Nanoparticle synthesis using phytochemical precursors – Instilling beneficial properties with green methodologies for pollution remediation

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Declaration

I, Adam Truskewycz (candidate) certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

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Summary

Nanotechnology has become an emerging platform for the treatment of organic and inorganic contaminants. Nanoparticles high surface area to volume ratio enhances their capacity for reactions and interactions with pollutants to proceed at an elevated rate compared to larger particles. Concerns pertaining to their toxicity and suitability for environmental release have prevented many studies from progressing to commercial products. However, considerable toxicology research has reinforced that nanoparticles synthesized with non-toxic ingredients can be biocompatible and be used for therapeutics. The research conducted during this PhD candidature was focused on developing nanomaterials with pollution remediation capacities from ingredients which are ubiquitous in the environment, derived from natural sources or show environmental biocompatibility. With this approach, nanoparticles with future commercial application for site remediation are possible.

Due to iron's large intrinsic presence within many soil types, its potential for environmental biocompatibility is heightened. Iron nanoparticles were created in this study by reducing iron salt precursors with aqueous plant extracts. Green tea extract, rich in antioxidants was initially used for this approach and resulted in iron oxide nanoparticles with the capacity to rapidly degrade concentrated dye mixtures (99.1 % of 500 ppm concentrated dye mixture) with the aid of hydrogen peroxide to facilitate a Fenton-like degradation mechanism. This process generated nanoparticles with a tendency to stick together, required acidic conditions and removal rates were increased at elevated temperatures. Due to the downfalls of requiring acidic conditions, hydrogen peroxide and the particles containing agglomerated chains of particles, the focus of further experimentation was directed to adsorption studies.

In the second research chapter, iron nanoparticles were generated using differing plant extracts to investigate the influence of diverse phytochemical mixtures on the morphology of iron nanoparticles. The generated nanoparticles were then assessed for their capacity to adsorb hexavalent chromium (Cr\(^{6+}\)) from aqueous solutions. Nanoparticle morphology differed significantly between the different plant extract reducing agents. No significant links between reducing powers, antioxidant concentration, phenolic concentration, protein concentration, reducing sugar concentration and iron chelating capacity were indicative of specific nanoparticle morphologies. However, high antioxidant concentrations did not result in optimal morphologies (chains of agglomerated particles) and small, monodisperse nanoparticles were shown to possess the greatest Cr\(^{6+}\) adsorption capacities (up to 96.2 % of 50 ppm Cr\(^{6+}\) solution).
Silicon dioxide is a routinely used adsorbent material for the removal of environmental contaminants as it has high adsorption capacity and is environmentally benign. Within chapter 4 *Helianthus annuus* extract was incorporated into a reaction vessel with an aminosilicon precursor and subjected to high temperatures (200°C) and pressures. The resulting silicon nanoparticles possessed increased surface area compared to controls. Their concentrated mixed dye removal was apparent after 5 mins and showed extraordinary removal capacities between 416.67 – 714.29 mg dye adsorption per g of adsorbent. These positively charged silanol nanoparticles showed little bacterial toxicity and are therefore likely to be suitable for environmental applications.

In summary, a sustainable, natural approach for instilling beneficial properties to iron and silicon nanoparticles using plant extracts has been developed. The capacity for these nanoparticles to remove pollutants from aqueous solutions is substantial and upon further refinement, may be suitable for environmental remediation applications.

**Preface**

This thesis is structured in 5 Chapters. Chapter 1 contains the introduction and includes a background on iron nanoparticles for site remediation. The main body of this thesis consists of journal papers presented as Chapters 2, 3 and 4 along with a general discussion and conclusion which is presented in Chapter 5.

The Chapters 2 and 3 cover the synthesis of iron nanoparticles using plant extracts and shows insight as to their capacity to remediate concentrated dye mixtures and hexavalent chromium.

Chapter 4 deviates from the synthesis of iron nanoparticles and shows how plant extracts can be used to enhance the properties of silicon nanoparticles for the remediation of concentrated dye mixtures.

Chapter 5 ties all the chapters together and shows the progression of research and understanding throughout the candidature. Conclusions and future perspectives are summarised and highlighted at the end of this chapter.
Papers published or under peer-review/submitted from this thesis


Chapter 1

Literature review

Iron nanoparticles for contaminated site remediation and environmental preservation

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Andrew S Ball contributed to the planning of the reviews focus, preparation of the manuscript and evaluation of Chapter 1.

