiction (except that nature already created CRISPR proteins), but it touches upon another field which is very much alive, namely nanobiotechnology. Nanobiotechnology regards the study of nanomaterials and their interaction with biological systems to develop, for example, new drugs with targeted delivery, bio imaging, bio sensors or slow releasing fertilizers. Since it is a novel field the term is not widely accepted, and it seems more related to medicine than anything else. So, because of the paramount importance of food production and the advances of nanomaterial use within agriculture, I hereby suggest and here forth will use the term Nano agriculture, regarding the use of nanoscience for improvement of food production.

2 Background

After the advent of technologies capable of working on the nanoscale in the 1980s had started nanotechnology and our quest for understanding nanoscopic size phenomena, we have become aware of nanoparticles´ possibilities and danger. However, it is still a young field of investigation and there are great gaps in our knowledge about nanoparticles´ properties, functions and diversity. Further, to understand their interaction with biological organisms and biological systems becomes immensely complex, giving researchers a formidable task. Here I will present some of the current knowledge about nanoparticles and their interactions with plants.

2.1 Natural occurrence of nanoparticles

There is no doubt that nanoparticles have been around since the beginning. Evolution of life has occurred in constant contact with nanoparticles and some think that inorganic nanoparticles has actually been part of creating life, i.e. the first organic molecules, and even the stars(Strambeanu et al., 2015). In the case of stars, it is of course a logical inevitability, since the accretion of matter must go from the very small (sub atomic and atomic) through the medium small (nano) to the gigantic (micro and macro). Interestingly, natural nanomaterials have been overlooked, apart from colloid scientist (colloids are according to IUPAC definition materials with at least on dimension between one nm and one µm), until recently. It was overlooked until the nanotechnology revolution appeared, and concerns for the nanomaterials inevitable spread in nature was raised. In that way a new field of geoscience was born (Nature´s Nanostructures). If one omits the organic nanomaterials one can divide natural nanomaterials into: Mineral nanoparticles (minerals of Nano size), nanominerals (minerals found exclusively of Nano size) and amorphous nanoparticles (Nature´s Nanostructures). It has been found that these nanomaterials are an integral and vital part of geochemistry cycles. For example, atmosphere chemistry is expected to be highly influenced by nanoparticles (Rubasinghege et al., 2010). For example, TiO_2 nanoparticles can in the atmosphere, with their photocatalytic activity decompose ozone (Nicolas et al., 2009).

The most frequently attributed terrestrial source of inorganic nanoparticles is volcanoes and they are the most well-known source of nanoparticles (Tepe and Bau, 2014). Yet, it is not amply studied and the types of nanoparticles we can expect, from different types of volcanoes, are very complex and not recognized. Strambeanu et al. 2015 goes as far as to say: “It must be specified that most metal and non-metal ores in Earth’s crust were formed throughout the geological eras, because of volcanic activity. Consequently, any chemical element existing as such or in one of its combinations may be found in the atmosphere under the form of nanoparticles during eruptions or immediately after them”. I would prefer a more cautionary outlook, but surely there is a formidable multitude of different nanoparticles being produced by volcanic activity.

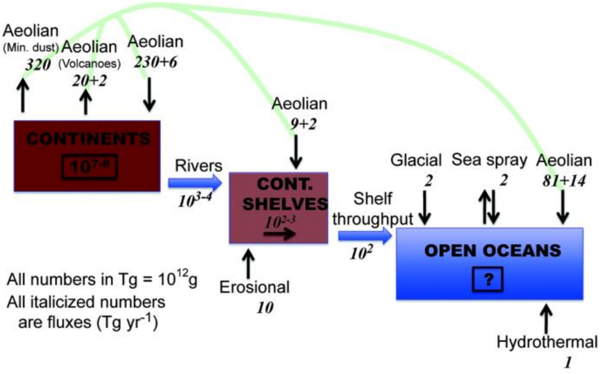


Figure 1. A scheme of estimated amounts of natural nanoparticle distribution in Tg per year. Reproduced *with permis*sion from CRC press.

Terrestrial nanoparticle generation by wind erosion, particularly in deserts, is even greater in quantity. Hochella et al (2015) has made estimations on the global cycles of nanoparticle generation in nature and constructed the schematic image reproduced here as figure 1 (Hochella et al., 2015). The estimation of 230 Tg of mineral nano dust being brought up into the atmosphere is an incomprehensible amount of nanoparticles, constantly on the move around the planet.

It could be, that Hochella et al. are actually underestimating the global nanomaterial cycles. In Nature´s Nanostructures Hochella et al. explain their model and it seems to be focused on geophysical processes while omitting a lot of the geochemical and biochemical processes. We know that metal nanoparticles are readily formed from its bulk constituents; Glover R.D. et al. (2011) has shown that normal copper and silver ware, leave behind traces of its self in the form of nanoparticles(Glover et al., 2011). At the same time, there is a large array of inorganic and organic processes and combinations thereof that can generate nanoparticles, in the bottom up way. All condensation of solids from solution goes via nucleation through formation of nanoparticles towards bulk materials. Sharma V.K et al. (2015) have written a good short review that provides an introduction to these processes in nature (Sharma et al., 2015b).

Although it has been poorly investigated we know that nano-sized particles of different compounds are formed in soil (Monreal and Schnitzer, 2008, Fedotov et al., 2009, Fedotov et al., 2008, Fedotov and Rudometkina, 2011). It is likely that root exudates stand for a substantial part of the formation of these compounds and particles (Tikhonovich et al., 2011, Seisenbaeva et al., 2013). It is known that plants release up to 40 % of assimilated carbon into the rhizosphere as a wide range of different substances (Vranova et al., 2013). Graystone et al. (1996) made a categorization of root excretion substances, namely, (1) water soluble low molecular weight substances that are released by diffusion, (2) high molecular weight substances that can be released against electrochemical and chemical potential through metabolic processes, (3) lysates which are cell debris from autolysis, (4) gases such as ethylene and carbon dioxide (CO_2), (5) and mucilage which is composed mainly from polysaccharides and polygalacturonic acids which are high molecular substances that form a gelatinous film around the roots (Grayston et al., 1997). Apart from sustaining and altering the rhizosphere microbial community some of these excretions have a direct effect on nutrients in soil. Chelators or siderophores such as mugenoic acid and avenic acid bind to minerals, increasing their solubility and mobility in the soil solution (Harsh P. Bais, 2006) *(Mench and Martin, 1991, H.M., 1988)*. There is also prove of acidification, precipitation and oxidation-reduction processes apparent from root exudates in soil (H.M., 1988). Most of these chemical interactions are believed to result in nano-sized particles for easy uptake into plants. Manceau A. et al. (2008) reported that *Phragmites australis* can reduce toxic Cu^+ and Cu^{+2} to Cu^0 nanostructures as a mode to withstand higher levels of Cu (Manceau et al., 2008). Many have investigated into the possibilities of phytomining i.e. *Msuya F.A. et al (2000)* calculated that, according to their results, it would be economically sane to harvest gold nanoparticles out of carrots grown with aid of added chelating agents(Msuya et al., 2000). Sharma N.C. et al. 2007 used extended x-ray absorption fine structure analysis (EXAFS) and x-ray absorption near-edge structure analysis (XANES) to investigate where the reduction of gold and silver salts occurred and formed elemental particles in *Sesbania* seedlings. They found that the reduction took place in the root-cells and that the particles were then transported symplastically and deposited throughout the plant (Sharma et al., 2007). Gardea-Torresdey J.L. et al. 2005 did a similar study on gold in the form of $KAuCl_4$ together with different concentrations of thiocyanate NH_4SCN in the plant *Chilopsis linearis* and found that the reduction took place in both roots and shoots, also that thiocyanate at a certain concentration increased deposition of nano-gold-particles in the roots by 595 %(Gardea-Torresdey et al., 2005). Haverkamp R.G and Marshall A.T. 2009 have conducted an additive investigation, with the same instrumental techniques, showing that there is a limit to silver deposition within *Brassica juncea* plants. They also investigated the uptake of silver nanoparticles from solution into the *Brassica juncea* plants and found that they did not take up any silver that was not in ion form, although they did not report on any size of the nanoparticles used(Haverkamp and Marshall, 2009).

2.2 Transport of nanoparticles in nature

How nanoparticles, both natural and engineered, move in water bodies, soil and air has shown to be a very difficult matter to investigate. There are no methods available that can measure *in situ*, i.e. all methods rely on sampling, giving rise to sampling bias and other artefacts. Our best way of understanding the fate of nanoparticles in nature is to measure movements in controlled environments and then use mathematical modelling to predict their behaviour under natural conditions. A fairly recent review by Baalousha et al. (2016) has described the struggles of creating accurate models (Baalousha et al., 2016). Some nanoparticles dissolve, under which conditions do they dissolve? Some nanoparticles aggregate and precipitate others adsorb to soil particles. Yet again, under which conditions? It is an immeasurable complexity that arises, when you also start to factor in the biosphere. How can nanoparticles enter the food chain? Only the first step of transport from soil and water to plants has proven to be incredibly diverse (Grillo et al., 2015). Some particles are taken up by some plants but not by others and vice versa. We have to consider, not only, the infinity of particle types but also the vast array of different soils and plants that will affect the particles transport. Schwab et al. have written a review that covers the subject well, also describing the problematics and possibilities of today´s techniques for studying nanoparticle transfer from environment to plants (Schwab et al., 2016). With new techniques evolving, especially at synchrotron source facilities, we are advancing our knowledge substantially (Castillo-Michel et al., 2017). At least the most important nanoparticles that are predicted to become prevalent in nature or that are being considered for use in agriculture, such as gold nanoparticles, silver nanoparticles and titania nanoparticles etc.; are getting investigated in different conditions with different plant models (Rico et al., 2011, Zuverza-Mena et al., 2017, Pradas del Real et al., 2016, Tripathi et al., 2017).

2.3 Concerns over the environmental effects of nanoparticles

Even though life on earth always has been surrounded with nanomaterials, there is reason to be concerned over the anthropogenic production of nanomaterials. We have the ability to create materials that do not exist in nature, and we do (Hochella et al., 2015). We do create these materials because they possess certain qualities and properties and we need to consider what effect these properties have in the environment. This is why the research on nanoparticle transport in nature, described above is of such importance. There is a great multitude of nanotoxicological research performed in lab conditions, showing that most nanoparticles are toxic at high concentrations (Tripathi et al., 2017). The research journal Nanotoxicology at the publishing house Francis and Taylor had an impact factor of 7.914 for the year 2015, which, shows a pronounced focus on a rather new and narrow field of research. But what concentrations will nanoparticles have in the environment (Holden et al., 2014)? Which particles are at risk of reaching toxic levels and where will these concentrations occur (Kah et al., 2012, Miseljic and Olsen, 2014)? It has been shown that humans who are exposed to great quantities of titania nanoparticles are severely affected health wise (Pelclova et al., 2017). It seems that the method of Pelclova et al. (2017), to monitor toxicity in people that are exposed to nanoparticles, will be the most accurate and alarming method, to bring about safety and security measures regarding nanoparticles. The variety and different conditions possible cannot possibly be covered by laboratory experiments and we will in practicality reside on the detrimental effects to occur, for us to detect them. The difficulty will be to predict these detrimental effects, to know what to look for and where. For example Tou et al. (2017) showed with SP-ICPMS and TEM that the amount of metal nanoparticles in sludge has hitherto been underestimated; and that the metal nanoparticle levels in the sludge around shanghai was above the regulated amounts, previously undetected by standard measurements (Tou et al., 2017). Some evidence suggest that adverse effects appear in soil communities (McKee and Filser, 2016). The global anthropogenic and industrial production of nanoparticles is in the ball park of hundredths of Tg year^{-1} (Hendren et al., 2011). These quantities are mainly constituted of a few nanomaterials, i.e. TiO_2, CeO_2, Carbon nanotubes and nano silver (Hochella et al., 2015). We can expect more and different types of nanoparticles to be released in the future, both intentionally and unintentionally.

2.4 Successful nano agriculture

It is difficult to assess how many agro-products, on the market, that are using nanomaterials. Some fertilizers and pesticides were containing nano-technological ingredients before nanotechnology became a hot topic and some might contain it without it being proclaimed. It seems as if the agrochemical companies are afraid that nanotechnology will suffer the same fate with public relations as gene technology has and the formulations used to produce agrochemicals are of course proprietary and business secrets (Kah, 2015). On top of just the beneficial functions of nanomaterials in formulations there are also findings of plant beneficial effects from nanoparticles alone. In research literature one can find a variety of review and research articles on positive effects of nanoparticles on plants, such as combating pathogens(Alghuthaymi et al., 2015, Servin et al., 2015), increasing growth(Cui et al., 2013b, Yang et al., 2007, Juhel et al., 2011, Khodakovskaya et al., 2013, Kumar et al., 2013, Servin et al., 2015) and enhancing tolerance to abiotic stresses(Pei et al., 2010, Davar Zareii et al., 2014, Tripathi et al., 2017, Ashkavand et al., 2015, Zaimenko et al., 2014).

Biology is a nanoscale phenomenon; its machinery i.e. proteins and nucleic acids etc. are nanoscale molecules and this makes them reactive to nanoparticles and vice versa. It is known that nanoparticles practically always form a layer of protein, dubbed protein corona, around them in organisms; and this protein corona affects the uptake and toxicity of nanoparticles(Gräfe et al., 2016, Lundqvist et al., 2011, Zanganeh et al., 2016, Cedervall et al., 2007). Hence, the effects induced by nanoparticles are complex and very fascinating. The effects need to be studied case by case; these interactions cannot be generalized between all nanoparticles and all organisms.

2.5 Synthesis of nanoparticles

To thoroughly cover the topic of all the ways to make nanoparticles I think we would need a couple of more doctoral theses. A great introduction to the subject, covering at least the wet chemistry, can be found in the review by Cushing et al (2004) (Cushing et al., 2004).Nevertheless, first of all we can divide nanoparticle production into two categories, namely: i) top down production, where bulk materials are gradually broken into smaller parts; ii) bottom up production where nanoparticles are grown from atoms and molecules. Top down production is mostly used on industrial scales because it is a cheaper way of creating nanomaterials if the starting bulk is cheap. However it is a much cruder way and if the goal is to create uniform particles with tuneable properties, then the bottom up approach is the way to go. I will briefly describe some of the most common bottom up methods. Only the first two, sol-gel and co-precipitation, has actually been used in the papers included in the thesis.

2.5.1 Sol-gel methods

Sol-gel technology dates back as far as to mid. 19th century when Ebelmen was working with the hydrolysis of $SiCl_4$ complexed with alcohols (Ebelmen, 1846). Although, it wasn't really until about 100 years later, the sol-gel method became widely used in the way interesting for nanoparticle synthesis, when it was spear headed by Donald C. Bradley. He was working with development of alkoxides, the most common precursor of sol-gel synthesis, and the conversion thereof into metal oxides. Sol-gel technology stretches much further than to just nanoparticle synthesis and is also used for producing films, ceramics and gels etc. However, for whatever possible product, the general outline for a sol-gel synthesis still follows a couple of easy steps, that is:

- Creation of a sol, a solution of particles, monomers of the alkoxides or metal precursor. With generation of small enough particles it will appear to be a solution.
- Gelation of the sol can appear spontaneous over time or be induced by some degree of drying. It means that the particles or monomers assemble into a network/polymer, commonly bridged by oxygen; this considerably increases the viscosity of the sol.
- Drying and removal of the solvent. If the gel has been casted, monolithic shapes can be created. If the gel is dried above the critical point an aerogel is produced and if it is dried thermally the product will be a xerogel (a compacted version of the original gel).

In sol-gel synthesis the precursor is a major governing factor. It requires a substantial knowledge of chemistry to predict the result of a particular synthesis. Sol-gel has been extensively used for production of silica nanoparticles(Rahman and Padavettan, 2012), titania nanoparticles(Macwan et al., 2011), Copper oxide nanoparticles(Wang et al., 2016b) and iron oxides(Masthoff et al., 2016, Cui et al., 2013a) etc. It is a versatile and usually low temperature synthesis at relatively mild pH values. These gentle conditions allow for the introduction of organic molecules to produce inorganic organic hybrid nanocomposites; which permit practically an infinity of materials to be produced, with different properties.

2.5.2 Coprecipitation

Coprecipitation is a method of synthesis that involves bringing a substance of moderate solubility to a supersaturated state. It is a multistep process that is not truly understood. The first step is nucleation, a process driven by supersaturation and governed by surface tension. According to classical nucleation theory the critical size of a cluster of atoms, to form a thermodynamically stable crystal that does not re-dissolve, can be predicted by:

$$r_{crit} = \frac{2\gamma v}{k_b T \ln S} \quad (1)$$

Where γ is the surface energy, v is the volume, k_b is Boltzmann´s constant, T is temperature and S is supersaturation. The term in the denominator can be exchanged for the chemical potential difference between bulk solid and bulk liquid, $\Delta\mu$(Roduner, 2006), that goes towards zero at the phase transition point, or it can be exchanged for free energy of the bulk crystal, $\Delta G_{v,}$ which can be written as(Thanh et al., 2014):

$$\Delta G_v = \frac{-k_b T \ln(S)}{v} \quad (2)$$

As the size of particles increases the free energy of bulk crystal into greater negative values and the surface free energy grow more positive, as can be seen in figure 2 and as is described by equation three.

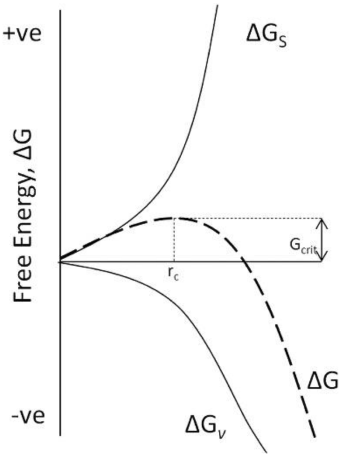


Figure 2. Free energy as a function of particle size. ΔG_s denotes the surface free energy, ΔG_v denotes the bulk free energy curve, the dashed line with ΔG is the curve for the total free energy of the particle and r_c is the critical size described by equation 1. Reprint from (Thanh et al., 2014) Creative Commons CC-BY.

Since the bulk free energy scales as r^3 and the surface free energy scales as r^2, the negative bulk free energy will dominate the bigger particles. In small clusters or particles the surface free energy dominates.

$$\Delta G = \Delta G_v + \Delta G_s = \frac{4\pi r^3 \rho \Delta\mu}{3} + 4\pi r^2 \gamma \quad (3)$$

Equation three explains the relationships illustrated in figure 2, where r is the radius of particle, ρ is the density of the bulk liquid, Δμ is the chemical potential between bulk solid and bulk liquid and γ is the surface free energy. If one studies equation one and two, one can see that increased temperature and supersaturation and increases the bulk free energy of the particle and hence drives the relationship towards a stable particle; and lower surface free energy alters the critical size needed. Increasing the supersaturation from S=2 to S=4 leads to an increase in nucleation by as much as 10^{70}(Kwon and Hyeon, 2011). To create really monodisperse and small nanoparticles this is what should be achieved, a quick nucleation rate, so that many nuclei are formed and consequently consume all the monomers before they are allowed to grow big.

After nucleation, the second step is growth, a process that is limited by reaction and diffusion. The precipitating salt can be limited by its reaction order but the most contributing limiter is usually the diffusion i.e. the mobility of ions in the solution. With an adaptation of Fick´s law to nanoparticles, equation four can model a diffusion limited growth and equation five models a reaction limited growth (Thanh et al., 2014).

$$\frac{\mathrm{dr}}{dt} = \frac{D_v}{r}(C_b - C_i) \quad (4)$$

$$\frac{\mathrm{dr}}{dt} = K_v(C_b - C_i) \quad (5)$$

Where r is the radius of the particle, C_b is the bulk concentration of monomers, C_i is the concentration of monomers at the interface between particle and solution, D_v is the diffusion coefficient and K_v is the reaction constant.

Because of the fact that small particles have a higher solubility than those of larger size, notably so in the sub 20 nanometre range, the smallest particles will dissolve and instead feed the larger particles with material. This is Ostwald ripening explained in simple terms, however it is not a simple phenomenon and it is indeed a subject of investigation yet(Kuo and Hwang, 2013, Madras and McCoy, 2002b, Madras and McCoy, 2002a, Oskam et al., 2002, Yec and Zeng, 2014). Other forms of ripening can also occur, i.e. digestive ripening and coalescence. Digestive ripening is the inverse of Ostwald ripening, meaning that the larger particles are consumed for the benefit of smaller particles. It is a substantially rarer phenomenon. Coalescence is when particles join together to form a new single crystal(Zheng et al., 2009).

A possible, third and final, step of co-precipitation is termination of growth by capping. Adding a so called capping agent, a surfactant or an organic ligand, halts further growth of the crystal and also stabilizes it in solution and prevents aggregation of the particles. This is a very common and sometimes crucial method in nanoparticles production, applied not only in co-precipitation synthesis.