All the co-authors give full consent to Adam Truskewycz to present these above papers for examination towards the Degree of Doctor of Philosophy.

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Part I – Literature Review

Chapter 1 - Iron Nanoparticles for Contaminated Site Remediation and Environmental Preservation

Iron Nanoparticles for Contaminated Site Remediation and Environmental Preservation

Adam Truskewycz, Sayali Patil, Andrew Ball, and Ravi Shukla

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10.1 What Are Nanoparticles?

A nanoparticle (NP) can be defined as any entity that has at least one dimension between 1–100 nm in size. To put their size into perspective, 1 nm is 1 billionth of a meter (approximately half the width of a DNA double helix). Nanoparticles are of great scientific interest, as they exhibit unique properties that are not observed at the atomic and molecular levels or in the bulk form of the materials. At the nano-size scale, the so called quantum effects dictate multiple properties of materials that include but are not limited to melting point, fluorescence, electrical conductivity, and magnetic permeability, and
chemical reactivity changes as a function of the size of the particles. At the nano-scale, environments are quite different from what is experienced at larger dimensions. In bulk materials, tightly packed atoms react with neighbouring atoms of the same type and behave as clusters/bulk solids, whereas at the nano-scale, the specific behaviour of individual atoms has a larger role to play in interactions (Halperin 1986). It is this size range that makes nanoparticles unique for numerous real-world applications (Daniel and Astruc 2004, Agrawal et al. 2013, Weerathunge et al. 2014, Arakha et al. 2015, Carnovale et al. 2016).

Nanoparticles can be organic (e.g., cholesterol, rhovanil, rhodiarome), inorganic (nanoscale zero-valent iron [nZVI], gold and silver nanoparticles), or a combination of both (e.g., yolk–shell nanoparticle@MOF Petalous Heterostructures) (Liu et al. 2014b, Destrée and Nagy 2006). Their small size and subsequent increased surface area-to-volume ratio allow them to tightly pack together to make smooth, uniform surfaces or, if intermolecular attractive forces can be combated, they can act as individual reactive entities with high surface area-to-volume ratio and elevated reactive properties.

They can exist naturally (e.g., mineral composites, biogenic magnetite) as incidental entities (e.g., combustion products, sand blasting) or as engineered nanoparticles (e.g., nZVI, carbon nanotubes) (Cameotra and Dhanjal 2012). The current use of nanoparticles spans many disparate disciplines from drug delivery in medical applications to conductors of electricity for electronic devices to their use in paint to act as UV light blockers for enhancing the life of paint (Allen et al. 2002) and for enhanced contaminant degradation efficiencies in the environmental remediation field (O’Carroll et al. 2013).

10.2 Environmental Pollution Due to Anthropogenic Activities

Since the industrial revolution, increased population growth and heavy reliance on machinery have led to unprecedented economic growth and development. While previously this has been carried out with little regard to the environment, the concept of environmental preservation has accelerated over the last decade within developing as well as first-world countries. The focus on climate change and human health has led to a plethora of scientific research aimed at tackling the issues related to anthropogenic activities such as design and development of smart nanomaterials/chemicals and their impact on the environment, including their ecotoxicity and the potential health implications they pose for both environmental and human health. Furthermore,
technologies that have shown promise in terms of decontaminating the environment are constantly being refined and optimized.

Pollution from human activities often finds its way into the environment in excessive concentrations, and this can cause rapid, significant, and prolonged ecological harm. Persistent organic pollutants and heavy metals are among the most serious environmental polluters (Zhu et al. 2015). Unlike most naturally occurring environmental impacts, these anthropogenic pollutants are particularly recalcitrant to environmental means of detoxification (bioremediation). Remediation, particularly bioremediation (natural attenuation), may be regarded as protracted when it comes to appropriate land restoration for commercial development purposes, animal and human health, and ecosystem regeneration (Prakash et al. 2013).

10.3 Iron in the Environment

Iron is everywhere—inside animal cells (part of the hemoglobin protein responsible for transporting oxygen around the body) (Gutteridge 1986), within plant cells (responsible for processes from respiration to photosynthesis) (Kim and Guerinot 2007), and within microbial cells (required for many processes, including apoptosis and electron shuttling) (Breuer et al. 2014). It is the fourth most abundant element found within the earth’s crust (Figure 10.1) and is present in many different forms. It is responsible for the dark red, yellow, and orange sands in the Australian outback and the dark black sands on the south coast of Indonesia’s Java Island and Elba island in Italy (Jamiesona et al. 2006, Perfumo et al. 2011).

Despite the huge prevalence of iron in natural ecosystems, metallic iron is seldom found naturally, as it readily reacts with oxygen to form iron oxides. There are a number of iron oxides falling under the categories of oxides, hydroxides, or oxide hydroxides; these may include the following (Génin et al. 2001; Schwertmann 2008) (Figure 10.2).

- Goethite $\alpha$-FeO(OH)
- Lepidocrocite $\gamma$-FeO(OH)
- Akaganeite $\beta$-FeO(OH)
- Schwertmannite $\text{Fe}_{16}\text{O}_{16}(\text{SO}_4)_{2}(\text{OH})_{12} \cdot n\text{H}_2\text{O}$
- Feroxyhite $\delta$-FeOOH
- High pressure FeOOH
- Ferrihydrite $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$
- Bernalite $\text{Fe}(\text{OH})_3 \cdot n\text{H}_2\text{O}$
• Ferrous hydroxide Fe(OH)$_2$
• Green rusts Fe$_{14}$Fe$_{12}$ (OH)$_{12}$$^{2+}$ • [SO$_{4}^{2-}$, $n$H$_2$O]$^{2-}$ & Fe$_{16}$Fe$_{14}$ (OH)$_{16}$$^{2+}$ • [C$_2$O$_4^{2-}$, 3 H$_2$O]$^{2-}$
• Hematite α–Fe$_2$O$_3$
• Magnetite Fe$_3$O$_4$(Fe$^{2+}$Fe$^{3+}$ O$_4$)
• Maghemite γ–Fe$_2$O$_3$
• β–Fe$_2$O$_3$
• ε–Fe$_2$O$_3$
• Wustite Fe$^{2+}$O

**FIGURE 10.1**
Abundance of elements in the continental crust. (Adapted from Skinner, B. J. 1999. Earth resources (minerals/metals/ores/geochemistry/mining), PNAS, Proceedings of the National Academy of Sciences 76(9): 4212–4217)

Iron oxides naturally exhibit low solubility in water; however, decreasing crystal size, reduction of Fe$^{2+}$, and the presence of ligands creating Fe complexes can increase their solubility. Furthermore, iron$^{III}$ oxides have surfaces with OH ions attached that adsorb many anions, which makes them encapsulated, thereby limiting their mobility in soils.

The iron oxides routinely used for nanoparticle research for site remediation are mainly Goethite α–FeO(OH), Magnetite Fe$_3$O$_4$ (Fe$^{2+}$Fe$^{3+}$ O$_4$), Hematite α–Fe$_2$O$_3$, and Maghemite γ–Fe$_2$O$_3$ (Figure 10.2). However, nZVI (metallic iron) nanoparticles tend to have the largest focus in research for environmental remediation (Hua et al. 2012).
The form and stability of iron is reliant on a variety of physicochemical and environmental conditions. Under aerobic conditions where soil is not affected by extreme acidity or alkalinity, Fe²⁺ ions oxidize to Fe³⁺ ions. These Fe³⁺ ions will then try to achieve stability by forming Fe₃O₄ oxides. The stability of these species is not readily affected by protonation but may be dissolved and recycled by biotic reduction or complexation with organic ligands. Under anaerobic conditions, Fe³⁺ in oxides can be reduced to Fe²⁺ via electron transfers resulting from the oxidation of biomass (Schwertmann 2008).

**FIGURE 10.2**


**10.4 Iron Nanoparticles: Potential Use in Remediation**

Iron addition is a current treatment for the degradation of a wide range of recalcitrant environmental pollutants, with millimetric iron and permeable reactive barriers being routinely used. As reactions between iron and pollutants occur at the surface of iron, it is logical that an increased surface area-to-volume ratio would favour elevated degradation
rates. Studies show that nanoparticles exhibit significant increase in degradation rates compared to their millimetric counterparts (Lien and Zhang 2001).