2.5.3 Hydrothermal synthesis

Hydrothermal synthesis, or solvothermal if the solvent is other than water, is a method where the reaction is performed in a closed vessel (The term stems from the field of geology and the study of hydrothermal vents). The closed vessel allows for temperatures and pressure to build up and bring the solvent to a supercritical state. In the supercritical state of a liquid there is no surface tension between solids and the liquid. This changes the rules of nucleation and allows for otherwise insoluble compounds to be dissolved and subsequently nucleated into new particle compounds. The supercritical state is not necessary in all cases. It is also possible to perform novel syntheses only using elevated temperature and pressure to increase solubility (Cushing et al., 2004).

Hydrothermal synthesis was first used in the nineteenth century to reproduce the conditions in the crust where water is present in the formation of minerals but it soon developed into an important commercial method. Firstly in the extraction of aluminium from bauxite in 1892, later in synthesis of important inorganic materials most importantly quartz crystals and zeolites (Byrappa and Yoshimura, 2001). Today, hydro and solvothermal approaches are being used for production of a myriad of things like for example: Titania catalysts(Liu et al., 2014), zinc oxides (Kołodziejczak-Radzimska and Jesionowski, 2014), zeolites (still)(Johnson and Arshad, 2014), rare earth oxides(Xu et al., 2013), metal organic frameworks(Lee et al., 2013), alloys(Howard et al., 2017), chitin materials (Wysokowski et al., 2017), carbon quantum dots(Wang et al., 2017) and things like biomass conversion(Jain et al., 2016) etc.

2.5.4 Gas phase synthesis

For electronic devices that require very high purity, gas phase synthesis might be the preferred method of nanoparticle production. Chemical vapour deposition is an extensively used method to produce thin films, which involves heating up precursors to a vapour and subsequently allowing nucleation onto a substrate. It can be performed under various pressures but most commonly today it is performed under vacuum around 0.1-0.01 Pascal. When producing particles instead of film it is called chemical vapour condensation.

The method permits many types of particles to be produced and it is particularly good for creating doped particles with different elemental mixtures. Successfully synthesised nanoparticle materials from gas phase synthesis include: Carbon nanotubes(Eveleens and Page, 2017), iron core with iron oxide shell particles(Choi et al., 2002), platinum and silver(Maicu et al., 2014), vanadate and titania(Cha et al., 2014), aluminium magnesium alloys etc. (Karbalaei Akbari et al., 2015).

2.5.5 Micro emulsion synthesis

Microemulsions are mixtures of hydrophobic solutions and hydrophilic solutions that creates nano droplet of one side the other. Commonly it is stabilized with a monolayer of surfactants, molecules that carry both a hydrophobic moiety and a hydrophilic moiety, in the interface between the two solutions. The micro emulsions can be used in inorganic nanoparticle synthesis by combining two, with reactants of an insoluble salt in the nano droplets. In this manner small amounts of reactants will be brought together when bubbles meet, creating small particles. Further, the prevention of growth can be accomplished by a surfactant that cover the particle in its formation.

The technique is considered to give considerable control over size and size dispersity of the particles (López-Quintela, 2003, López-Quintela and Rivas, 1993). It used for the production of a wide variety of compounds such as: catalysts (Boutonnet and Sanchez-Dominguez, 2017, Tojo et al., 2017, Henam Sylvia et al., 2017), core shell particles(Han et al., 2016), rare earth nanoparticles (Lotfi and Khorrami, 2016), starch nanoparticles (Chin et al., 2014) and aluminium hydroxide flakes (Xu et al., 2014), to name a few.

2.5.6 Biomimetic nanoparticles synthesis

Biomimicry is the science of imitating life (the etymology originates from old Greek: Bios meaning life and mimesis meaning imitation). Scientists are looking to nature for design solutions, production methods and energy savings etc. Most of these problems that humanity is facing, in improving our materials, our energy consumption and production and our way of life, have been solved better and more efficiently by organisms through evolution.

Nanoparticle synthesis is no exception, many organisms have been found to produce nanoparticles. Most famously magneto tactic bacteria, discovered in 1975 by Blakemore (Blakemore, 1975), that produce magnetite nanoparticles to navigate in the magnetic field of the earth(Bazylinski D et al., 1995). These nanoparticles are produced inside a protein called magnetosomes and this way of allowing and controlling growth of nanoparticles inside a protein cage as template has since been reproduced in viruses, peptides, proteins and DNA(Capek, 2015). Genetic engineering can allow for new templates and gives us infinity of possibilities for producing nanoparticles of different types and shapes. Many organisms in the plant kingdom, fungal kingdom and prokaryote phyla have been found to reduce metal cations to their elemental or oxide form and produce nanoparticles (Jeevanandam et al., 2016). For example the synthesis of metal nanoparticles using plant extracts as reducing agents is considered as green chemistry (meaning environmentally friendly) and has become a common research area(Akhtar et al., 2013, Dauthal and Mukhopadhyay, 2016). Using bacteria for nanoparticles synthesis is also considered a green chemistry route. A wide variety of particles have been demonstrated to be possible (Iravani, 2014), such as: Silver nanoparticles(Klaus et al., 1999), gold nanoparticles (Konishi et al., 2006), magnetite nanoparticles(Lee et al., 2004), palladium nanoparticles(De Windt et al., 2005), selenium nanospheres (Oremland et al., 2004), titanium dioxide (Vishnu Kirthi et al., 2011) and zinc oxide (Jayaseelan et al., 2012).

2.6 Characterization of nanoparticles

As our knowledge and experience about nanomaterials has developed, the way we study them has improved and become more and more sophisticated. This has also improved the way we see nano particles. The importance of different characteristics is becoming apparent. Not only size influences materials´ effects but also in synergy with size do other properties i.e. surface charge, surface area, porosity, crystal structure, density, shape, composition, chirality and magnetism. Many of these properties are normally overlooked in studies of nanoparticle and plant interactions, which could be one of the explanations for the rather large discrepancies in results that reoccur within the field of nanoagriculture. I will briefly introduce some of the most common techniques used for studying nanoparticles and discuss their benefits and drawbacks.

2.6.1 Electron microscopy

Electron microscopy has arguably been the most important investigation technique for modern science. It was developed in the 1930s and since then it has helped us see, understand and improve, materials and biology. To the field of nanoagriculture it has been crucial and few research papers would be published without studying the material with an electron microscope (Chandra et al., 2015). No other visualization techniques allow better resolution on electron conductive and inorganic materials. Even with organic materials it is still hard to match the resolution of electron microscopes with light microscopy or scanning probe microscopy techniques, *vide infra*.

Another benefit with electron microscopy techniques is that it is easy to simultaneously acquire elemental composition information by energy dispersive x-ray spectroscopy (EDX or EDS) or electron energy loss spectroscopy (EELS) on a transmission electron microscope (TEM).

There is also the possibility to use electron diffraction to study the structure of materials making the TEM a very powerful tool for characterisation of nanoparticles.

Although, there are drawbacks, for example that it requires extensive sample treatment for biological samples and a lot also for inorganic samples (Schrand et al., 2010). This is because the electron beam is a very destructive probe, which is, of course, something that makes it questionable as probe that gives result representative of reality. The samples also need to be electron conductive and for TEM they also need to be very thin, ca 50-100 nm depending on electron density of the material. The electron beam also requires vacuum to be able to operate effectively and this as well affects the sample and possibly skews results. For example when studying the size of <10 nm nanoparticles that are non-conductive with SEM, which is fairly common, one would have to sputter (coat) the particles in an electron conductive metal. This would then alter the surface structure and change the size of the particles, albeit by a somewhat certain degree.

2.6.2 X-ray techniques

The use of x-ray to study materials would be the second contender for the most important advent for advancement of modern science. X-ray diffraction was developed during the early 20th century and was spearheaded by Max von Laue and a father and son both by the name of William Bragg. All three of them received Nobel prizes rather shortly after their discoveries, which were about the relationship between the unit cell (the smallest repeating unit) of the crystal and the so called diffraction pattern created by allowing x-rays to shine through the crystal. The wavelengths of x-rays are in the order of 1 Angstrom (A, $1*10^{-10}$ meters) which also is the same order of distances between atoms in molecules and compounds. This is why x-rays are so useful for studying materials. Today we divide x-rays into two categories soft and hard, with soft being x-rays longer than 1 A and hard shorter than 1 A. The full x-ray range is not fully defined because of confusions between x-rays and gamma rays but it is about 0.1 A to 100 A. The hard x-rays penetrate materials better while soft x-rays are absorbed by air and must be attenuated in vacuum. Hard x-rays can interact with molecular bonds and hence create radiation sickness and cancers. This feature also makes x-rays very useful in materials characterization, as it, through spectroscopy, can provide us with information about what chemical bonds are present and what is the coordination of atoms. There is a plethora of spectroscopy techniques that utilizes x-rays to study nanoparticles i.e. x-ray photo electron spectroscopy (XPS)(Prieto et al., 2012), x-ray emission spectroscopy (XES)(Hirsch et al., 2015), x-ray absorption spectroscopy (XAS)(Hirsch et al., 2015, López-Moreno et al., 2010), small and wide angle x-ray scattering spectroscopy (SAXS/WAXS)(Li et al., 2016b) and x-ray fluorescence spectroscopy (XRF)(Filez et al., 2014). It is also possible to put a detector screen behind the sample so that an x-ray microscopy image can be created. This can be a very powerful technique since it can be combined with XRF and/or XAS to give chemical information in the image. Only one problem appears for studying nanoparticles, which is that, because of the diffraction limit and the wavelength of the x-rays the spatial horizontal resolution is at best

50 nm. In this aspect TEM with EELS is superior with its ~ 0.1-1 nm resolution.

2.6.3 Light scattering techniques

Light scattering techniques such as dynamic light scattering (DLS) and nano tracking analysis (NTA) are designed to study the hydro dynamic size of particles. This means that they measure the size of the particles in dispersion, including the layers of molecules adsorbed to its surface, which depends on the charge of the surface and what solvent and solution the particles are dispersed in. Both DLS and NTA rely on Stokes-Einstein equation (6) to calculate the size of the particles by measuring the Brownian motion.

$$\mathrm{D}_t = \frac{TK_b}{3\pi\eta d} \quad (6)$$

Stokes-Einstein equation where the D_t is the diffusion constant or speed of movement, T is the absolute temperature, K_b is the Boltzmann constant, η is the viscosity of dispersant and d is the diameter of the spherical particle. By controlling temperature and knowing the viscosity, the spherical size can be calculated after measuring the diffusion constant. In DLS one measures the D_t by detecting the constructive interference specs from the whole population of particles and over time auto correlate these specs that will oscillate in intensity, the faster the oscillation the smaller the particle. This will generate an average of the whole population and a dispersity index. In NTA instead a video is recorded, usually of the particles being pumped by in front of the camera as they are being irradiated by a laser. In this way, positions of individual particles are constantly recorded and subsequently can have their size calculated. This creates a data set showing the true size distribution. Both of these techniques can also be combined with z-potential measurements. The z-potential is very important since it is a measure of the surface charge on the particles, which influences the particles behaviours such as aggregation, adsorption, reactivity and transferability.

In comparison DLS is slightly more robust and can measure particles over a bigger size range ca. 1-10000 nm. The nano tracking analysis has an approximate range of 20-2000 nm however the data provided has higher value. There are other sources of information that goes into greater depths of the functionality of light scattering (Xu, 2015, Moore and Cerasoli, 2010).

2.6.4 Single particle inductively coupled plasma mass spectrometry (SP-ICP-MS)

One of the most versatile instruments for measuring nanoparticles is SP-ICP-MS. It can measure large range of sizes of about 20 nanometres to several micrometres (although Perkin-Elmer claims that their new NexION 2000 can measure any size of particle with its ultrafast data acquisition). Particles can be measured in any matrices and in any state, solid, liquid or gas. The detection limit is very low allowing for detection of very low amount in the parts per quadrillion (10^{-15}) range and multiple components can be measured at the same time(Laborda et al., 2014). The technique however needs to be complemented with TEM, SPM and or X-ray diffraction and scattering to get an as complete picture as possible, since SP-ICPMS doesn´t give information about crystal phase or shape of the particles.

2.6.5 Scanning probe microscopy

Scanning probe microscopy (SPM) is a plethora of microscopy techniques that utilises a tactile probe cantilever that is scanned over the surface. The first to be invented was the scanning tunnelling microscope in 1981, by Gerd Binning, Heinrich Rohrer and Christoph Gerber at IBM in Zurich (Chen, 2007). Scanning tunnelling microscopy is also the scanning probe microscope technique that renders the highest resolution, with 0.01 nm in the z plane and 0.1 nm in the x-y plane. It relies on the quantum tunnelling effect of electrons to sense the bias voltage of the sample to the tip. The current between the tip and the sample is amplified in a negative feedback loop to the piezo electric control of the cantilever that holds the tip in an equilibrium distance from the sample. The changes of tips z-position can then be read out as the actual topography of the sample surface. (It is by this technique that the world nano car championship is followed that was mentioned in the introduction). Atomic force microscopy (AFM) is slightly different with the cantilever tip being oscillated, in z-direction, above the sample and hence by detecting the bonding (atomic force) with the surface and the current position of the tip when the bond occurs the topography is recorded from the scan. As mentioned earlier many different techniques have been developed beyond these two and have been review well already (Kasai and Bhushan, 2008, Wiesendanger, 1994). This is one big advantage, that auxiliary to studying particles size, shape and surface, SPM can be combined with measuring chemical bonding, surface reactivity, magnetism and conductance etc. Another benefit is that amorphous materials can be studied as well as ordered materials and in the case of AFM it can be done in ambient atmosphere in both liquid and air. This allows for studying more natural conditions with less bias than under vacuum. Nanoparticle coronas and interaction with proteins is theoretically possible to study in real time under natural conditions.

2.6.6 Gas sorption

By lowering the temperature of a sample contained in a chamber with a gas, usually nitrogen, a layer of the gas will condensate onto the sample. Consequently, by measuring how much gas has adsorbed to the sample, through manometry, one can, by calculation, determine the surface area of the sample. The calculations depend on models on how adsorption and desorption will occur. The most common model is Brunauer-Emmet-Teller (BET) theory that explains how multilayers should behave mathematically. However this model has been questioned for its functionality of porous materials (Sing, 2001). If the material in question is porous Xenon nuclear magnetic resonance spectroscopy is a superior option (Cros et al., 2000).

2.6.7 Thermo gravimetric analysis

The thermal decomposition of solids is a useful characterising property. Thermo gravimetric analysis (TGA) is workhorse for the chemistry lab. Albeit not the most common method for nanoparticle research it is still of interest since the temperature of decomposition is reduced as particles size is reduced. It is also useful because of the possibility to quantify different components of materials.

2.7 Detection of nanoparticles

To detect and analyse nanoparticles in complex matrices is a formidable task. Since the defining property of sub 100 nm size makes them invisible to many standard techniques such as light microscopy, the ways of detection is limited. These method largely correlates with the methods in the chapter on characterization, *vide supra.* The most promising is SP-ICP-MS that can both measure size and composition of particles, with the drawback that the matrice needs to be dissolved. The methods that rely on imaging must also be accompanied with some chemical detection method, since there are many other nano sized structures that can result in false positives. Other methods like gas sorption and dynamic light scattering is largely useless for studying nanoparticles *in vivo* or *in situ*, unless it is possible to reliably extract the particles. When it is possible, one must take into consideration that the extraction process might affect the particles and that the concentration and species might not reflect the nature of the particles *in situ.*

Since the use of nano particles has become so widespread there is a great need for development of techniques that can measure nano particles in complex matrices. Some quite creative methods has been proposed. For example, Mudanyali et al (2013) developed an on-chip microscopy method using self-assembled nanolenses around individual particles to enhance scattering (Mudanyali et al., 2013). That allows for wide field imaging and detection of nanoparticles that they proved to work on polystyrene particles and virus particles. Corredor et al. (2015) established a colorimetric method that measures both catalytic activity of the particles and their quantity (Corredor et al., 2015). However, they failed to show how this method would differentiate between different particles and other natural catalysers. Othman et al. (2017) created a membrane with surface-confined ligands with tailored affinity towards CeO_2 also relying on a redox reaction that leads to a change in optical properties that easily can be measured. With the development of enhanced resolution dark-field microscopy, that achieves resolution around 90 nm, we get closer to something useful (Vainrub et al., 2006). Avellan et al (2017) used this technique together with hyperspectral imaging to detect and confirm gold nanoparticles in root cells (Avellan et al., 2017). They also used x-ray tomography and could successfully image and detect agglomerations of gold nanoparticles in and around roots by normalizing the greyscale levels to air and compare histograms to particles in gel. This was of course made easier by the fact that gold is a heavy element, with, for example, titania or aluminium oxide it would be much more challenging.

A possibility that seems underestimated, or perhaps underused, at least in the plant interaction field, is radioactive or rare isotope labelling. Either by attaching isotopes to particles or incorporating them into the particles, we can achieve easy and robust detection of nanoparticles. There are many ways of preparing radioactive nanoparticles: Neutron radiation of existing particles, performing the synthesis with radioactive isotopes or by adsorbing radioactive isotopes onto the particles (Yin et al., 2017, Yu et al., 2015). After, the particles can be detected by spectrometry, scintillation counting, ICP-MS and imaged by autoradiography, positron emission spectroscopy, single photon emission computed tomography and secondary ion mass spectroscopy (SIMS). The latter has a spatial imaging resolution of 50 nm and very high sensitivity, making it a top notch alternative for this type of research. The draw-back is that it is labour intensive and low through put.

2.8 Suggestion for guidelines and standards

When studying nanoparticles in complex systems it quickly becomes clear that it is difficult to get at complete picture of what is going on. Most nanoparticles have very complex chemistry themselves and trying to discern driving factors in a complex environment becomes difficult. It is not uncommon to see conflicting results in the literature (Yang et al., 2007, Mattiello et al., 2015, Feizi et al., 2013). At this point it is impossible to know what the reasons for these discrepancies are. Is it because of differences in the particles used or in how they were used? For the research community to even begin to acquire this knowledge we must strive for high standards of experiments and guidelines should be formulated. This, off course, is something that must be worked out by the community. Similar, to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) a high standard should be set. Few publications actually follow the MIQE to a full extent but the guidelines probably have improved the quality of data in publications since (Bustin et al., 2009). Similarly, we could put forward a list of demands, for nanoparticle research, including particle characteristics, for example: Zeta potential (in water and relevant medium), hydrodynamic size (in water and relevant medium), size (measured by electron microscopy and SP-ICP-MS), crystallite size, crystal structure, surface area and if possible other characteristics such as magnetism, chirality and shape. Also adaptation and application must be thoroughly described. For studying toxicity and effect, standardisation of the biological conditions could also be put forward. For example what are the relevant concentrations (preferably in particle per ml) in hydroponic system or a system with soil? At which developmental stage should toxicity be measured? Should nanoparticle exposure be constant or transient?

3 Titanium dioxide (anatase) (paper I)

3.1 Background

Titania (TiO_2) nanoparticles have high refractive index, are white, UV-absorbing, photocatalytic and can be designed in a variety of sizes, morphologies and crystal structures. Since titanium is the ninth most common element with 0.63 mass percent, production of titania nanoparticles is relatively cheap (1997). The particles are used as food colorant, paint pigments, UV-protection cream and anti-fouling agent, just to name a few applications.

3.2 Synthesis

Titania nanoparticles can be synthesized in a myriad of ways. In industrial context the sulfate and chloride processes are the most common (Ramos-Delgado Norma et al., 2016).

In the sulfate process ilmenite ($FeTiO_3$) is acid digested with sulphuric acid (H_2SO_4) to produce titanium sulfate ($TiOSO_4$). The titanium sulfate is then hydrolysed under heating to 109° C. which forms a gel with H_2SO_4. To the gel seed crystals are added to initiate crystallisation, different seed will generate different crystal structures.

In the chloride process the starting materials require higher purity and rutile or titanium slags are commonly used. The titania is chlorinated in presence of graphite to produce titanium chloride ($TiCl_4$) and CO_2. Then the $TiCl_4$ is oxidized with oxygen to produce TiO_2 and chloride. The chloride process is a greener process that creates less waste and reuses the chlorine, however only rutile phase can be created.

Less common on industrial scale, because of the more expensive precursors, alkoxides, is the sol-gel process. However, it uses much less energy and produces high purity materials with great variety, such as: monoliths, hybrid materials, porous materials, thin films, powders and fibers.

Additionally there are many other ways of producing titania nanoparticles, such as: Precipitation, microemulsion, hydrothermal, solvothermal, electrochemical and biological synthesis (Gupta and Tripathi, 2012), which allow even greater versatility of the resulting materials possible.

3.3 Interactions with plant growth promoting rhizobacteria

Titania is known to have phosphate affinity, to the extent that it´s been used for chromatography of phosphorylated compounds (Jaoude et al., 2012, Sano and Nakamura, 2004, Pinkse et al., 2004). This affinity also extends to phospholipids (Rossetti et al., 2006). Which explains why titania also has affinity to bacteria (Palmqvist et al., 2015, McGivney et al., 2017). It isn´t overtly preposterous to say that titania nanoparticles should affect the rhizosphere.

3.3.1 Titania nanoparticle influence on growth and root colonization

To assess potential toxicity of titania nanoparticles to the plant growth promoting rhizobacteria *Bacillus amyloliquefaciens,* the bacteria were cultivated together with the particles in a plate reader. The absorbance was measured continuously. Somewhat surprisingly, the titania nanoparticles induced an increased growth. The absorbance of the titania treatment increased faster and to a higher degree, se figure 3.1.

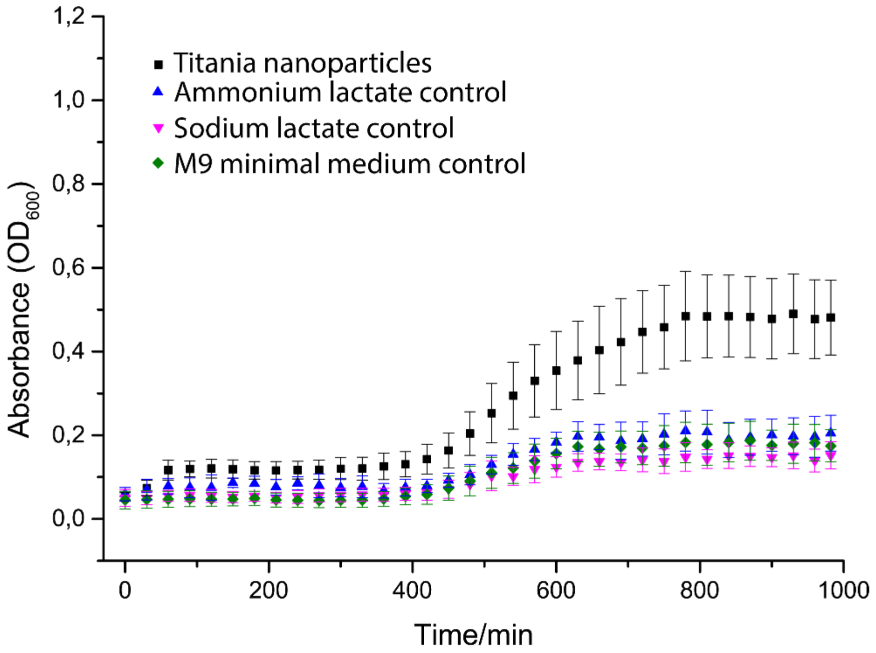


Figure 3.1. Growth curve of Bacillus amyloliquefaciens grown in a plate reader as measured by absorbance over time. The bacteria were grown in minimal medium M9 together with titania nanoparticles, noted as ■*, or with lactate controls noted with* ▲ *and* ▼*. Negative control with just M9 is noted with* ♦*.*

Measuring absorbance is a good way of estimating concentration of bacteria, however it relies upon rather big assumptions in this case. We measured the absorbance of the particles themselves and it was practically zero but still it was not possible to exclude the possibility that the particles together with the bacteria somehow increased absorbance without increasing the number of bacteria. It could be that they were just aggregating and precipitating on the bottom of the well and in this manner increased absorbance. Hence we decided to use other ways of quantifying the bacteria. First we tried to do serial dilutions of the cultures from the plate, to do plate counting of colony forming units. Plate counting did not work well in this case, because of bacterial clustering, *vide infra*. Instead we managed to transform the bacteria to produce the enhanced green fluorescent protein (EGFP). Having fluorescent excitable bacteria makes quantification more robust. We used this to measure the amount of bacteria that grows on roots in a hydroponic system with or without bacteria, by washing the roots and measuring the amount of bacteria in the rinsing water. Even though it would be expected that a higher degree of bacteria should still adhere to the roots after washing, higher concentrations of bacteria was found in the rinse water of titania treatment, see figure 3.2.

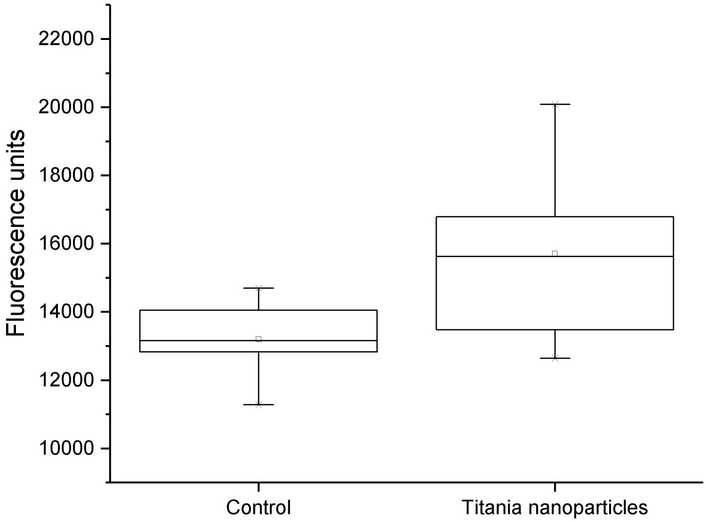


Figure 3.2.Amount of bacteria washed off of roots grown in hydroponic system with MS-media with or without titania nanoparticles.

The same plant roots where also studied by scanning confocal fluorescence microscopy to get a visualization of the amount of bacteria colonizing the roots. Indeed it was clearly visible that greater amount of bacteria colonized the roots in presence of titania nanoparticles.

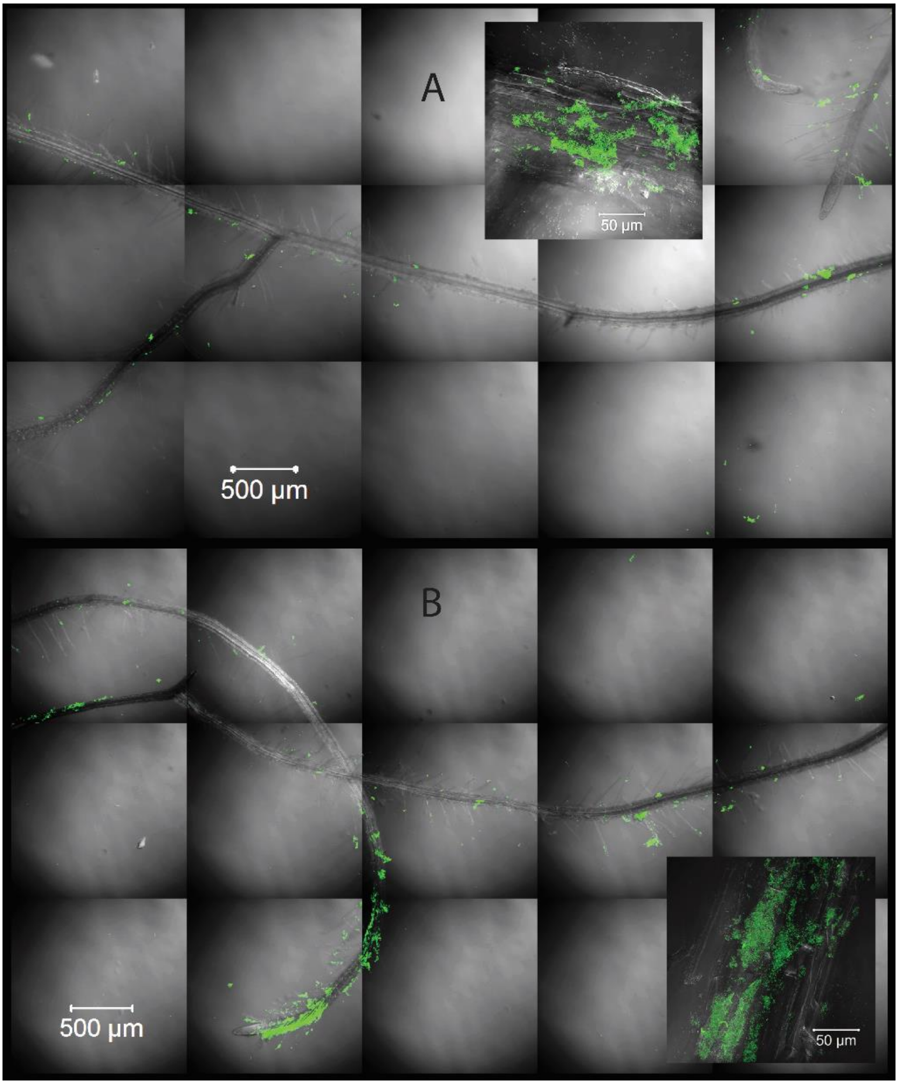


Figure 3.3. Roots of Brassica napus colonized by Bacillus amyloliquefaciens 5113 that has been transformed to produce EGFP. Hence what as shown in green colour is from the fluorescence channel, showing the presence of bacteria. The inserts are images with greater magnification (630 x) that were taken from randomized positions of the roots.

3.3.2 Clustering

The phospholipid affinity of the particles, leads to them, adhering in aggregates, on the surface of bacteria (McGivney et al., 2017). These aggregates can then act as glue to bind the bacteria to each other. Which was clearly seen when *Bacillus amyloliquefaciens* 5113 was cultivated in media containing titania nanoparticles. The standard method of plate counting colony forming units did not work because the bacteria clustered together and formed abnormally large colonies. In figure 3.2 four plates of bacteria cultivated without titania nanoparticles can be seen compared to four plates with bacteria that was, even though the amount of bacteria is higher in the titania treatment, *vide supra*, there are much fewer colonies.

Figure 4.2. Photograph of plates from an attempt to do plate counting of colony forming units. The four plates on the left are control which have been cultivated without titania nanoparticles in LB-media. The four plates on the left where grown together with 50 mg/ml titania nanoparticles in LB-media.

Interestingly, these structures showed smooth surfaces in SEM. The bacterial colonies were encapsulated together with a layer of titania on the surface. These capsules could be mechanically broken and the bacteria could be seen on the inside, see figure 3.3.

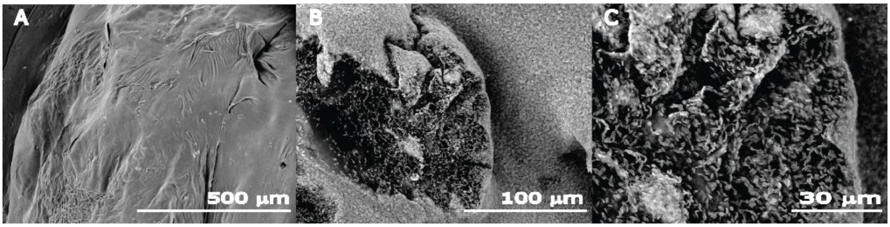


Figure 3.3. Bacillus amyloliquefaciens cultivated in LB-media together with titania nanoparticles, as seen in in SEM. These are the taken from the cultures in figure 3.1. that has orange colour. Panel A. shows the clusters before mechanical rupture of the surface layer, panel B. and C. show where the cluster surface have been ruptured and the bacteria inside.

3.3.3 Plant protection

To study if the increased aggregation and growth of bacteria on plant roots had any effect on the ability of *Bacillus amyloliquefaciens* to reduce disease severity, we inoculated *Brassica napus* plants with *Alternaria brassicicola.* The disease severity was assessed and then measured by quantifying the amount of fungi in the plant tissue with quantitative polymerase chain reaction (q-PCR). The fungal content in the leaves was lowest in the treatment with titania and bacteria but the difference was not statistically significant compared to bacteria only and interestingly neither to titania only (figure 3.4).

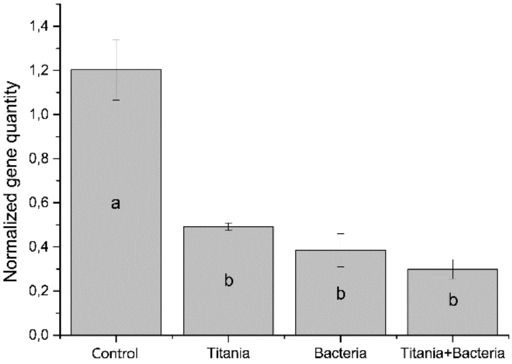


Figure 3.4. Quantified amount of Alternaria brassicicola in the leaves of inoculated Brassica napus by q-PCR. Different letters signifies statistically significant difference.

It is possible that with greater number of replicates the trend, of lower amount of fungi when bacteria is cultivated together with titania nanoparticles, would have solidified. Due to difficulties in growing enough plants to have sufficient amount of fungi the amount of replicates were low. However the assessed disease severity showed that the titania did not reduce the disease symptoms compared to the bacteria alone. Also, the disease severity of titania without bacteria did not show a statistically significant difference to control, although it was lower (figure 3.5).

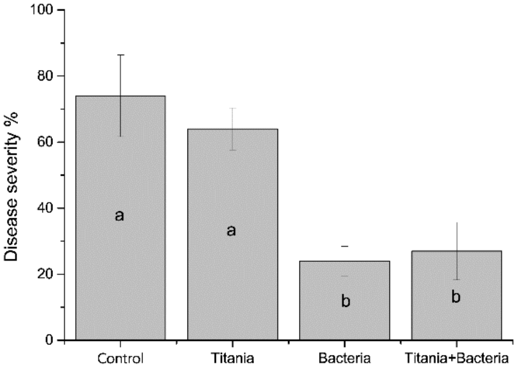


Figure 3.5. Disease severity assessment as described by (Van der Ent et al., 2009). Different letters means statistically significant difference.

4 Iron oxide (maghemite) (paper II)

4.1 Background

Iron oxides are ubiquitous in nature. So far, ten, of all fourteen known iron oxides have been found in nature and many have stable nano particulate stages (Guo and Barnard, 2013). Maghemite usually forms topotactically from magnetite and other iron oxides. It occurs mostly in soils and is more stable than magnetite and even more stable than hematite when in nano form (Guo and Barnard, 2013).

4.2 Synthesis

Synthesis of maghemite nanoparticles can be performed in many ways, such as: Co-precipitation, sol-gel synthesis, micro emulsion, flow injection, flame spray pyrolysis and oxidation of magnetite (Shokrollahi, 2017). For our biological experiments we used maghemite nanoparticles synthesised with a sol-gel method (Zhang et al., 2011). We used sol-gel because it allowed us to easily produce large quantities, which was needed for plant treatments. Further, this particular method that introduces yttrium also stabilizes the maghemite structure and prevents transformation into hematite.

4.3 Properties

Maghemite is ferromagnetic at room temperature and becomes super paramagnetic when in nano form below 300 K. The structure of maghemite is the same spinel structure of magnetite, the difference is that maghemite lacks any Fe(II). The morphology of maghemite nanoparticles is versatile and largely depends on the morphology of its precursor when they have been created topotactically. The size of particles can be anything from a few nm to macro sized. From the synthesis of (Zhang et al., 2011) the particles are 5 nm according to TEM studies. Also the powder-XRD of the particles and Sherrer calculation demonstrate a primary particles size of 3.8 nm (figure 4.1). However when suspended in plant nutrient solution the particles aggregate and the hydrodynamic size demonstrate a different pattern (figure 4.2).

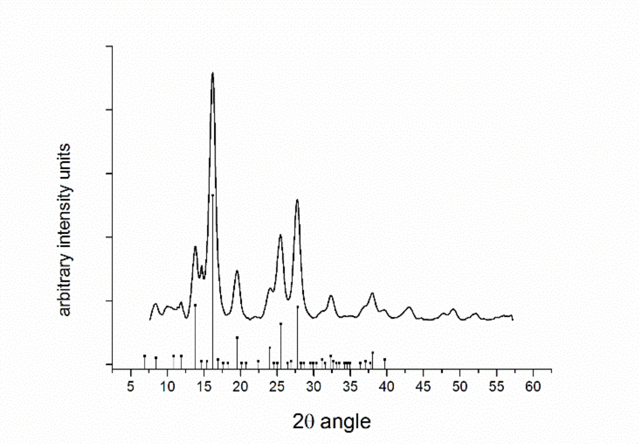


Figure 4.1. Powder diffractogram of maghemite nanoparticles. The points with drop line annotate standard peaks for maghemite. A crystallite size of 3.8 nm was calculated with Sherrer equation from the largest peak at 16.197 2θ degrees.

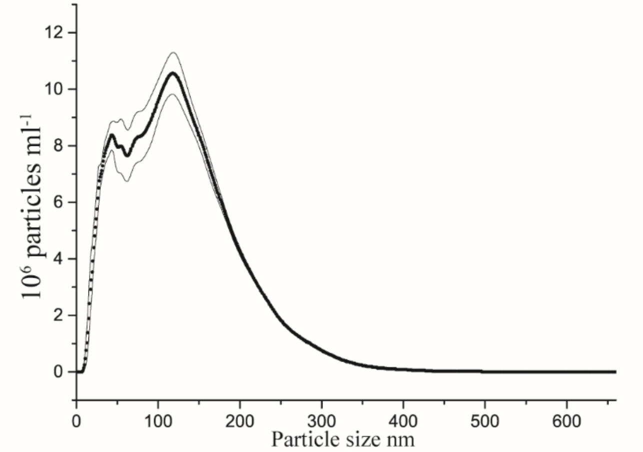


Figure 4.2. Hydrodynamic size of maghemite nanoparticles in plant nutrient solution as measured by nano tracking analysis with Nanosight.

The aggregation also becomes evident when looking at the maghemite nano powder in SEM. Large aggregates with hierarchical structure clearly show build up from smaller particles (figure 4.3).

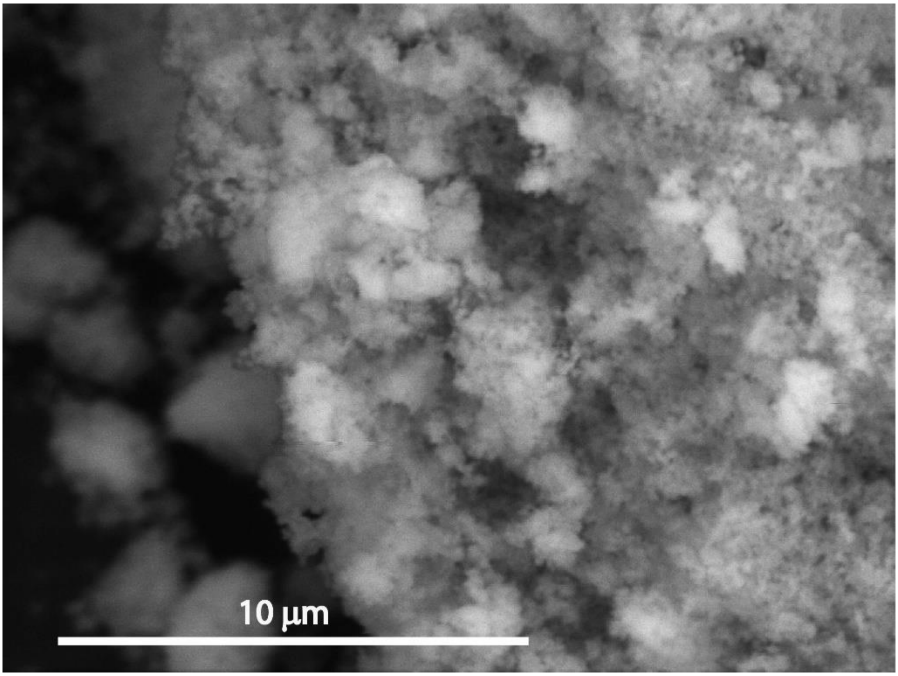


Figure 4.3. Maghemite nanoparticle powder imaged under SEM.

Nanozymes

The term nanozymes was coined by F. Manea et al. in 2004 and refers to nanoparticles that exhibit enzyme like activities. It is a very promising field of science and technology since using nanoparticles, instead of organic enzymes, is robust, cheap and recyclable. The iron oxides have been some of the most prominent nanozymes. They have both peroxidase and catalase activity and have been used in applications such as: hydrogen peroxide detection, glucose detection, immunoassays, aptasensors and DNA-detection (Wei and Wang, 2013, Roy et al., 2016, Chen et al., 2012).

4.4 Interactions with plants

Iron oxide nanoparticles and their interaction with plants have been studied several times. Some seminal research papers from the early years of nano agriculture was about iron oxide nanoparticles (Zhu et al., 2008). Sometimes only for learning about the interaction with plants (Wang et al., 2011, Li et al., 2016a, Li et al., 2017). Sometimes to study the possibility to use them as fertilizer (Ghafariyan et al., 2013, Ghazi Harsini et al., 2014, Rui et al., 2016). Since maghemite particles have enzymatic properties it is possible that these properties will have an effect in plants. The maghemite nanoparticles have been shown to have a catalase like activity, meaning that they will break down hydrogen peroxide to water and oxygen by an oxidation-reduction reaction. This should mean that the nanoparticles can be beneficial under abiotic stress situations, such as drought, when large amounts of hydrogen peroxide is produced in plant tissues (Moran et al., 1994, Zhang and Kirkham, 1994).

4.4.1 Growth promotion

As mentioned earlier, maghemite nanoparticles have been suggested for use as easily available iron fertilizer (Rui et al., 2016). Iron is an important micronutrient for plants and its availability is usually highly dependent on soil pH (Colombo et al., 2014). A soil pH of 6.5 is generally recommended because iron is insoluble at higher pH, while at lower pH than that availability of potassium is instead lowered. Usually the plants counteract lack of iron by the release of siderophores, sometimes in a mutualistic relationship with bacteria that produce them. The siderophores can chelate iron and hence solvate it. If the plants instead get iron in form of particles that follow the flow of water into the xylem the plant doesn’t have to waste resources on producing siderophores or nurturing bacteria.

We wanted to investigate the enzymatic effect of maghemite nanoparticles *in planta*. So we introduced the particles in addition to chelated iron. The control was fully nourished. Interestingly, we found that adding the maghemite nanoparticles increased the growth of leaves and that the chlorophyll content was increased compared to the control (figure 4.4).