Not only have exceptional pollutant degradation rates been shown in laboratory scale tests, but there are other benefits for particles to enter the nano-scale. An example of this lies with groundwater contamination. Contaminated groundwater is locked away underneath complex geographical matrices and is particularly hard to access for remediation purposes (Figure 10.3). Pumping and treating is time intensive and costly, as installation of permeable reactive barriers requires costly excavation, and they can only be installed in shallow aquifers. Chemical treatment methods are often toxic to the environment and can be costly. Emulsified iron nanoparticles and other coated iron nanoparticles have been successfully applied to in vitro groundwater remediation projects for deep aquifers without the need to excavate or transport to treatment plants (Cook 2009). Due to iron’s high abundance in the earth’s crust and nZVI’s ability to rapidly form iron oxide following passivation in aerobic environments, its potential environmental compatibility, particularly for groundwater remediation applications, is of great interest.

**FIGURE 10.3**

Groundwater dynamics to consider when applying EnZVI to a contaminated site.
10.5 Different Types of Iron Nanoparticles

In the field of nanoparticle research, there are a number of different types of iron (Figure 10.2) and iron-based nanoparticles, each with their own inherent properties. Magnetism, high coercivity, low Curie temperature, and reactivity are parameters that may be sought after, and optimizing one or all of these properties is of significant interest for researchers. In the environmental remediation field, reactivity with recalcitrant pollutants and minimization of passivation are of high importance.

10.5.1 Nanoscale Zero-Valent Iron (Fe⁰)

Metallic iron, elemental iron, or nano-scaled zero valent iron is ferromagnetic in nature and does not possess a charge. Its structure is made up of only iron atoms and is unstable under aerobic conditions. It will therefore oxidize readily unless coatings are applied. The majority of research conducted with metallic iron nanoparticles uses nZVI with an oxide shell to prevent further rapid oxidation (Fan et al. 2015).

10.5.2 Magnetite (Fe₃O₄)

Magnetite is a ferromagnetic species of iron (superparamagnetic when nanoparticle size is smaller than 15 nm) when it is below its Curie temperature (858K). It has an inverse cubic iron oxide spinel structure and is a mixed valence metal possessing Fe²⁺ and Fe³⁺ states. It exhibits strong magnetism and is black in colour but unstable under aerobic conditions and will readily oxidize into maghemite (Laurenzi 2008, Zhang and Olin 2011, Virkutyte and Varma 2013). Therefore, magnetite is synthesized under anaerobic conditions.

10.5.3 Maghemite (γ-Fe₂O₃)

Maghemite is ferrimagnetic below its Curie temperature (645K). Its structure has a meta stable cubic iron oxide spinel structure and possesses Fe³⁺ charged states. It is magnetic and reddish brown in appearance and is often the product of weathered magnetite. It may also be produced by heating other iron oxides (Laurenzi III 2008, Zhang and Olin 2011, Virkutyte and Varma 2013).

10.5.4 Hematite (α-Fe₂O₃)

Hematite is a weak ferromagnetic or antiferromagnetic iron species. It possesses a hexagonal closed-packed corundum, like maghemite, only with Fe³⁺ charged states. It is
very stable under aerobic conditions and has a dark red colour when ground or a black/grey colour if coarsely crystalline. Hematite can be formed from magnetite and maghemite upon heating to temperatures above 300°C and between 370–600°C, respectively (Laurenzi III 2008, Virkutyte and Varma 2013, Pereira et al. 2013).

10.5.5 Goethite (α-FeO(OH))

Goethite is an antiferromagnetic iron oxide that is very insoluble, particularly in acidic media. It possesses an orthorhombic unit cell or exists as rhombohedral prisms when nanoparticles. They are thermodynamically stable. When iron(III) salts undergo hydrolysis, they form ferrihydrite. Upon exposure to higher temperatures (60°C) followed by environmental aging, they can form goethite (Madsen et al. 2009, Guo and Barnard 2011).

10.5.6 Lepidocrocite (γ-FeO(OH))

Lepidocrocite is generally regarded as paramagnetic; however, at extremely low temperatures (52 K), it can become antiferromagnetic (Hirt and Lanci 2011). It has an edge-shared octahedral structure and is relatively unstable under aerobic conditions favouring the formation of Goethite (Chesworth et al. 2008). Lepidocrocite can be formed by mixing solutions of iron salts (Fe³⁺ or Fe²⁺) with an oxidant through precipitation or hydrolysis reactions (Cui et al. 2012).