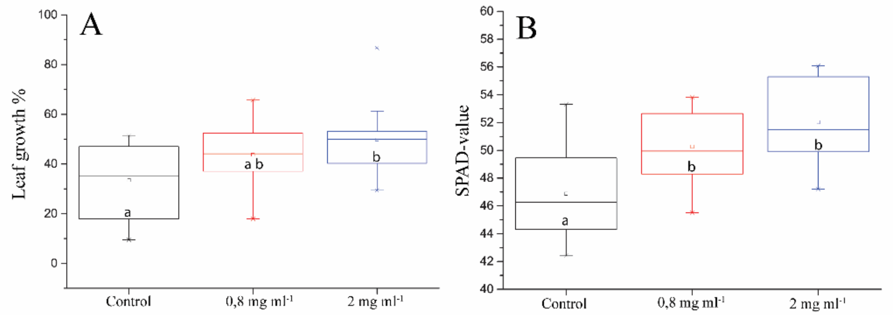


Figure 4.4. Measures of plant growth and health on Brassica napus treated with maghemite nanoparticles compared to control with full nutrition. Panel A shows leaf growth in percent after treatment with maghemite nanoparticles. Panel B. shows chlorophyll content of the leaves as measured with Konica SPAD-meter after treatment with maghemite nanoparticles. Different letters mean statistically significant differences.

4.4.2 Reduced oxidative stress

When plants are exposed to stress, hydrogen peroxide starts to accumulate in the cells to toxic levels (You and Chan, 2015). We hypothesized that maghemite nanoparticles can reduce the toxicity of abiotic stress by scavenging on hydrogen peroxide. So we irrigated *Brassica napus* with a nutrient solution with suspended maghemite nanoparticles for five days, then exposed the plants to drought for five days. Then we measured the hydrogen peroxide content in the leaves according to (Cheeseman, 2006). Indeed the hydrogen peroxide levels in the leaves of plants that had been irrigated with maghemite nanoparticles was lower than in control (figure 4.5).

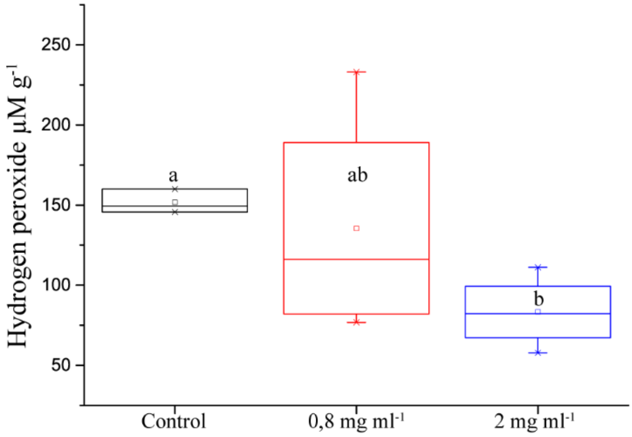


Figure 4.5. Hydrogen peroxide content of Brassica napus leaves after drought stress. Control is plants irrigated with nutrient solution, the other two were irrigated with the same nutrient solution with 0.8 mg ml^{-1} amd 2 mg ml^{-1} maghemite nanoparticles. Different letters mean statistically significant difference.

Since hydrogen peroxide is not only a toxic by-product but also an important signalling molecule (You and Chan, 2015), it was important to measure not only the amount of hydrogen peroxide but also the damage that it created. Hence we measured the amount of lipid peroxidation that had occurred in the leaf tissue, by measuring the proxy of malondialdehyde. Again the results supports our hypothesis since a reduced amount of damaged lipid was found (figure 4.6).

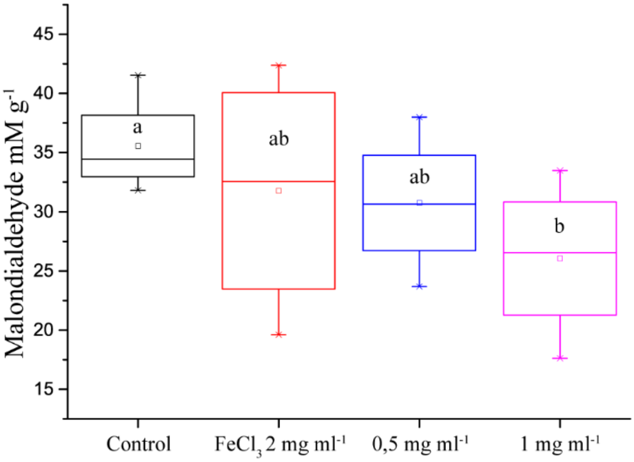


Figure 4.6. Malondialdehyde concentration in leaves, as a proxy for lipid peroxidation, in plants treated with maghemite nanoparticles in nutrient solution. Control plants where irrigated with the same nutrient solution, as where the positive control with Fe(III) ions at the same molar concentration as highest maghemite concentration. Different letters indicate statistically significant difference.

4.4.3 Maghemite nanoparticle uptake into plant tissues

It is interesting that iron oxide nanoparticle uptake by plants is still a controversial issue. It has been questioned by some of the most prominent scientists in the field (Zuverza-Mena et al., 2017). However, these conclusions seems based on research done with nanomaterials that does not reach below the plant root cell-wall limit of maximum 20 nm (Martínez-Fernández et al., 2016). That iron oxide nanoparticles can adhere to roots, is without doubt, but there is some evidence that they also are transported into above ground tissues (Grażyna Bystrzejewska-Piotrowska, 2012, Zhu et al., 2008). We discovered that, our 3 nm crystallite size nanoparticles, when administered through soil irrigation, increased the iron content of *Brassica napus* leaves, by 22 percent (figure 4.7). Interestingly, the iron content was reduced by adding the molar equivalent of Fe(III) ions showing that plants when presented with toxic levels of Fe(III) ions can block uptake. This indicates that the increased leaf content comes from particle uptake and not from dissolution of particles in the rhizosphere. To further corroborate this indication we measure the low temperature magnetization of the same leaves. We did, indeed, find a magnetic behaviour consistent with super paramagnetic iron oxide nanoparticles (figure 4.8).

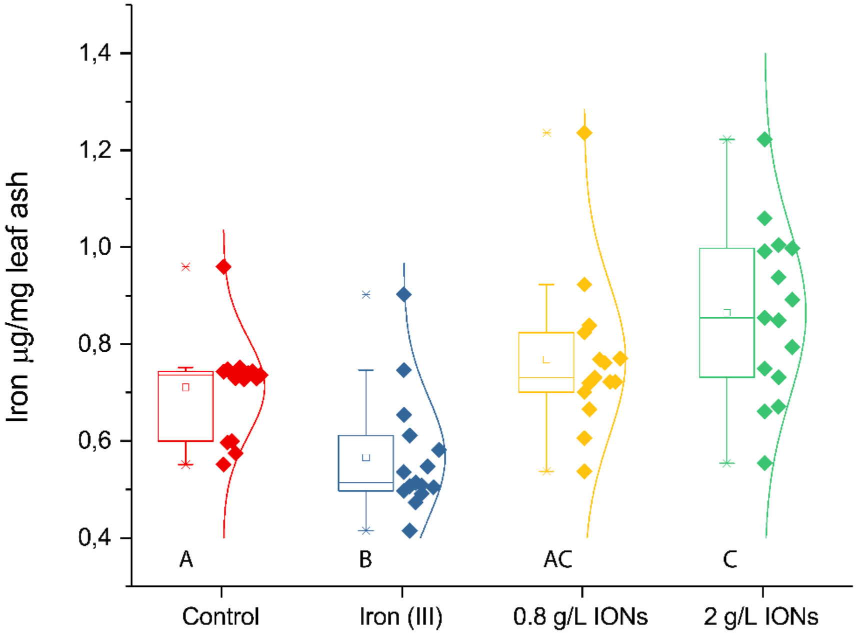


Figure 4.7. Iron content comparison of leaves, three weeks after treatment with iron oxide nanoparticles, as measured by ICP-AES of the ashed leaf tissue.

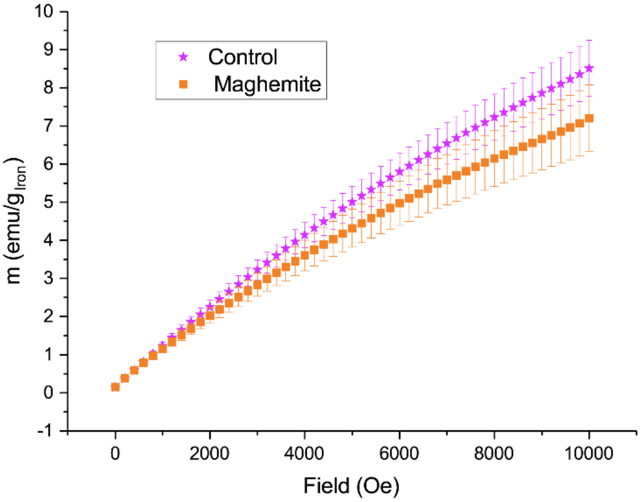


Figure 4.8. The low temperature magnetization of leaf ash from control plants and plant treated with 2 g/l of maghemite nanoparticles. The trend of lower magnetization at higher fields, because of blocked magnetizations when the atoms in the crystal is oriented in directions not aligned with the field, and higher remnant magnetizations at zero field is characteristic for super paramagnetic nanoparticles.

5 Calcium carbonate (calcite) (paper III&IV)

5.1 Background

Calcium carbonate ($CaCO_3$) is one of humanities oldest material used. Its versatility and its fantastic chemistry with water and CO_2 has made it the perfect building material. The worlds´ oldest standing building, the great pyramid of Giza is made of calcium carbonate. Its low hardness (3 on Mohs scale) made it easy to cut into pieces and create sculptures and shapes; it also made it a great abrasive that do not scratch, used until this day. It´s beautiful white colour has inspired people to make paint for tens of thousands years and it is still used in paints today.

5.1.1 The fantastic chemistry of calcium carbonate

The word calcine is a verb defined by Oxford Dictionary as: “Reduce, oxidize or desiccate by roasting or strong heat”. To calcine has become synonymous with heating something, because of the ancient process of heating calcareous stones to over 875 degrees C. to produce quick lime (CaO). The CaO is unstable and will gradually react with CO_2 to convert back to $CaCO_3$ when cooled back to room temperature. It is also very hygroscopic and reacts fervently with water to produce heat and slaked lime ($Ca(OH)_2$). The slaked lime or lime slurry will react with CO_2 in the air as it dries to form $CaCO_3$ fulfilling the so called lime cycle. The slaked lime is an integral part of traditional plasters and mortars and in fact the word lime comes from old English lim, meaning glue, which might come from Old Norse (according to city guides in Visby); in modern Swedish lim is still the common word for glue.

Precipitated calcium carbonate (PCC)

In the calcination step it is possible to collect the CO_2 and then reintroduce it to the slaked lime. This will react $CaOH_2$ with CO_2 and precipitate the insoluble $CaCO_3$. By changing the temperature, pressure, stirring rate of gas flow it is possible the change the particles size and morphology in a formidable range, allowing for tailored particles to specific applications. It also purifies the calcium carbonate and removes impurities that always are present in lime stone.

Solution equilibrium

Calcium carbonate has very low solubility in neutral water, around $5.5x10^{-6}$ mol L^{-1} (Gal et al., 1996). At lower pH $CaCO_3$ naturally increases solubility and consequently the solubility increases from increased partial pressure of CO_2, as $CO_2 + H_2O \rightleftharpoons H_2CO_3$ and $H_2CO_3 \rightleftharpoons HCO_3^- + H^+$ and $H^+ + CO_3^{2-} \rightleftharpoons HCO_3^-$. Calcium bicarbonate ($Ca(HCO_3)_2$) is very soluble and in fact only present in solution.

5.1.2 A biomaterial

One of the common sources traditionally used to make quick lime was sea shells. Calcium carbonate is produced by molluscs to form their shells. Other sea organisms also produce $CaCO_3$ i.e. the protist foraminifera and the algae coccolithotrophs. The large deposits of $CaCO_3$ that can be found around the world, most notably the Mediterranean Sea basin, are formed from millions of years of life and death and the subsequent deposition on the sea bed. These organisms biosynthesize $CaCO_3$ and form hybrid materials with proteins that display surprising strength (Rae Cho et al., 2016).

Biocompatible

As nature has shown for millions of years calcite and aragonite crystals are compatible with life. Precipitated calcium carbonate is considered safe for human consumption. It is registered as a white colorant with E-number 170 but it is also used as nutritional additive and antacid or drug matrix filler in drug tablets(Gal and Nussinovitch, 2007, Wagner-Hattler et al., 2017).

5.1.3 Modern use

Already in 1841 did the English company John E. Sturgess Ltd. commercialize the production of PCC. The white powder found its way into tooth pastes and linoleum floors. Eventually it was discovered that it strengthened rubbers (Sadeghi Ghari et al., 2014, Sadeghi Ghari and Jalali-Arani, 2016) and plastics(Piekarska et al., 2016) and when paper production became alkaline in the 1950´s, both ground calcium carbonate (GCC) and PCC became standard in production of paper and remains so until this day(Nypelö et al., 2011). It´s use in environmental protection is more recent, both as absorbent in fume towers or as mitigation of acidifying lakes (Vinoba, 2013). Calcium carbonate is, of course, still part of plaster and concrete production (Sekkal and Zaoui, 2017), same as for glues (Donate-Robles et al., 2014) and paints (Karakaş et al., 2015). High end plastics and composites also take advantage of the strengthening properties of calcium carbonate (He and Gao, 2017). As the reader might notice, most of these recent publications are concerned with improving old formulas and concepts with nano-sized calcium carbonate.

5.2 Synthesis

To synthesize nano calcium carbonate it is important to create a state of super saturation so that nucleation of the crystal is rapid. This creates many small nuclei which must then be prevented from growth. This is most commonly done by adding a capping agent, usually a surfactant; a molecule that binds to the surface and has both hydrophobic and hydrophilic moieties. The surfactant helps to create a stable suspension and stops the growth of the nanoparticles. However, most surfactants are incompatible with biological applications. Hence, other ways of preventing growth must be used, for instance, addition of spectator ions that will reduce the mobility of the precipitating salts or modification of the solvent.

5.2.1 Co-precipitation

Co-precipitation of calcium carbonate can be easily achieved by combining to solutions, one containing Ca^{2+} and the other containing CO_3^{2-}. As seen in equation 1, the nucleation rate will be governed by the degree of supersaturation. In figure 5.1 the hydrodynamic size distribution of calcium carbonate nanoparticles synthesized by co-precipitation in presence of Mg^{2+} can be seen.

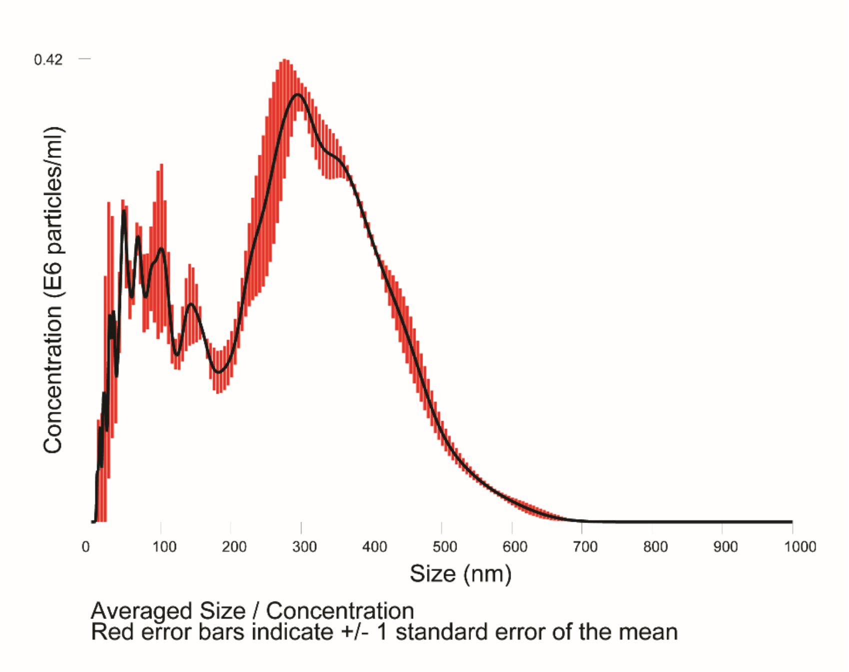


Figure 5.1. Hydrodynamic particles size distribution of calcium carbonate nanoparticles, as measured by nano tracking analysis. The modular size is 288 nm while the mean size is 275 nm.

Ostwald ripening will be a major factor in this type of synthesis unless the mobility of ion can be prevented. The most successful method to reduce mobility in water is by adding capping agents to calcium carbonate nanoparticles (Jiang et al., 2015, Sonawane et al., 2009, Zhao et al., 2016). This has also been discovered by nature and Bernard et al. found that lithostathine is a regulator of calcite crystal formation (Bernard et al., Gerbaud et al., 2000). Another way of reducing the growth is by adding other ions. Competing ions that reduce the mobility of ions that constitute the crystal to transfer from one to another or ions that incorporate into the crystal and change the surface charge and tension and hence the growth properties. For example, adding magnesium ions (Mg^{2+}) is a bioinspired way of reducing size of crystals (Berner, 1975, Bischoff, 1968, Long et al., 2014). By incorporating the more highly charged Fe^{3+} ion one can reduce the size substantially; in figure 5.2 the results of coprecipitation of calcium carbonate in the presence of Mg^{+} and Fe^{3+} at 2:1 and 1:1 molar ratio in relation to Ca^{2+}, respectively, are represented. In order to further lower the solubility of calcium carbonate, the carbonate was dissolved in 50% ethanol, into which the Ca^{2+} was titrated.

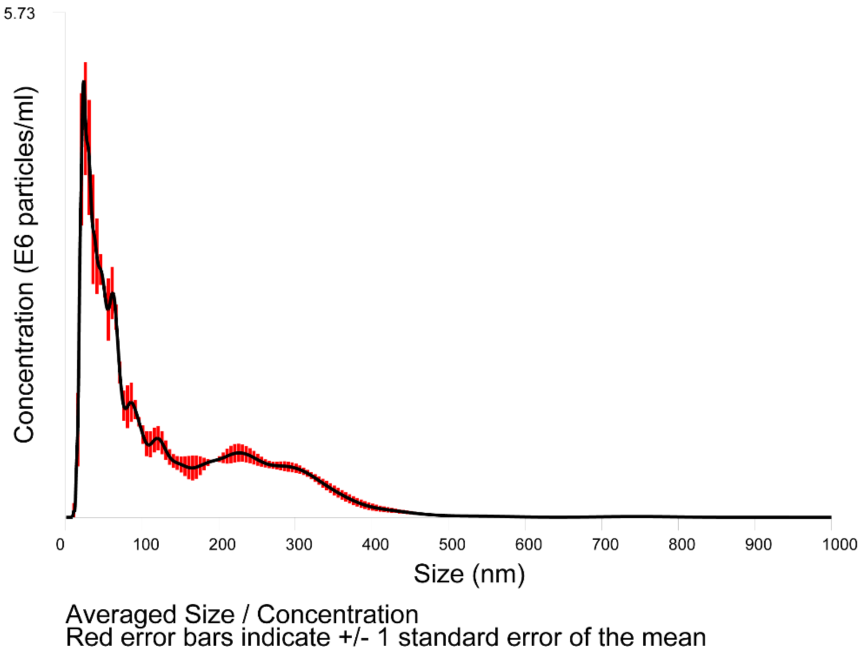
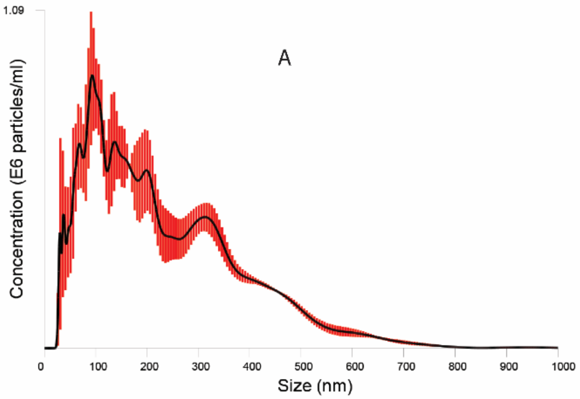


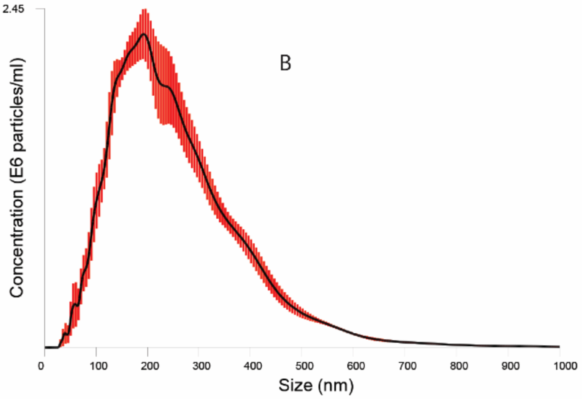
Figure 5.2. Hydrodynamic particles size distribution of calcium carbonate nanoparticles synthesized with coprecipitation in the presence of Mg^{2+} *and* Fe^{3+}*. The modular size is 26 nm, however the mean is 139 due to large polydispersity.*

5.2.2 Gas precipitation

If the goal is to reduce particle sizes, the same rules apply to gas solid reactions. The same processes of growth and Ostwald ripening govern the more common precipitation method of bubbling CO_2 through a solution of $Ca(OH^-)_2$. A supersaturated solution is desired to get a fast nucleation process that leads to many nuclei. Then the growth must be prevented by capping or ion transport hindrance. By comparing A, B and C in figure 5.3 one can discern the effect of adding a surfactant and an ion that changes the surface tension. In B one can see the effect of a surfactant that caps growth at a certain stage and forms a more monodisperse distribution and also increases stability of the suspension by preventing aggregation of particles. While in C the Fe^{3+} ions have changed the surface tension of the particles and reduced the affinity for binding new Ca^{2+} ions, these primary particles are smaller but in turn there is greater aggregation.



Averaged Size / Concentration
Red error bars indicate +/- 1 standard error of the mean



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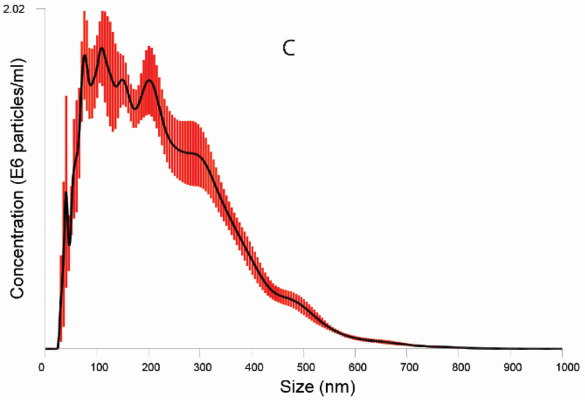

Averaged Size / Concentration
Red error bars indicate +/- 1 standard error of the mean

Figure 5.3. Hydrodynamic particles size distribution of calcium carbonate nanoparticles synthesized by gas-solid precipitation. Panel A shows an unmodified case with modular size of 81 nm and a mean of 236 nm. Panel B shows particle distribution when albumen has been added as surfactant with modular size of 173 nm and mean size of 258 nm. Panel C shows particle distribution from synthesis with 1:1 molar ratio between Ca^{2+} *and* Fe^{3+} *with a modular size of 95 nm and mean size of 230 nm.*

5.2.3 Sol-gel synthesis

To the best of our knowledge there has previously not been any sol-gel methods reported that can produce calcium carbonate nanoparticles. I believe that the included paper III is the first to do so(Palmqvist et al.). The method is illustrated by figure 5.7.

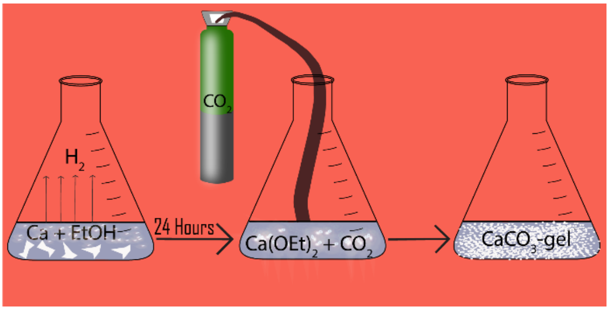

Figure 5.4. A schematic representation of the sol-gel method for calcium carbonate nanoparticle synthesis.

The first step is to react calcium with absolute ethanol. If a pure and colourless gel is desired it is important that the ethanol is kept completely dry, otherwise the Guerbet reaction can occur and form the yellow vinyl aldehyde. The second step is performed in ambient conditions because the formation of oxo complexes such as $Ca_6O_2(OC_2H_5)_8(C_2H_5OH)_{14}$ is desired and humidity is a prerequisite (Turova et al., 1993). It involves passing CO_2 through the calcium ethoxide under vigorous stirring. In our standard conditions with 0.8 grams of calcium and a CO_2 flow rate of 0.2 L/min at 50 bar the precipitation becomes visible after three minutes but we continued the reaction until the solution neutralised after approximately 15 min. The third optional step of gel formation occurs overnight if air humidity is suitable (figure 5.5). Weather it gelates or not the product consists of ultra-small nanoparticles as demonstrated in figure 5.6, 5.7 and 5.8.

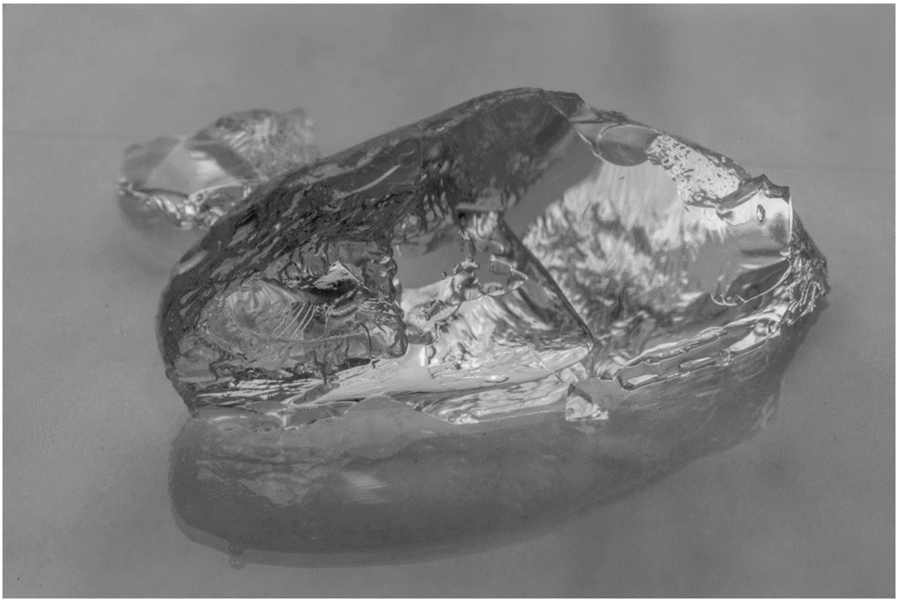

Figure 5.5. Photograph of calcium carbonate gel produced by calcium ethoxide CO_2 gas sol gel method.

Particle sizes and morphology

The particles produced, as measured by NTA in the mother liquor, have extremely small hydrodynamic sizes. When the particles are centrifuged and subsequently redispersed into water the hydrodynamic sizes are increased to a spectrum of considerably larger sizes (figure 5.6). In figure 5.7 a SEM image demonstrates the aggregation effect on the particles of centrifugation and desiccation. A hierarchical structure of small primary particles towards a micro aggregate. If the particles are not centrifuged but deposited onto a surface by evaporation of the ethanol a morphology typical of a desiccated gel can be seen (figure 5.8).

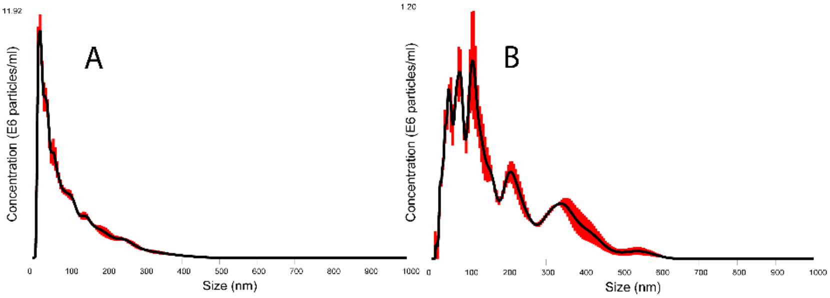

Figure 5.6. Hydrodynamic sizes of calcium carbonate nanoparticles synthesized through sol-gel method. Left panel A shows the particles as synthesized in the mother liquor ethanol. Right panel B shows the same particles after centrifugation and subsequent redistribution in ethanol.

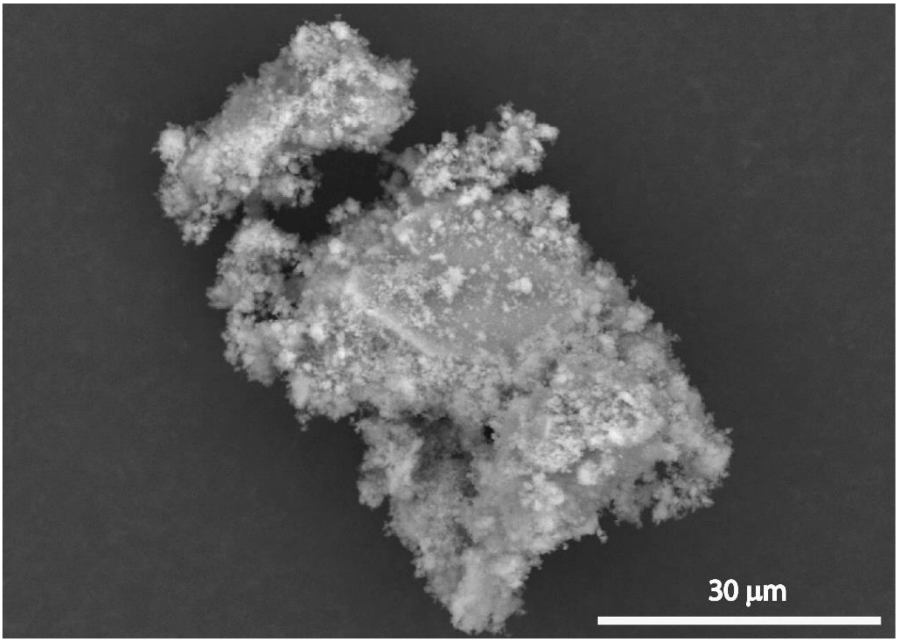

Figure 5.7. Calcium carbonate nanoparticles, synthesized by sol-gel, as seen by SEM after centrifugation and subsequent desiccation.

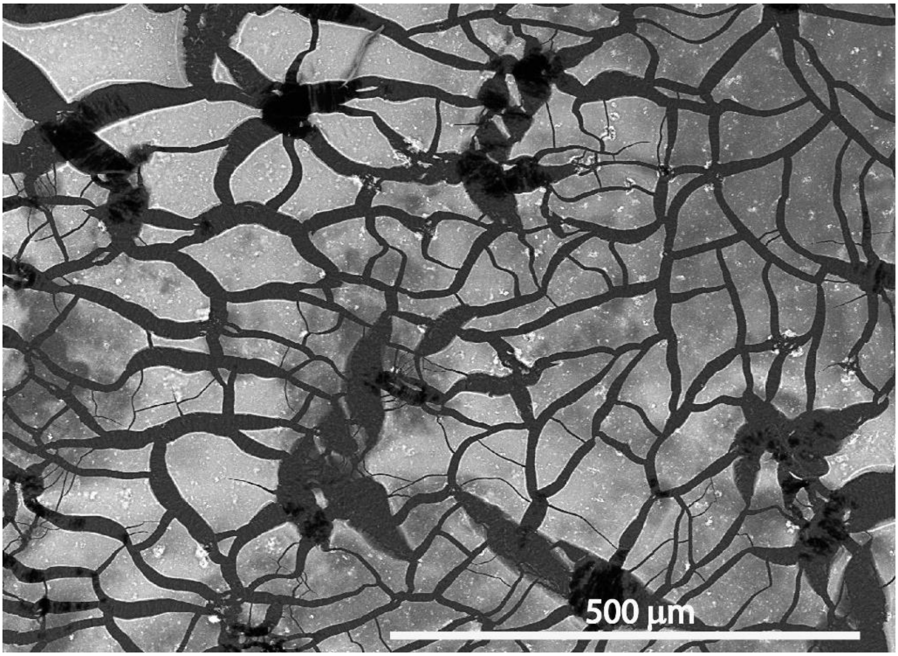

Figure 5.8. Calcium carbonate nanoparticles synthesized by sol-gel after evaporation of the mother liquor ethanol.

If all ethanol is evaporated, a fine white powder is revealed. This powder is made up of 5 nm crystallites as measured by XRD (figure 5.9) and TEM (figure 5.10).

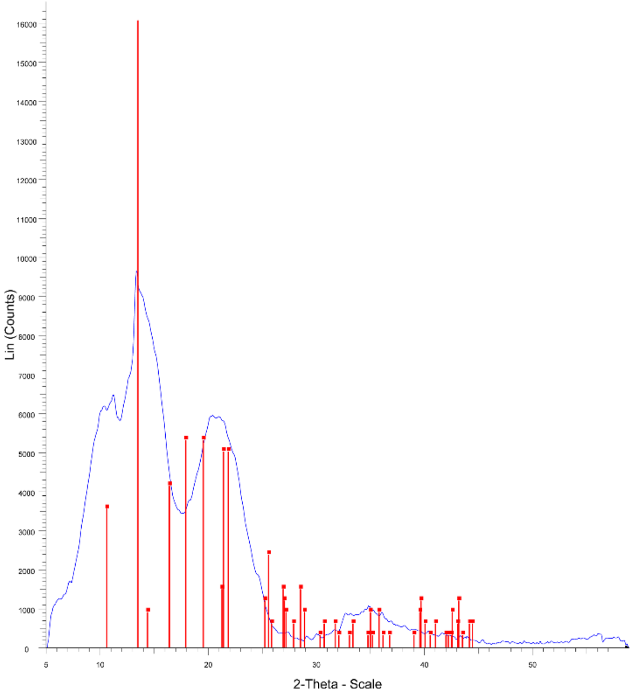

Figure 5.9. Diffractogram of calcium carbonate nanoparticles synthesised by sol-gel method. The red bars shows the reference peaks of synthesized calcite structure. The largest peak at thirteen degrees was used for Sherrer calculation which showed a crystallite size of five nm.

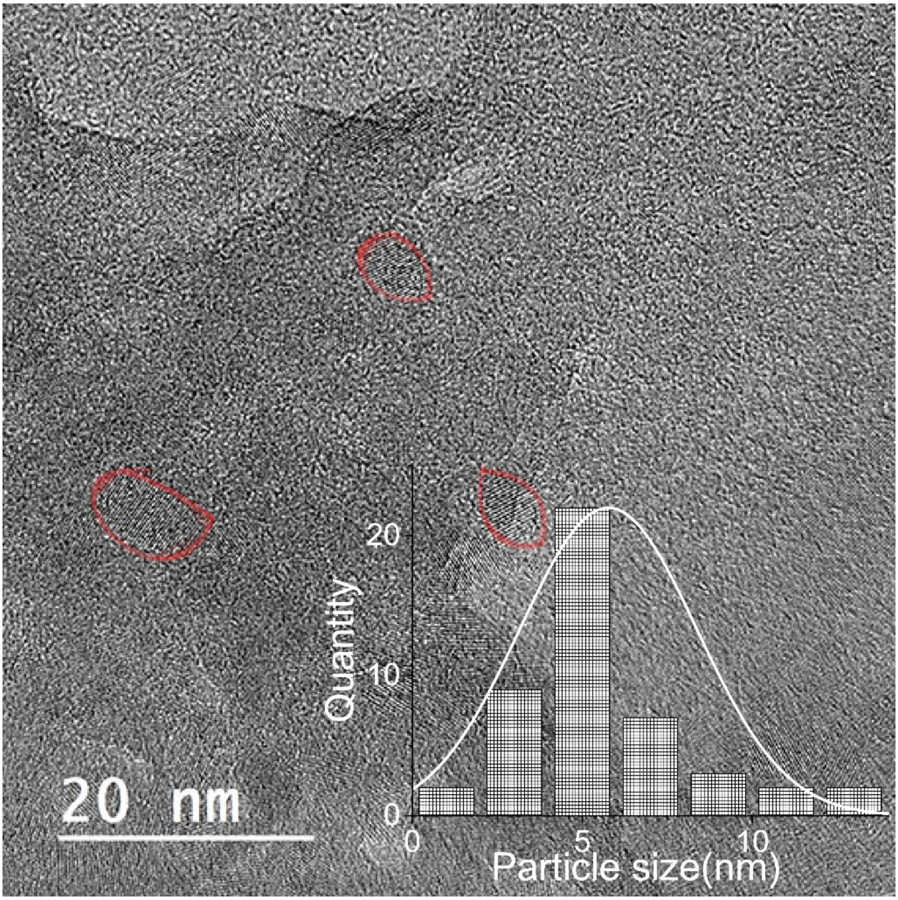

Figure 5.10. A selected TEM image of calcium carbonate nanoparticles synthesized by sol-gel method. The inset graph displays particle size distribution from several images (n:46).

Surface charge and zeta potential

Calcium carbonate nanoparticles are negatively charged particles by nature although there are some discrepancies in the literature (Moulin and Roques, 2003). A positive zeta potential is more commonly reported for calcium carbonate nanoparticles (Ulkeryildiz et al., 2016, Zhang et al., 2016). When we measured the particles synthesized by sol-gel, we found that they had a high positive ζ-potential in water around 62 mV, but there was a distribution of potential in the sample showing both negative and positive elements; figure 5.11 shows the actual counts of the different charges.

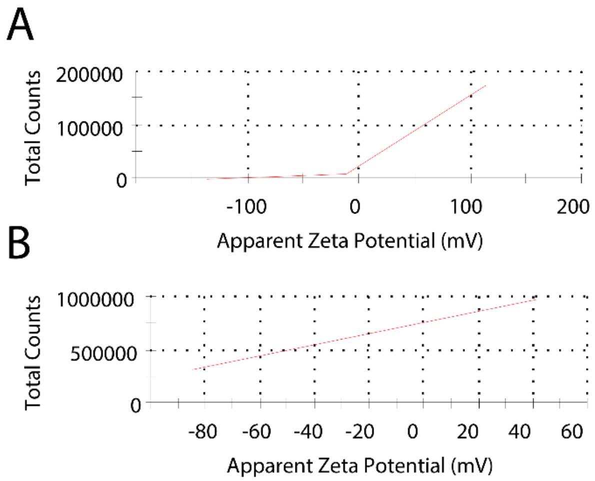

Figure 5. 11. Zeta potential of calcium carbonate nanoparticles produced *by calcium ethoxide CO_2 gas sol gel method. Panel A are the particles, as produced in ethanol suspension. Panel B are the same particles dried and resuspended in water.*

Surface area

Calcium carbonate nanoparticles can vary substantially in size and morphology. Typically 20-20000 nm in particle size and with the typical rhombohedral shape of calcite and the star shape of vaterite and many other shapes. This leads to a great variety of surface area. The particles prepared by sol gel demonstrated a surface area of 22 m^2/g that is a rather typical value for calcium carbonate particles of micron size but small for nanoparticles (Kirboga and Öner, 2017, Wang et al., 2016a).

Thermal decomposition

The thermal decomposition of sol gel calcium carbonate nanoparticles, showed an expected reduction of temperature of decomposition in TGA. In figure 5.12. these results of TGA are displayed and one can see that the calcium carbonate starts to decompose at 600° C. which is at least 100° lower than usual(Singh and Singh, 2007).

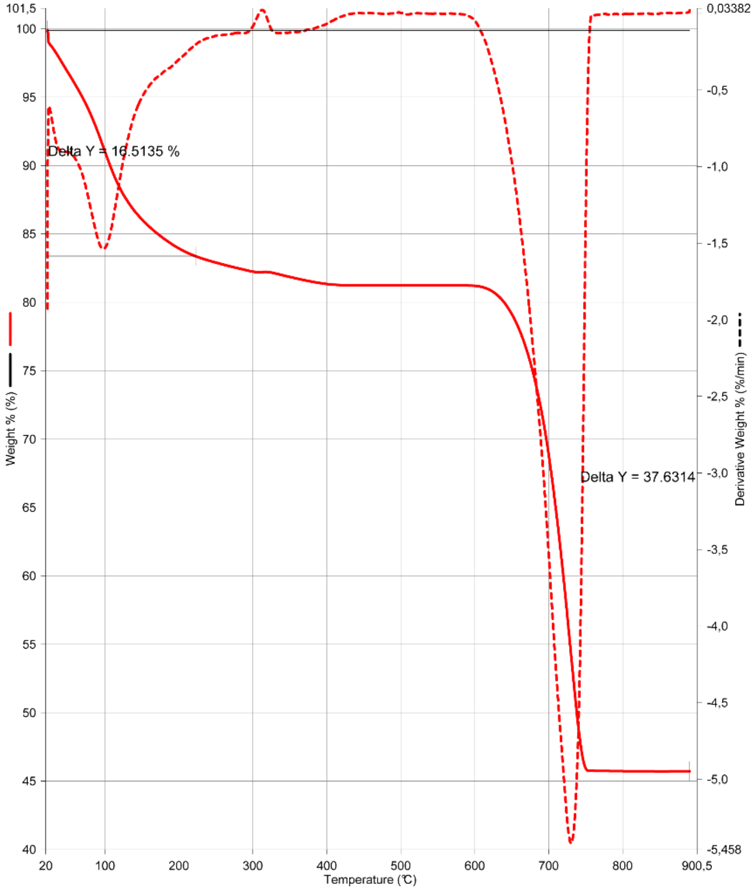

Figure 5.12. Differential and absolute weight loss graph for the decomposition of calcium carbonate synthesized by sol gel method.

5.3 Calcium carbonate hybrid materials

One interesting aspect of the sol-gel method of calcium carbonate nanoparticles synthesis is that it is easily modified to produce hybrid materials. Hybrid materials are compound materials with organic and inorganic constituents. Since the synthesis is performed in ethanol hydrophobic organic molecules can be dissolved, prior to the nucleation of the inorganic calcium carbonate. It is possible to incorporate even the most hydrophobic molecules that generally are insoluble in ethanol, by dissolving it in a less polar solvent like hexane or toluene and add it to the calcium ethoxide solution before reaction with CO_2. We have incorporated carotene, ibuprofen, tetracycline, doxorubicin, Isopropyl β-D-1-Thiogalactopyranoside into the matrix of calcium carbonate particles. The result is a powder nano composite material. According to our studies with TEM the powders consist of globular structures in the sub-micron size range, see figure 5.13.

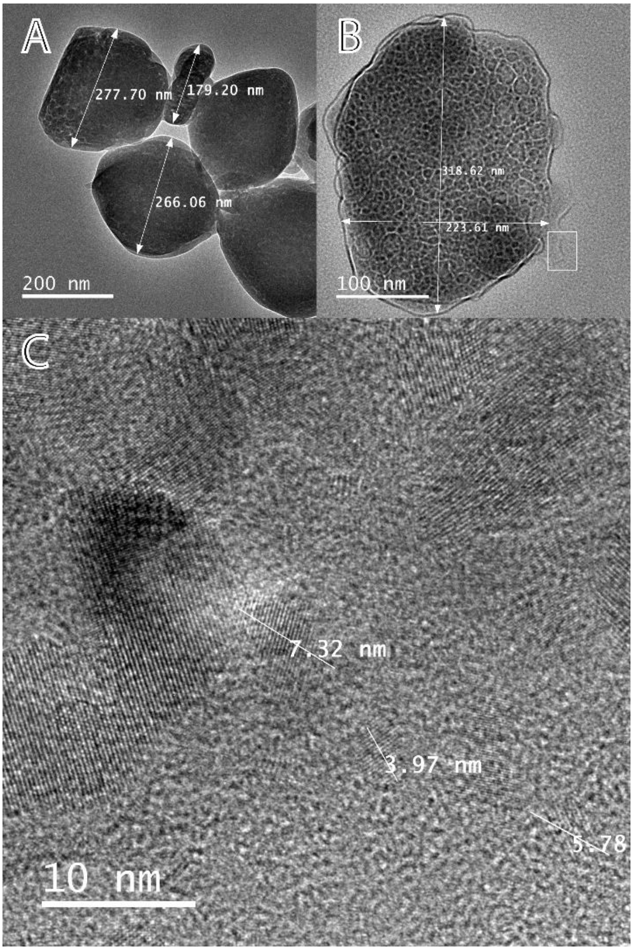

Figure 5.13. Carotene calcium carbonate nanocomposite particles as imaged with TEM. The sizes of the particles can be seen in panel A and B. The small square in panel B shows the area where panel C image is taken from. Panel C demonstrate the calcium carbonate crystallites imbedded into the hybrid material.

These materials can then be used for release of hydrophobic molecules into hydrophilic environment; control or triggering of release can be achieved by pH regulation or alternatively strong calcium ligands such as phosphate. We demonstrated the effect by releasing carotene at pH 1. The nanocomposite powder was first dispersed in the liquid. After approximately five hours one could see colloid formation and aggregation of carotene as the calcium carbonate was dissolved. After another 24 hours the carotene aggregates had begun precipitate towards the bottom (figure 5.14).

Figure 5.134. Release of carotene into acidic water from the hybrid nanocomposite powder produced with calcium carbonate sol-gel synthesis.

5.4 Interactions with plants and effect on aphid feeding

It is known that calcium ions play an important signalling role in plants innate defence system against sap sucking insects, i.e. *Hemiptera (Will et al., 2013, van Bel and Will, 2016)*. Many plant genera are known to store calcium carbonate (Bauer et al., 2011, Nitta et al., 2006, Okazaki et al., 1986, Zindler-Frank, 1995). The purpose of this behaviour has not been discerned. One possible function could be increased tolerance to biotic and abiotic stress. We have hypothesise that introduction of calcium carbonate nanoparticles into the phloem could create a latent supercharge of the calcium signalling. The signalling relies upon calcium ions coming through the cell wall as it has been ruptured by the aphid stylet. According to the latest model, the aphids tries to prevent this by three main mechanisms, i) sealing the cell wall puncture with a polymer, gel saliva, to prevent calcium ions to enter the sieve tube element from the apoplast or cell walls, ii) releasing calcium binding proteins (Will et al., 2013), iii) break down of phloem proteins (Furch et al., 2015). Hence presence of calcium carbonate nanoparticles in sieve elements should disturb mechanism two. Furthermore if it is possible to stabilize the particles with a protein corona that is broken down by mechanism three a controlled release mechanism can be created.

5.4.1 Plant responses to calcium carbonate nanoparticles

If calcium carbonate nanoparticles are to be used in agriculture to prevent aphid infestations it is important to assess the effect of the particles on the plants themselves. It can be expected that it should have if any a positive effect. Calcium is an important macro nutrient and plants need large amounts. Agricultural liming with calcium carbonate is a common practice to increase the pH of soils, which tend to acidify especially when fertilized with ammonia.

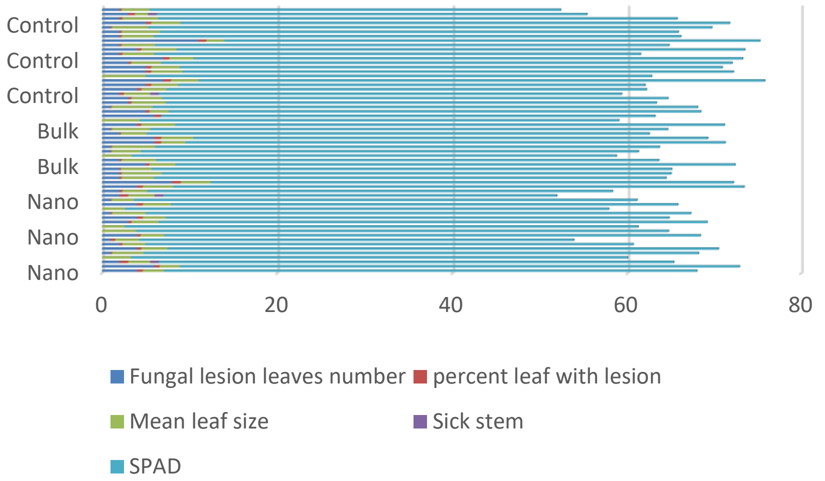

Figure 5.14. Display of plant features measured after treatment with calcium carbonate nanoparticles. Exclusively the fungal lesions had statistically significant difference compared to control and bulk control (P=0.045).

From our tests we could not see any effect on growth, chlorophyll content or morphology of the plants compared to plants fertilized with 60 mg L^{-1} calcium. No change in appearance could be perceived. The results can be seen in figure 5.11.

5.4.2 Aphid response to plants treated with calcium carbonate nanoparticles

Studying aphid feeding behaviour by electropenetrography gives detailed information about the process. It is possible to see how much time each aphid spends on certain behaviours such as penetration, salivation and actual feeding. We tried many times, with many different particles, to use this method for discovering effects of calcium carbonate nanoparticles on aphid feeding.

However, no statistically significant effects have been found. The method is far from being high through put. Our maximum sample size is eight per group and it is quite common to loose aphids during the experiment. The variation within groups are also very big, bigger than the average for some parameters. Hence, to succeed with this method a meta-analysis must be made. That combines many experiments, with the same particles, repeated over time.

Future possibilities

Since all our experiments are inconclusive we can neither confirm nor reject the hypothesis. Hence more experiments could be done to investigate this idea that could lead to a new environmentally friendly way of preventing phloem sucking pests. As mentioned, *vide supra*, the electropenetrography is an information rich method that could be used with enough repetition. However, before that, it is suggestable to investigate other parameters and interactions first: i) The particle uptake should be verified. We struggled for a long time to synthesize particles that would be smaller than the generally known barrier of approximately 20 nm. To prove their uptake turned out to be difficult with ICP-MS. A possible alternative would be to synthesize them with radioactive calcium isotopes that could be more easily detected in plant tissue. ii) The particles´ ability to adsorb calcium from solution should be measured, to be able to predict adsorption of calcium ions at the relevant concentrations, *in planta*. iii) If a protein coating should be used the characterization of the coating must be performed. Using AFM to visualize the particles and measuring young modulus to see coating versus particle core should be a possibility. Also these particles should be smaller than the uptake barrier. iiii) If particles are coated or not they must be tested for their interaction with aphid saliva. It is possible to extract aphid saliva and perform *in vitro* tests. Will the particles adsorb the saliva proteins that aphids use to bind calcium ions? Will the particles interact with the enzyme that the aphids use to break down plant defence callose?

There are many questions to address before any conclusions can be drawn upon this hypothesis. Possibly, it would require multiple PhD theses to answer these questions.

References

1997. 21 - Titanium, Zirconium and Hafnium. *Chemistry of the Elements (Second Edition).* Oxford: Butterworth-Heinemann.

AKHTAR, M. S., PANWAR, J. & YUN, Y.-S. 2013. Biogenic Synthesis of Metallic Nanoparticles by Plant Extracts. *ACS Sustainable Chemistry & Engineering,* 1**,** 591-602.

ALGHUTHAYMI, M. A., ALMOAMMAR, H., RAI, M., SAID-GALIEV, E. & ABD-ELSALAM, K. A. 2015. Myconanoparticles: synthesis and their role in phytopathogens management. *Biotechnology & Biotechnological Equipment,* 29**,** 221-236.

ANDRESEN, T. L., THOMPSON, D. H. & KAASGAARD, T. 2010. Enzyme-triggered nanomedicine: Drug release strategies in cancer therapy (Invited Review). *Molecular Membrane Biology,* 27**,** 353-363.

ASHKAVAND, P., TABARI, M., ZARAFSHAR, M., TOMÁŠKOVÁ, I. & STRUVE, D. 2015. Effect of SiO2 nanoparticles on drought resistance in hawthorn seedlings. *Forest Research Papers.*

AVELLAN, A., SCHWAB, F., MASION, A., CHAURAND, P., BORSCHNECK, D., VIDAL, V., ROSE, J., SANTAELLA, C. & LEVARD, C. 2017. Nanoparticle Uptake in Plants: Gold Nanomaterial Localized in Roots of Arabidopsis thaliana by X-ray Computed Nanotomography and Hyperspectral Imaging. *Environmental Science & Technology,* 51**,** 8682-8691.

BAALOUSHA, M., CORNELIS, G., KUHLBUSCH, T. A. J., LYNCH, I., NICKEL, C., PEIJNENBURG, W. & VAN DEN BRINK, N. W. 2016. Modeling nanomaterial fate and uptake in the environment: current knowledge and future trends. *Environmental Science: Nano,* 3**,** 323-345.

BAUER, P., ELBAUM, R. & WEISS, I. M. 2011. Calcium and silicon mineralization in land plants: Transport, structure and function. *Plant Science,* 180**,** 746-756.

BAZYLINSKI D, A., FRANKEL R, B., HEYWOOD B, R., MANN, S., KING J, W., DONAGHAY P, L. & HANSON A, K. 1995. Controlled biomineralization of magnetite (Fe?3?O?4?) and greigite (Fe?3?S?4?) in a magnetotactic bacterium. *Applied and environmental microbiology (Print),* 61**,** 3232-3239.

BERNARD, J.-P., ADRICH, Z., MONTALTO, G., DE CARO, A., DE REGGI, M., SARLES, H. & DAGORN, J.-C. Inhibition of nucleation and crystal growth of calcium carbonate by human lithostathine. *Gastroenterology,* 103**,** 1277-1284.

BERNER, R. A. 1975. Role of Magnesium in Crystal-Growth of Calcite and Aragonite from Sea-Water. *Geochimica Et Cosmochimica Acta,* 39**,** 489-&.

BISCHOFF, J. L. 1968. Kinetics of Calcite Nucleation - Magnesium Ion Inhibition and Ionic Strength Catalysis. *Journal of Geophysical Research,* 73**,** 3315-&.

BLAKEMORE, R. 1975. Magnetotactic bacteria. *Science,* 190**,** 377-379.

BOBO, D., ROBINSON, K. J., ISLAM, J., THURECHT, K. J. & CORRIE, S. R. 2016. Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date. *Pharmaceutical Research,* 33**,** 2373-2387.

BOUTONNET, M. & SANCHEZ-DOMINGUEZ, M. 2017. Microemulsion droplets to catalytically active nanoparticles. How the application of colloidal tools in catalysis aims to well designed and efficient catalysts. *Catalysis Today,* 285**,** 89-103.

BUSTIN, S. A., BENES, V., GARSON, J. A., HELLEMANS, J., HUGGETT, J., KUBISTA, M., MUELLER, R., NOLAN, T., PFAFFL, M. W., SHIPLEY, G. L., VANDESOMPELE, J. & WITTWER, C. T. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry,* 55**,** 611-622.

BYRAPPA, K. & YOSHIMURA, M. 2001. 1 - Hydrothermal Technology—Principles and Applications. *Handbook of Hydrothermal Technology.* Norwich, NY: William Andrew Publishing.

CAPEK, I. 2015. Introduction. *DNA Engineered Noble Metal Nanoparticles.* John Wiley & Sons, Inc.

CASTILLO-MICHEL, H. A., LARUE, C., PRADAS DEL REAL, A. E., COTTE, M. & SARRET, G. 2017. Practical review on the use of synchrotron based micro- and nano- X-ray fluorescence mapping and X-ray absorption spectroscopy to investigate the interactions between plants and engineered nanomaterials. *Plant Physiology and Biochemistry,* 110**,** 13-32.

CEDERVALL, T., LYNCH, I., LINDMAN, S., BERGGÅRD, T., THULIN, E., NILSSON, H., DAWSON, K. A. & LINSE, S. 2007. Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proceedings of the National Academy of Sciences,* 104**,** 2050-2055.

CHA, W., CHIN, S., PARK, E., YUN, S.-T. & JURNG, J. 2014. Photocatalytic performance of V2O5/TiO2 materials prepared by chemical vapor condensation and impregnation method under visible-light. *Powder Technology,* 258**,** 352-357.

CHANDRA, S., CHAKRABORTY, N., DASGUPTA, A., SARKAR, J., PANDA, K. & ACHARYA, K. 2015. Chitosan nanoparticles: A positive modulator of innate immune responses in plants. *Scientific Reports,* 5, 15195.

CHEESEMAN, J. M. 2006. Hydrogen peroxide concentrations in leaves under natural conditions. *Journal of Experimental Botany,* 57, 2435-2444.

CHEN, C. J. 2007. - Introduction to Scanning Tunneling Microscopy: Second Edition.

CHEN, H. & YADA, R. 2011. Nanotechnologies in agriculture: New tools for sustainable development. *Trends in Food Science & Technology,* 22, 585-594.

CHEN, Z., YIN, J.-J., ZHOU, Y.-T., ZHANG, Y., SONG, L., SONG, M., HU, S. & GU, N. 2012. Dual Enzyme-like Activities of Iron Oxide Nanoparticles and Their Implication for Diminishing Cytotoxicity. *ACS Nano,* 6, 4001-4012.

CHIN, S. F., AZMAN, A. & PANG, S. C. 2014. Size controlled synthesis of starch nanoparticles by a microemulsion method. *Journal of Nanomaterials,* 2014.

CHOI, C. J., TOLOCHKO, O. & KIM, B. K. 2002. Preparation of iron nanoparticles by chemical vapor condensation. *Materials Letters,* 56, 289-294.

COLOMBO, C., PALUMBO, G., HE, J.-Z., PINTON, R. & CESCO, S. 2014. Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. *Journal of Soils and Sediments,* 14, 538-548.

CORREDOR, C., BORYSIAK, M. D., WOLFER, J., WESTERHOFF, P. & POSNER, J. D. 2015. Colorimetric Detection of Catalytic Reactivity of Nanoparticles in Complex Matrices. *Environmental Science & Technology,* 49, 3611-3618.

CROS, F., KORB, J. P. & MALIER, L. 2000. Spectroscopic Mesopore Size Characterization and Diffusion Measurement in Closed Porosity by Xenon NMR. *Langmuir,* 16, 10193-10197.

CUI, H., LIU, Y. & REN, W. 2013a. Structure switch between α-Fe2O3, γ-Fe2O3 and Fe3O4 during the large scale and low temperature sol–gel synthesis of nearly monodispersed iron oxide nanoparticles. *Advanced Powder Technology,* 24, 93-97.

CUI, H., YANG, G. C., JIANG, J., ZHANG, P. & GU, W. 2013b. Biological effects of PAS TiO2 sol on disease control and photosynthesis in cucumber (Cucumis sativus L.). *Australian Journal of Crop Science,* 7, 99-103.

CUSHING, B. L., KOLESNICHENKO, V. L. & O'CONNOR, C. J. 2004. Recent Advances in the Liquid-Phase Syntheses of Inorganic Nanoparticles. *Chemical Reviews,* 104, 3893-3946.

DAUTHAL, P. & MUKHOPADHYAY, M. 2016. Noble Metal Nanoparticles: Plant-Mediated Synthesis, Mechanistic Aspects of Synthesis, and Applications. *Industrial & Engineering Chemistry Research,* 55, 9557-9577.

DAVAR ZAREII, F., ROOZBAHANI, A. & HOSNAMIDI, A. 2014. Evaluation the Effect of Water Stress and Foliar Application of Fe Nanoparticles on

Yield, Yield Components and Oil Percentage of Safflower (Carthamus Tinctorious L.). *International Journal of Advanced Biological and Biomedical Research,* 2**,** 1150-1159.

DE WINDT, W., AELTERMAN, P. & VERSTRAETE, W. 2005. Bioreductive deposition of palladium (0) nanoparticles on Shewanella oneidensis with catalytic activity towards reductive dechlorination of polychlorinated biphenyls. *Environmental Microbiology,* 7**,** 314-325.

DONATE-ROBLES, J., LIAUW, C. M. & MARTÍN-MARTÍNEZ, J. M. 2014. Flow micro-calorimetry and diffuse reflectance Fourier transform infrared spectroscopy studies in filled polyurethane adhesives by using dimethyl adipate as a model compound. *International Journal of Adhesion and Adhesives,* 48**,** 43-50.

EBELMEN, J.-J. 1846. *Annalen,* 57.

EVELEENS, C. A. & PAGE, A. J. 2017. Effect of ammonia on chemical vapour deposition and carbon nanotube nucleation mechanisms. *Nanoscale,* 9**,** 1727-1737.

FEDOTOV, G. N., BYSTROVA, O. N. & MARTYNKINA, E. A. 2009. On the nanostructural organization of soils. *Doklady Chemistry,* 425**,** 69-72.

FEDOTOV, G. N., PUTLYAEV, V. I., RUDOMETKINA, T. F. & ITKIS, D. M. 2008. Nanostructural Organization of Soils. *Doklady Chemistry,* 422**,** 265-268.

FEDOTOV, G. N. & RUDOMETKINA, T. F. 2011. Effects of Nanostructural Organization of Soil Gels on the Fractional Composition of Soil Humic Substances. *Doklady Chemistry,* 439**,** 183-186.

FEIZI, H., KAMALI, M., JAFARI, L. & REZVANI MOGHADDAM, P. 2013. Phytotoxicity and stimulatory impacts of nanosized and bulk titanium dioxide on fennel (Foeniculum vulgare Mill). *Chemosphere,* 91**,** 506-511.

FILEZ, M., POELMAN, H., RAMACHANDRAN, R. K., DENDOOVEN, J., DEVLOO-CASIER, K., FONDA, E., DETAVERNIER, C. & MARIN, G. B. 2014. In situ XAS and XRF study of nanoparticle nucleation during O3-based Pt deposition. *Catalysis Today,* 229**,** 2-13.

FURCH, A. C. U., VAN BEL, A. J. E. & WILL, T. 2015. Aphid salivary proteases are capable of degrading sieve-tube proteins. *Journal of Experimental Botany,* 66**,** 533-539.

GAL, A. & NUSSINOVITCH, A. 2007. Hydrocolloid Carriers with Filler Inclusionfor Diltiazem Hydrochloride Release. *Journal of Pharmaceutical Sciences,* 96**,** 168-178.

GAL, J.-Y., BOLLINGER, J.-C., TOLOSA, H. & GACHE, N. 1996. Calcium carbonate solubility: a reappraisal of scale formation and inhibition. *Talanta,* 43**,** 1497-1509.

GARDEA-TORRESDEY, J. L., RODRIGUEZ, E., PARSONS, J. G., PERALTA-VIDEA, J. R., MEITZNER, G. & CRUZ-JIMENEZ, G. 2005. Use of ICP and XAS to determine the enhancement of gold phytoextraction by Chilopsis linearis using thiocyanate as a complexing agent. *Analytical and Bioanalytical Chemistry,* 382**,** 347-352.

GERBAUD, V., PIGNOL, D., LORET, E., BERTRAND, J. A., BERLAND, Y., FONTECILLA-CAMPS, J.-C., CANSELIER, J.-P., GABAS, N. &

VERDIER, J.-M. 2000. Mechanism of Calcite Crystal Growth Inhibition by the N-terminal Undecapeptide of Lithostathine. *Journal of Biological Chemistry,* 275**,** 1057-1064.

GHAFARIYAN, M. H., MALAKOUTI, M. J., DADPOUR, M. R., STROEVE, P. & MAHMOUDI, M. 2013. Effects of Magnetite Nanoparticles on Soybean Chlorophyll. *Environmental Science & Technology,* 47**,** 10645-10652.

GHAZI HARSINI, M., HABIBI, H. & HOSEIN TALAEI, G. 2014. *Effect of nano iron foliar application on quantitative characteristics of new line of wheat*.

GLOVER, R. D., MILLER, J. M. & HUTCHISON, J. E. 2011. Generation of Metal Nanoparticles from Silver and Copper Objects: Nanoparticle Dynamics on Surfaces and Potential Sources of Nanoparticles in the Environment. *ACS Nano,* 5**,** 8950-8957.

GRAYSTON, S. J., VAUGHAN, D. & JONES, D. 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology,* 5**,** 29-56.

GRAŻYNA BYSTRZEJEWSKA-PIOTROWSKA, M. A., ROMUALD STĘBOROWSKI, HALINA POLKOWSKA-MOTRENKO, BOŻENA DANKO, JUSTYNA RYNIEWICZ 2012. Application of neutron activation for investigation of Fe3O4 nanoparticles accumulation by plants. *Nukleonika,* 57**,** 427.

GRILLO, R., ROSA, A. H. & FRACETO, L. F. 2015. Engineered nanoparticles and organic matter: A review of the state-of-the-art. *Chemosphere,* 119**,** 608-619.

GRÄFE, C., WEIDNER, A., LÜHE, M. V. D., BERGEMANN, C., SCHACHER, F. H., CLEMENT, J. H. & DUTZ, S. 2016. Intentional formation of a protein corona on nanoparticles: Serum concentration affects protein corona mass, surface charge, and nanoparticle–cell interaction. *The International Journal of Biochemistry & Cell Biology,* 75**,** 196-202.

GUO, H. & BARNARD, A. S. 2013. Naturally occurring iron oxide nanoparticles: morphology, surface chemistry and environmental stability. *Journal of Materials Chemistry A,* 1**,** 27-42.

GUPTA, S. M. & TRIPATHI, M. 2012. A review on the synthesis of TiO2 nanoparticles by solution route. *Central European Journal of Chemistry,* 10**,** 279-294.

H.M., U. N. C. R. 1988. The role of root exudates in nutrient acquisition. *Advances in Plant Nutrition,* 3**,** 79-114.

HAN, D. Y., WANG, C. Q., LI, D. D. & CAO, Z. B. 2016. NiO/ZnO Core-Shell Nanoparticles In Situ Synthesis Via Microemulsion Method. *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry,* 46**,** 794-797.

HARSH P. BAIS, T. L. W., LAURA G. PERRY, SIMON GILROY, AND JORGE M.VIVANCO 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology,* 57**,** 233-266.

HAVERKAMP, R. G. & MARSHALL, A. T. 2009. The mechanism of metal nanoparticle formation in plants: limits on accumulation. *Journal of Nanoparticle Research,* 11**,** 1453-1463.

HE, H. & GAO, F. 2017. Resin modification on interlaminar shear property of carbon fiber/epoxy/nano-CaCO3 hybrid composites. *Polymer Composites,* 38**,** 2035-2042.

HENAM SYLVIA, D., HENAM PREMANANDA, S. & THIYAM DAVID, S. 2017. Green synthesized urchin like Pt/Cu bimetallic photonanocatalysts: Understanding composition effect. *Materials Research Bulletin,* 86**,** 277-286.

HENDREN, C. O., MESNARD, X., DRÖGE, J. & WIESNER, M. R. 2011. Estimating Production Data for Five Engineered Nanomaterials As a Basis for Exposure Assessment. *Environmental Science & Technology,* 45**,** 2562-2569.

HIRSCH, O., KVASHNINA, K. O., LUO, L., SÜESS, M. J., GLATZEL, P. & KOZIEJ, D. 2015. High-energy resolution X-ray absorption and emission spectroscopy reveals insight into unique selectivity of La-based nanoparticles for CO2. *Proceedings of the National Academy of Sciences,* 112**,** 15803-15808.

HOCHELLA, M. F., SPENCER, M. G. & JONES, K. L. 2015. Nanotechnology: nature's gift or scientists' brainchild? *Environmental Science: Nano,* 2**,** 114-119.

HOLDEN, P. A., KLAESSIG, F., TURCO, R. F., PRIESTER, J. H., RICO, C. M., AVILA-ARIAS, H., MORTIMER, M., PACPACO, K. & 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.

HOWARD, D. P., MARCHAND, P., MCCAFFERTY, L., CARMALT, C. J., PARKIN, I. P. & DARR, J. A. 2017. High-Throughput Continuous Hydrothermal Synthesis of Transparent Conducting Aluminum and Gallium Co-doped Zinc Oxides. *ACS Combinatorial Science,* 19**,** 239-245.

IRAVANI, S. 2014. Bacteria in Nanoparticle Synthesis: Current Status and Future Prospects. *International Scholarly Research Notices,* 2014**,** 18.

JAIN, A., BALASUBRAMANIAN, R. & SRINIVASAN, M. P. 2016. Hydrothermal conversion of biomass waste to activated carbon with high porosity: A review. *Chemical Engineering Journal,* 283**,** 789-805.

JAOUDE, M. A., RANDON, J., BORDES, C., LANTERI, P. & BOIS, L. 2012. A design of experiment approach to the sol-gel synthesis of titania monoliths for chromatographic applications. *Analytical and Bioanalytical Chemistry,* 403**,** 1145-1155.

JAYASEELAN, C., RAHUMAN, A. A., KIRTHI, A. V., MARIMUTHU, S., SANTHOSHKUMAR, T., BAGAVAN, A., GAURAV, K., KARTHIK, L. & RAO, K. V. B. 2012. Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy,* 90**,** 78-84.

JEEVANANDAM, J., CHAN, Y. S. & DANQUAH, M. K. 2016. Biosynthesis of Metal and Metal Oxide Nanoparticles. *ChemBioEng Reviews,* 3**,** 55-67.

JIANG, J., MA, Y., ZHANG, T., LIANG, Z. & CUI, Z. 2015. Morphology and size control of calcium carbonate crystallized in a reverse micelle system with switchable surfactants. *RSC Advances,* 5**,** 80216-80219.

JOHNSON, E. B. G. & ARSHAD, S. E. 2014. Hydrothermally synthesized zeolites based on kaolinite: A review. *Applied Clay Science,* 97–98**,** 215-221.

JUHEL, G., BATISSE, E., HUGUES, Q., DALY, D., VAN PELT, F. N. A. M., O'HALLORAN, J. & JANSEN, M. A. K. 2011. Alumina nanoparticles enhance growth of Lemna minor. *Aquatic Toxicology,* 105**,** 328-336.

KAH, M. 2015. Nanopesticides and Nanofertilizers: Emerging Contaminants or Opportunities for Risk Mitigation? *Frontiers in Chemistry,* 3**,** 64.

KAH, M., BEULKE, S., TIEDE, K. & HOFMANN, T. 2012. Nanopesticides: State of Knowledge, Environmental Fate, and Exposure Modeling. *Critical Reviews in Environmental Science and Technology,* 43**,** 1823-1867.

KARAKAŞ, F., VAZIRI HASSAS, B. & ÇELIK, M. S. 2015. Effect of precipitated calcium carbonate additions on waterborne paints at different pigment volume concentrations. *Progress in Organic Coatings,* 83**,** 64-70.

KARBALAEI AKBARI, M., DERAKHSHAN, R. & MIRZAEE, O. 2015. A case study in vapor phase synthesis of Mg–Al alloy nanoparticles by plasma arc evaporation technique. *Chemical Engineering Journal,* 259**,** 918-926.

KASAI, T. & BHUSHAN, B. 2008. Applications of Scanning Probe Methods in Chemical Mechanical Planarization. *In:* BHUSHAN, B., TOMITORI, M. & FUCHS, H. (eds.) *Applied Scanning Probe Methods X: Biomimetics and Industrial Applications.* Berlin, Heidelberg: Springer Berlin Heidelberg.

KHODAKOVSKAYA, M. V., KIM, B.-S., KIM, J. N., ALIMOHAMMADI, M., DERVISHI, E., MUSTAFA, T. & CERNIGLA, C. E. 2013. Carbon Nanotubes as Plant Growth Regulators: Effects on Tomato Growth, Reproductive System, and Soil Microbial Community. *Small,* 9**,** 115-123.

KIRBOGA, S. & ÖNER, M. 2017. Investigating the effect of ultrasonic irradiation on synthesis of calcium carbonate using Box-Behnken experimental design. *Powder Technology,* 308**,** 442-450.

KLAUS, T., JOERGER, R., OLSSON, E. & GRANQVIST, C. G. 1999. Silver-based crystalline nanoparticles, microbially fabricated. *Proceedings of the National Academy of Sciences of the United States of America,* 96**,** 13611-13614.

KOŁODZIEJCZAK-RADZIMSKA, A. & JESIONOWSKI, T. 2014. Zinc Oxide—From Synthesis to Application: A Review. *Materials,* 7**,** 2833.

KONISHI, Y., TSUKIYAMA, T., OHNO, K., SAITOH, N., NOMURA, T. & NAGAMINE, S. 2006. Intracellular recovery of gold by microbial reduction of AuCl 4 - ions using the anaerobic bacterium Shewanella algae. *Hydrometallurgy,* 81**,** 24-29.

KUMAR, V., GULERIA, P., KUMAR, V. & YADAV, S. K. 2013. Gold nanoparticle exposure induces growth and yield enhancement in Arabidopsis thaliana. *Sci Total Environ,* 461–462.

KUO, C. L. & HWANG, K. C. 2013. Does Morphology of a Metal Nanoparticle Play a Role in Ostwald Ripening Processes? *Chemistry of Materials,* 25**,** 365-371.

KWON, S. G. & HYEON, T. 2011. Formation Mechanisms of Uniform Nanocrystals via Hot-Injection and Heat-Up Methods. *Small,* 7**,** 2685-2702.

LABORDA, F., BOLEA, E. & JIMÉNEZ-LAMANA, J. 2014. Single Particle Inductively Coupled Plasma Mass Spectrometry: A Powerful Tool for Nanoanalysis. *Analytical Chemistry,* 86**,** 2270-2278.

LEE, H., PURDON, A. M., CHU, V. & WESTERVELT, R. M. 2004. Controlled assembly of magnetic nanoparticles from magnetotactic bacteria using microelectromagnets arrays. *Nano Letters,* 4**,** 995-998.

LEE, Y.-R., KIM, J. & AHN, W.-S. 2013. Synthesis of metal-organic frameworks: A mini review. *Korean Journal of Chemical Engineering,* 30**,** 1667-1680.

LI, J., HU, J., MA, C., WANG, Y., WU, C., HUANG, J. & XING, B. 2016a. Uptake, translocation and physiological effects of magnetic iron oxide (γ-Fe2O3) nanoparticles in corn (Zea mays L.). *Chemosphere,* 159**,** 326-334.

LI, J., HU, J., XIAO, L., GAN, Q. & WANG, Y. 2017. Physiological Effects and Fluorescence Labeling of Magnetic Iron Oxide Nanoparticles on Citrus (Citrus reticulata) Seedlings. *Water, Air, & Soil Pollution,* 228**,** 52.

LI, T., SENESI, A. J. & LEE, B. 2016b. Small Angle X-ray Scattering for Nanoparticle Research. *Chemical Reviews,* 116**,** 11128-11180.

LIU, N., CHEN, X., ZHANG, J. & SCHWANK, J. W. 2014. A review on TiO2-based nanotubes synthesized via hydrothermal method: Formation mechanism, structure modification, and photocatalytic applications. *Catalysis Today,* 225**,** 34-51.

LONG, X., MA, Y. & QI, L. 2014. Biogenic and synthetic high magnesium calcite – A review. *Journal of Structural Biology,* 185**,** 1-14.

LÓPEZ-MORENO, M. L., DE LA ROSA, G., HERNÁNDEZ-VIEZCAS, J. A., PERALTA-VIDEA, J. R. & GARDEA-TORRESDEY, J. L. 2010. X-ray Absorption Spectroscopy (XAS) Corroboration of the Uptake and Storage of CeO2 Nanoparticles and Assessment of Their Differential Toxicity in Four Edible Plant Species. *Journal of Agricultural and Food Chemistry,* 58**,** 3689-3693.

LÓPEZ-QUINTELA, M. A. 2003. Synthesis of nanomaterials in microemulsions: formation mechanisms and growth control. *Current Opinion in Colloid & Interface Science,* 8**,** 137-144.

LÓPEZ-QUINTELA, M. A. & RIVAS, J. 1993. Chemical Reactions in Microemulsions: A Powerful Method to Obtain Ultrafine Particles. *Journal of Colloid and Interface Science,* 158**,** 446-451.

LOTFI, R. & KHORRAMI, S. A. 2016. Calcination temperature and molar ratio effects of nanosized CeO2 and La2O3/γ-Al2O3 synthesized within reverse micelles of surfactant/cyclohexane/water microemulsion. *Journal of Ceramic Processing Research,* 17**,** 942-946.

LUNDQVIST, M., STIGLER, J., CEDERVALL, T., BERGGÅRD, T., FLANAGAN, M. B., LYNCH, I., ELIA, G. & DAWSON, K. 2011. The Evolution of the Protein Corona around Nanoparticles: A Test Study. *ACS Nano,* 5**,** 7503-7509.

MACWAN, D. P., DAVE, P. N. & CHATURVEDI, S. 2011. A review on nano-TiO2 sol–gel type syntheses and its applications. *Journal of Materials Science,* 46**,** 3669-3686.

MADRAS, G. & MCCOY, B. J. 2002a. Denucleation rates during Ostwald ripening: Distribution kinetics of unstable clusters. *The Journal of Chemical Physics,* 117**,** 6607-6613.

MADRAS, G. & MCCOY, B. J. 2002b. Ostwald ripening with size-dependent rates: Similarity and power-law solutions. *The Journal of Chemical Physics,* 117**,** 8042-8049.

MAICU, M., SCHMITTGENS, R., HECKER, D., GLÖß, D., FRACH, P. & GERLACH, G. 2014. Synthesis and deposition of metal nanoparticles by gas condensation process. *Journal of Vacuum Science & Technology A: Vacuum, Surfaces, and Films,* 32**,** 02B113.

MANCEAU, A., NAGY, K. L., MARCUS, M. A., LANSON, M., GEOFFROY, N., JACQUET, T. & KIRPICHTCHIKOVA, T. 2008. Formation of metallic copper nanoparticles at the soil-root interface. *Environmental Science and Technology,* 42**,** 1766-1772.

MARCHESAN, S. & PRATO, M. 2013. Nanomaterials for (Nano)medicine. *ACS Medicinal Chemistry Letters,* 4**,** 147-149.

MARTÍNEZ-FERNÁNDEZ, D., BARROSO, D. & KOMÁREK, M. 2016. Root water transport of Helianthus annuus L. under iron oxide nanoparticle exposure. *Environmental Science and Pollution Research,* 23**,** 1732-1741.

MASTHOFF, I.-C., KRAKEN, M., MENZEL, D., LITTERST, F. J. & GARNWEITNER, G. 2016. Study of the growth of hydrophilic iron oxide nanoparticles obtained via the non-aqueous sol–gel method. *Journal of Sol-Gel Science and Technology,* 77**,** 553-564.

MATTIELLO, A., FILIPPI, A., POŠĆIĆ, F., MUSETTI, R., SALVATICI, M. C., GIORDANO, C., VISCHI, M., BERTOLINI, A. & MARCHIOL, L. 2015. Evidence of Phytotoxicity and Genotoxicity in Hordeum vulgare L. Exposed to CeO2 and TiO2 Nanoparticles. *Frontiers in Plant Science,* 6.

MCGIVNEY, E., HAN, L., AVELLAN, A., VANBRIESEN, J. & GREGORY, K. B. 2017. Disruption of Autolysis in Bacillus subtilis using TiO2 Nanoparticles. 7**,** 44308.

MCKEE, M. S. & FILSER, J. 2016. Impacts of metal-based engineered nanomaterials on soil communities. *Environmental Science: Nano,* 3**,** 506-533.

MENCH, M. & MARTIN, E. 1991. Mobilization of cadmium and other metals from two soils by root exudates of Zea mays L., Nicotiana tabacum L. and Nicotiana rustica L. *Plant and Soil,* 132**,** 187-196.

MISELJIC, M. & OLSEN, S. 2014. Life-cycle assessment of engineered nanomaterials: a literature review of assessment status. *Journal of Nanoparticle Research,* 16**,** 1-33.

MONREAL, C. & SCHNITZER, M. 2008. Soil Organic Matter in Nano-Composite and Clay Fractions, and Soluble Pools of the Rhizosphere. *Revista de la ciencia del suelo y nutrición vegetal,* 8**,** 48-55.

MOORE, J. & CERASOLI, E. 2010. Particle Light Scattering Methods and Applications A2 - Lindon, John C. *Encyclopedia of Spectroscopy and Spectrometry (Second Edition).* Oxford: Academic Press.

MORAN, J. F., BECANA, M., ITURBE-ORMAETXE, I., FRECHILLA, S., KLUCAS, R. V. & APARICIO-TEJO, P. 1994. Drought induces oxidative stress in pea plants. *Planta,* 194**,** 346-352.

MOULIN, P. & ROQUES, H. 2003. Zeta potential measurement of calcium carbonate. *Journal of Colloid and Interface Science,* 261**,** 115-126.

MSUYA, F. A., BROOKS, R. R. & ANDERSON, C. W. N. 2000. Chemically-induced uptake of gold by root crops: Its significance for phytomining. *Gold Bulletin,* 33**,** 134-137.

MUDANYALI, O., MCLEOD, E., LUO, W., GREENBAUM, A., COSKUN, A. F., HENNEQUIN, Y., ALLIER, C. P. & OZCAN, A. 2013. Wide-field optical detection of nanoparticles using on-chip microscopy and self-assembled nanolenses. *Nat Photon,* 7**,** 240-247.

NICOLAS, M., NDOUR, M., KA, O., D'ANNA, B. & GEORGE, C. 2009. Photochemistry of Atmospheric Dust: Ozone Decomposition on Illuminated Titanium Dioxide. *Environmental Science & Technology,* 43**,** 7437-7442.

NITTA, I., KIDA, A., FUJIBAYASHI, Y., KATAYAMA, H. & SUGIMURA, Y. 2006. Calcium carbonate deposition in a cell wall sac formed in mulberry idioblasts. *Protoplasma,* 228**,** 201-208.

NYPELÖ, T., ÖSTERBERG, M. & LAINE, J. 2011. Tailoring Surface Properties of Paper Using Nanosized Precipitated Calcium Carbonate Particles. *ACS Applied Materials & Interfaces,* 3**,** 3725-3731.

OKAZAKI, M., SETOGUCHI, H., AOKI, H. & SUGA, S. 1986. Application of soft X-ray microradiography to observation of cystoliths in the leaves of various higher plants. *The botanical magazine = Shokubutsu-gaku-zasshi,* 99**,** 281-287.

OREMLAND, R. S., HERBEL, M. J., BLUM, J. S., LANGLEY, S., BEVERIDGE, T. J., AJAYAN, P. M., SUTTO, T., ELLIS, A. V. & CURRAN, S. 2004. Structural and Spectral Features of Selenium Nanospheres Produced by Se-Respiring Bacteria. *Applied and Environmental Microbiology,* 70**,** 52-60.

OSKAM, G., HU, Z., PENN, R. L., PESIKA, N. & SEARSON, P. C. 2002. Coarsening of metal oxide nanoparticles. *Physical Review E,* 66**,** 011403.

PALMQVIST, N. G. M., BEJAI, S., MEIJER, J., SEISENBAEVA, G. A. & KESSLER, V. G. 2015. Nano titania aided clustering and adhesion of beneficial bacteria to plant roots to enhance crop growth and stress management. *Scientific Reports,* 5**,** 10146.

PALMQVIST, N. G. M., NEDELEC, J.-M., SEISENBAEVA, G. A. & KESSLER, V. G. Controlling nucleation and growth of nano-CaCO3 via CO2 sequestration by a calcium alkoxide solution to produce nanocomposites for drug delivery applications. *Acta Biomaterialia*.

PEI, Z. F., MING, D. F., LIU, D., WAN, G. L., GENG, X. X., GONG, H. J. & ZHOU, W. J. 2010. Silicon Improves the Tolerance to Water-Deficit Stress Induced by Polyethylene Glycol in Wheat (Triticum aestivum L.) Seedlings. *Journal of Plant Growth Regulation,* 29**,** 106-115.

PELCLOVA, D., ZDIMAL, V., KACER, P., ZIKOVA, N., KOMARC, M., FENCLOVA, Z., VLCKOVA, S., SCHWARZ, J., MAKEŠ, O., SYSLOVA, K., NAVRATIL, T., TURCI, F., CORAZZARI, I., ZAKHAROV, S. & BELLO, D. 2017. Markers of lipid oxidative damage in the exhaled breath condensate of nano TiO2 production workers. *Nanotoxicology,* 11**,** 52-63.

PIEKARSKA, K., SOWINSKI, P., PIORKOWSKA, E., HAQUE, M. M. U. & PRACELLA, M. 2016. Structure and properties of hybrid PLA nanocomposites with inorganic nanofillers and cellulose fibers. *Composites Part A: Applied Science and Manufacturing,* 82**,** 34-41.

PINKSE, M. W. H., UITTO, P. M., HILHORST, M. J., OOMS, B. & HECK, A. J. R. 2004. Selective Isolation at the Femtomole Level of Phosphopeptides from Proteolytic Digests Using 2D-NanoLC-ESI-MS/MS and Titanium Oxide Precolumns. *Analytical Chemistry,* 76**,** 3935-3943.

PRADAS DEL REAL, A. E., CASTILLO-MICHEL, H., KAEGI, R., SINNET, B., MAGNIN, V., FINDLING, N., VILLANOVA, J., CARRIÈRE, M., SANTAELLA, C., FERNÁNDEZ-MARTíNEZ, A., LEVARD, C. & SARRET, G. 2016. Fate of Ag-NPs in Sewage Sludge after Application on Agricultural Soils. *Environmental Science & Technology,* 50**,** 1759-1768.

PRIETO, P., NISTOR, V., NOUNEH, K., OYAMA, M., ABD-LEFDIL, M. & DÍAZ, R. 2012. XPS study of silver, nickel and bimetallic silver–nickel nanoparticles prepared by seed-mediated growth. *Applied Surface Science,* 258**,** 8807-8813.

RAE CHO, K., KIM, Y.-Y., YANG, P., CAI, W., PAN, H., KULAK, A. N., LAU, J. L., KULSHRESHTHA, P., ARMES, S. P., MELDRUM, F. C. & DE YOREO, J. J. 2016. Direct observation of mineral-organic composite formation reveals occlusion mechanism. *Nature communications* [Online], 7. Available: http://europepmc.org/abstract/MED/26732046
http://europepmc.org/articles/PMC4729825?pdf=render
http://europepmc.org/articles/PMC4729825
https://doi.org/10.1038/ncomms10187 [Accessed 2016/01//].

RAHMAN, I. A. & PADAVETTAN, V. 2012. Synthesis of silica nanoparticles by sol-gel: size-dependent properties, surface modification, and applications in silica-polymer nanocomposites—a review. *Journal of Nanomaterials,* 2012**,** 8.

RAMOS-DELGADO NORMA, A., GRACIA-PINILLA MIGUEL, Á., MANGALARAJA RAMALINGA, V., O'SHEA, K. & DIONYSIOU DIONYSIOS, D. 2016. Industrial synthesis and characterization of nanophotocatalysts materials: titania. *Nanotechnology Reviews.*

RICO, C. M., MAJUMDAR, S., DUARTE-GARDEA, M., PERALTA-VIDEA, J. R. & GARDEA-TORRESDEY, J. L. 2011. Interaction of nanoparticles with edible plants and their possible implications in the food chain. *J Agric Food Chem,* 59.

RODUNER, E. 2006. Thermodynamics of Finite Size Systems. *Nanoscopic Materials: Size-Dependent Phenomena.* The Royal Society of Chemistry.

ROSSETTI, F. F., TEXTOR, M. & REVIAKINE, I. 2006. Asymmetric Distribution of Phosphatidyl Serine in Supported Phospholipid Bilayers on Titanium Dioxide. *Langmuir,* 22**,** 3467-3473.

ROY, A., SAHOO, R., RAY, C., DUTTA, S. & PAL, T. 2016. Soft template induced phase selective synthesis of Fe2O3 nanomagnets: one step towards peroxidase-mimic activity allowing colorimetric sensing of thioglycolic acid. *RSC Advances,* 6**,** 32308-32318.

RUBASINGHEGE, G., ELZEY, S., BALTRUSAITIS, J., JAYAWEERA, P. M. & GRASSIAN, V. H. 2010. Reactions on Atmospheric Dust Particles: Surface Photochemistry and Size-Dependent Nanoscale Redox Chemistry. *The Journal of Physical Chemistry Letters,* 1**,** 1729-1737.

RUI, M., MA, C., HAO, Y., GUO, J., RUI, Y., TANG, X., ZHAO, Q., FAN, X., ZHANG, Z., HOU, T. & ZHU, S. 2016. Iron Oxide Nanoparticles as a Potential Iron Fertilizer for Peanut (Arachis hypogaea). *Frontiers in Plant Science,* 7**,** 815.

SADEGHI GHARI, H. & JALALI-ARANI, A. 2016. Nanocomposites based on natural rubber, organoclay and nano-calcium carbonate: Study on the structure, cure behavior, static and dynamic-mechanical properties. *Applied Clay Science,* 119**,** 348-357.

SADEGHI GHARI, H., SHAKOURI, Z. & SHIRAZI, M. M. A. 2014. Evaluation of microstructure of natural rubber/nano-calcium carbonate nanocomposites by solvent transport properties. *Plastics, Rubber and Composites,* 43**,** 177-186.

SANO, A. & NAKAMURA, H. 2004. Chemo-affinity of Titania for the Column-switching HPLC Analysis of Phosphopeptides. *Analytical Sciences,* 20**,** 565-566.

SCHRAND, A. M., SCHLAGER, J. J., DAI, L. & HUSSAIN, S. M. 2010. Preparation of cells for assessing ultrastructural localization of nanoparticles with transmission electron microscopy. *Nat. Protocols,* 5**,** 744-757.

SCHWAB, F., ZHAI, G., KERN, M., TURNER, A., SCHNOOR, J. L. & WIESNER, M. R. 2016. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants – Critical review. *Nanotoxicology,* 10**,** 257-278.

SEISENBAEVA, G. A., DANIEL, G., NEDELEC, J.-M. & KESSLER, V. G. 2013. Solution equilibrium behind the room-temperature synthesis of nanocrystalline titanium dioxide. *Nanoscale,* 5**,** 3330-3336.

SEKKAL, W. & ZAOUI, A. 2017. Enhancing the interfacial bond strength of cement nanocomposite with carbonate nanostructure. *Composites Part B: Engineering,* 124**,** 111-119.

SERVIN, A., ELMER, W., MUKHERJEE, A., DE LA TORRE-ROCHE, R., HAMDI, H., WHITE, J., BINDRABAN, P. & DIMKPA, C. 2015. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *Journal of Nanoparticle Research,* 17**,** 1-21.

SHARMA, N., OJHA, H., BHARADWAJ, A., PATHAK, D. P. & SHARMA, R. K. 2015a. Preparation and catalytic applications of nanomaterials: a review. *RSC Advances,* 5**,** 53381-53403.

SHARMA, N. C., SAHI, S. V., NATH, S., PARSONS, J. G., GARDEA-TORRESDE, J. L. & PAL, T. 2007. Synthesis of Plant-Mediated Gold Nanoparticles and Catalytic Role of Biomatrix-Embedded Nanomaterials. *Environmental Science & Technology,* 41**,** 5137-5142.

SHARMA, V. K., FILIP, J., ZBORIL, R. & VARMA, R. S. 2015b. Natural inorganic nanoparticles - formation, fate, and toxicity in the environment. *Chemical Society Reviews,* 44**,** 8410-8423.

SHOKROLLAHI, H. 2017. A review of the magnetic properties, synthesis methods and applications of maghemite. *Journal of Magnetism and Magnetic Materials,* 426**,** 74-81.

SING, K. 2001. The use of nitrogen adsorption for the characterisation of porous materials. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 187-188**,** 3-9.

SINGH, N. B. & SINGH, N. P. 2007. Formation of CaO from thermal decomposition of calcium carbonate in the presence of carboxylic acids. *Journal of Thermal Analysis and Calorimetry,* 89**,** 159-162.

SONAWANE, S. H., GUMFEKAR, S. P., MESHRAM, S., DEOSARKAR, M. P., MAHAJAN, C. M. & KHANNA, P. 2009. Combined Effect of Surfactant and Ultrasound on Nano Calcium Carbonate Synthesized by Crystallization Process. *International Journal of Chemical Reactor Engineering,* 7.

STRAMBEANU, N., DEMETROVICI, L. & DRAGOS, D. 2015. Natural Sources of Nanoparticles. *In:* LUNGU, M., NECULAE, A., BUNOIU, M. & BIRIS, C. (eds.) *Nanoparticles' Promises and Risks: Characterization, Manipulation, and Potential Hazards to Humanity and the Environment.* Cham: Springer International Publishing.

TAO, F. & SPIVEY, J. - Metal Nanoparticles for Catalysis. - P001.

TEPE, N. & BAU, M. 2014. Importance of nanoparticles and colloids from volcanic ash for riverine transport of trace elements to the ocean: Evidence from glacial-fed rivers after the 2010 eruption of Eyjafjallajökull Volcano, Iceland. *Science of The Total Environment,* 488–489**,** 243-251.

THANH, N. T. K., MACLEAN, N. & MAHIDDINE, S. 2014. Mechanisms of Nucleation and Growth of Nanoparticles in Solution. *Chemical Reviews,* 114**,** 7610-7630.

TIKHONOVICH, I. A., KRAVCHENKO, L. V. & SHAPOSHNIKOV, A. I. 2011. Root exudates as an important factor of formation of nanomolecular structures of the rhizosphere. *Russian Agricultural Sciences,* 37**,** 40-42.

TOJO, C., BUCETA, D. & LÓPEZ-QUINTELA, M. A. 2017. On metal segregation of bimetallic nanocatalysts prepared by a one-pot method in microemulsions. *Catalysts,* 7.

TOU, F., YANG, Y., FENG, J., NIU, Z., PAN, H., QIN, Y., GUO, X., MENG, X., LIU, M. & HOCHELLA, M. F. 2017. Environmental Risk Implications of Metals in Sludges from Waste Water Treatment Plants: The Discovery of Vast Stores of Metal-Containing Nanoparticles. *Environmental Science & Technology,* 51**,** 4831-4840.

TRIPATHI, D. K., SHWETA, SINGH, S., SINGH, S., PANDEY, R., SINGH, V. P., SHARMA, N. C., PRASAD, S. M., DUBEY, N. K. & CHAUHAN, D. K. 2017. An overview on manufactured nanoparticles in plants: Uptake, translocation, accumulation and phytotoxicity. *Plant Physiology and Biochemistry,* 110**,** 2-12.

TUROVA, N. Y., TUREVSKAYA, E. P., KESSLER, V. G., YANOVSKY, A. I. & STRUCHKOV, Y. T. 1993. Synthesis, crystal and molecular structure of calcium oxo ethoxide, [Ca6([small micro]4-O)2([small micro]3-OEt)4(OEt)4][middle dot]14EtOH. *Journal of the Chemical Society, Chemical Communications***,** 21-23.

ULKERYILDIZ, E., KILIC, S. & OZDEMIR, E. 2016. Rice-like hollow nano-CaCO3 synthesis. *Journal of Crystal Growth,* 450**,** 174-180.

WAGNER-HATTLER, L., SCHOELKOPF, J., HUWYLER, J. & PUCHKOV, M. 2017. Characterization of new functionalized calcium carbonate-polycaprolactone composite material for application in geometry-constrained drug release formulation development. *Drug Development and Industrial Pharmacy,* 43**,** 1669-1676.

VAINRUB, A., PUSTOVYY, O. & VODYANOY, V. 2006. Resolution of 90 nm (λ/5) in an optical transmission microscope with an annular condenser. *Optics Letters,* 31**,** 2855-2857.

VAN BEL, A. J. E. & WILL, T. 2016. Functional Evaluation of Proteins in Watery and Gel Saliva of Aphids. *Frontiers in Plant Science,* 7.

VAN DER ENT, S., VAN HULTEN, M., POZO, M. J., CZECHOWSKI, T., UDVARDI, M. K., PIETERSE, C. M. J. & TON, J. 2009. Priming of plant innate immunity by rhizobacteria and β-aminobutyric acid: differences and similarities in regulation. *New Phytologist,* 183**,** 419-431.

WANG, A., YANG, Y., ZHANG, X., LIU, X., CUI, W. & LI, J. 2016a. Gelatin-Assisted Synthesis of Vaterite Nanoparticles with Higher Surface Area and Porosity as Anticancer Drug Containers In Vitro. *ChemPlusChem,* 81**,** 194-201.

WANG, F., LI, H., YUAN, Z., SUN, Y., CHANG, F., DENG, H., XIE, L. & LI, H. 2016b. A highly sensitive gas sensor based on CuO nanoparticles synthetized via a sol-gel method. *RSC Advances,* 6**,** 79343-79349.

WANG, H., KOU, X., PEI, Z., XIAO, J. Q., SHAN, X. & XING, B. 2011. Physiological effects of magnetite (Fe3O4) nanoparticles on perennial ryegrass (Lolium perenne L.) and pumpkin (Cucurbita mixta) plants. *Nanotoxicology,* 5**,** 30-42.

WANG, X., CHENG, J., YU, H. & YU, J. G. 2017. Facile hydrothermal synthesis of carbon dots modified g-C3N4 for enhanced photocatalytic H2-evolution performance. *Dalton Transactions*.

WEI, H. & WANG, E. 2013. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. *Chemical Society Reviews,* 42, 6060-6093.

WIESENDANGER, R. 1994. Contributions of scanning probe microscopy and spectroscopy to the investigation and fabrication of nanometer-scale structures*. *Journal of Vacuum Science & Technology B: Microelectronics and Nanometer Structures Processing, Measurement, and Phenomena,* 12, 515-529.

WILL, T., FURCH, A. & ZIMMERMANN, M. 2013. How phloem-feeding insects face the challenge of phloem-located defenses. *Frontiers in Plant Science,* 4.

VISHNU KIRTHI, A., ABDUL RAHUMAN, A., RAJAKUMAR, G., MARIMUTHU, S., SANTHOSHKUMAR, T., JAYASEELAN, C., ELANGO, G., ABDUZ ZAHIR, A., KAMARAJ, C. & BAGAVAN, A. 2011. Biosynthesis of titanium dioxide nanoparticles using bacterium Bacillus subtilis. *Materials Letters,* 65, 2745-2747.

VRANOVA, V., REJSEK, K., SKENE, K. R., JANOUS, D. & FORMANEK, P. 2013. Methods of collection of plant root exudates in relation to plant metabolism and purpose: A review. *Journal of Plant Nutrition and Soil Science,* 176, 175-199.

WYSOKOWSKI, M., KAISER, S. & JESIONOWSKI, T. 2017. Hydrothermal Synthesis of Advanced Chitin-Based Materials. *In:* EHRLICH, H. (ed.) *Extreme Biomimetics.* Cham: Springer International Publishing.

XIA, Y., YANG, H. & CAMPBELL, C. T. 2013. Nanoparticles for Catalysis. *Accounts of Chemical Research,* 46, 1671-1672.

XU, D. X., LIAN, Z. W., FU, M. L., YUAN, B. L., SHI, J. W. & CUI, H. J. 2013. Advanced near-infrared-driven photocatalyst: Fabrication, characterization, and photocatalytic performance of beta-NaYF4:Yb3+,Tm3+@TiO2 core@shell microcrystals. *Applied Catalysis B-Environmental,* 142, 377-386.

XU, R. 2015. Light scattering: A review of particle characterization applications. *Particuology,* 18, 11-21.

XU, Z., YU, J., LOW, J. & JARONIEC, M. 2014. Microemulsion-Assisted Synthesis of Mesoporous Aluminum Oxyhydroxide Nanoflakes for Efficient Removal of Gaseous Formaldehyde. *ACS Applied Materials & Interfaces,* 6, 2111-2117.

YANG, F., LIU, C., GAO, F., SU, M., WU, X., ZHENG, L., HONG, F. & YANG, P. 2007. The Improvement of Spinach Growth by Nano-anatase TiO2 Treatment Is Related to Nitrogen Photoreduction. *Biological Trace Element Research,* 119, 77-88.

YEC, C. C. & ZENG, H. C. 2014. Synthesis of complex nanomaterials via Ostwald ripening. *Journal of Materials Chemistry A,* 2, 4843-4851.

YIN, Y., TAN, Z., HU, L., YU, S., LIU, J. & JIANG, G. 2017. Isotope Tracers To Study the Environmental Fate and Bioaccumulation of Metal-Containing Engineered Nanoparticles: Techniques and Applications. *Chemical Reviews,* 117, 4462-4487.

YOU, J. & CHAN, Z. 2015. ROS Regulation During Abiotic Stress Responses in Crop Plants. *Frontiers in Plant Science,* 6.

YU, B., ANTSIFEROVA, A., DEMIN, V. A., DEMIN, V. F. & KASHKAROV, P. 2015. The method of radioactive tracer for measuring the amount of inorganic nanoparticles in biological samples. *IOP Conference Series: Materials Science and Engineering,* 98**,** 012039.

ZAIMENKO, N. V., DIDYK, N. P., DZYUBA, O. I., ZAKRASOV, O. V., ROSITSKA, N. V. & VITER, A. V. 2014. *Enhancement of Drought Resistance in Wheat and Corn by Nanoparticles of Natural Mineral Analcite*.

ZANGANEH, S., SPITLER, R., ERFANZADEH, M., ALKILANY, A. M. & MAHMOUDI, M. 2016. Protein corona: Opportunities and challenges. *The International Journal of Biochemistry & Cell Biology,* 75**,** 143-147.

ZHANG, J. & KIRKHAM, M. B. 1994. Drought-Stress-Induced Changes in Activities of Superoxide Dismutase, Catalase, and Peroxidase in Wheat Species. *Plant and Cell Physiology,* 35**,** 785-791.

ZHANG, R., HUO, J.-H., PENG, Z.-G., LUO, W.-J., FENG, Q., WANG, J.-X. & ZHANG, J. 2016. Study on the interaction of CaCO3 nanoparticles and surfactant in emulsion phase transition and its molecular dynamics simulation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 511**,** 105-112.

ZHANG, X., REN, W. & CUI, H. 2011. A general ultra large scale strategy for low temperature sol–gel synthesis of nearly monodispersed metal ions doped γ-Fe2O3 nanoparticles. *Journal of Sol-Gel Science and Technology,* 58**,** 232-237.

ZHAO, L., ZHANG, Y., MIAO, Y. & NIE, L. 2016. Controlled synthesis, characterization and application of hydrophobic calcium carbonate nanoparticles in PVC. *Powder Technology,* 288**,** 184-190.

ZHENG, H., SMITH, R. K., JUN, Y.-W., KISIELOWSKI, C., DAHMEN, U. & ALIVISATOS, A. P. 2009. Observation of Single Colloidal Platinum Nanocrystal Growth Trajectories. *Science,* 324**,** 1309-1312.

ZHU, H., HAN, J., XIAO, J. Q. & JIN, Y. 2008. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. *Journal of Environmental Monitoring,* 10**,** 713-717.

ZINDLER-FRANK, E. 1995. Calcium, Calcium Oxalate Crystals, and Leaf Differentiation in the Common Bean (Phaseolus vulgaris L.)*. *Botanica Acta,* 108**,** 144-148.

ZUVERZA-MENA, N., MARTÍNEZ-FERNÁNDEZ, D., DU, W., HERNANDEZ-VIEZCAS, J. A., BONILLA-BIRD, N., LÓPEZ-MORENO, M. L., KOMÁREK, M., PERALTA-VIDEA, J. R. & GARDEA-TORRESDEY, J. L. 2017. Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review. *Plant Physiology and Biochemistry,* 110**,** 236-264.

Popular science summary

Nanomaterials have been around us since forever. We have created them and we are ourselves in a sense made out them. Yet it wasn´t until relatively recently that we started to fine tune materials at the nano scale and hence understand them. In this thesis, a small addition of new knowledge has been made, about the interactions of nanoparticles in biological systems. With a focus plants, a small selection of common nanoparticles have been studied for their interaction with biology. Titania nanoparticles have been studied for their interaction with biological membranes and how that can affect bacteria interaction with plants. Iron oxide nanoparticles have been investigated for their enzymatic mimic properties and how that can affect plants ability to handle stress. Calcium carbonate nanoparticles have been studied for use as a benign matrix for drugs and for their possible interaction with aphids in plant infestation events.

Populärvetenskaplig sammanfattning

Nanomaterial har alltid funnits i naturen. Vi människor har länge skapat nanomaterial, om än ovetandes, och i viss bemärkelse så består vi av dem eftersom proteiner är nanomaterial. Dock så är det inte förrän relativt nyligen som vi började manipulera material på nanonivå och därmed så har vi också börjat förstå betydligt mer om egenskaperna hos dess material. Den här avhandlingen bidrar med en liten utökning av denna förståelse genom att utforska nanopartiklars interaktioner med biologiska system och organismer. Med fokus på växter så har ett urval av vanligt förekommande nanopartiklar studerats. Titandioxid-nanopartiklar har undersökt för deras cellmembran-bindande egenskaper och hur detta kan påverka mikrobers interaktioner med växter. Järnoxid-nanopartiklar har studerats för deras förmåga att agera som enzymer och hur detta kan påverka växters förmåga att hantera stress. Kalciumkarbonat-nanopartiklar har studerats för deras förmåga att binda organiska molekyler för att sedan släppa dessa i vatten, samt för hur de skulle kunna interagera med bladlöss i växter.

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