10.5.7 Ferrihydrite

Ferrihydrite is an ordered iron(III) oxyhydroxide whose structure is yet to be determined. Some researchers believe it is made up of various poorly ordered Fe(III) oxyhydroxides (Carta et al. 2009). It is easily formed upon hydrolysis of iron(III) salts and is generally considered to be antiferromagnetic; however, a decrease in particle size increases the magnetism of the particles (Carta et al. 2009, Guo and Barnard 2011, Masina et al. 2015). 10.5.8 Amorphous Mixtures

Crystalline structures are easily formed at high temperatures; however, amorphous structures can be generated upon rapid cooling. Furthermore, impurities in nanoparticle structures may also impact regularly arranged atoms/entities, leading to amorphous nature of particles. Boron, oxide, and hydroxides may impact the crystallinity of nanoparticles and reduce their reactivity (Phu et al. 2011). Conversely, Yoo et al. (2007) stated that amorphous iron oxide nanoparticles possess larger catalytic and magnetic attributes.
10.6 Parameters for the Application of Iron Nanoparticles to Bioremediation

10.6.1 Size

In many cases, nanotechnology is just a scaled-down version of current technologies (Hameri 2012); however, by reducing entities to the nanoscale different forces, interactions and properties are observed. Atoms and molecules are nanoscale entities and their interactions within their environment are heavily reliant on other entities of similar size.

In the field of nanoparticle research, interactions between small entities are observed that are not experienced with micro or macro dimensions. Optical properties may differ, that is, gold nanoparticles are red in colour (Daniel and Astruc 2004), magnetism may become apparent (Carta et al. 2009, Guo and Barnard 2011, Masina et al. 2015), and so on. These interactions are often termed quantum size effects.

Long et al. (2015) indicated that nanoparticles with sizes of approximately 30 nm have superior quantum effects when used for catalysis, biology, and medical applications; however, Nurmi et al. (2005) reported that a particle size of less than 5 nm was optimal for maximizing the influence of quantum effects on the physical and chemical properties of nanoparticles. However, iron oxide nanoparticles with sizes between 10–150 nm will exhibit similar behaviour, as they possess a lower electron density to other non-oxide or non-semiconductive entities.

In the field of pollutant degradation for environmental preservation/restoration, iron nanoparticle size is particularly important. If the nanoparticle is too large, reactivity rates may be too slow to sufficiently catabolize the anthropogenic entity or may not be deliverable to the pollution source in the case of groundwater remediation where particles need to travel through complex geological matrices (Figure 10.3) to reach the pollutant (Patil et al. 2016). On the other hand, if the particle size is too small, nanoparticles are susceptible to rapid oxidation under aerobic conditions and may also react with entities found naturally in the environment. Due to their increased surface area, rapid oxidation and reactivity with other environmental constituents may exhaust the nanoparticles to the point where their metallic core is reduced in size so that insufficient concentrations of metallic iron remain to take part and complete remediation (Cundy et al. 2008).

Nanoparticles are classified as particles having at least one dimension between 1–100 nm (Jordan et al. 2005). In environmental remediation situations, a nanoparticle is a particle where all three dimensions are below 100 nm in diameter. Although it has been reported that small nanoparticles may react too fast for many site applications (Bardos et al. 2011),
there is no accepted “optimal” size for nanoparticle mediated remediation, as particles generated via different synthesis methods will possess variations in porosity and surface area, particle size distribution, and impurities. Furthermore, coatings may be added to stabilize or increase the reactivity of particles (Grieger et al. 2010).

Maleki et al. 2012 optimized parameters for generating nanoparticles with tuneable size characteristics. Different ratios of iron salts (Fe$^{3+}$ and Fe$^{2+}$) were reduced with ammonium hydroxide in conjunction with surfactants (toluene, cetyl trimethylammonium bromide (CTAB), and 1-butanol, respectively). The findings revealed that the reducing agent had little effect on size control; however, tweaking the water, surfactants, and iron salt concentration had an impact on particle size.

When the molar ratio of water to surfactant was increased, the porosity and size of the nanoparticles also increased. When the concentration of iron salts was increased, so was the size of the nanoparticles. This is due to a high nucleation rate and fast nucleus growth rate. Increases in reducing agent concentration have been linked with the formation of smaller nanoparticles. Maleki et al. (2012), however, found only a small correlation, with only 25%–30% size difference experienced.

### 10.6.2 Agglomeration

Nanoparticle chemistry, when used for water remediation, can be identified as surface science, where physical and chemical interactions/reactions occur at the solid and liquid interphases. Perhaps the greatest benefits of progressing from the macro scale to the micro scale and then to the nano-scale are the subsequent increase in particle surface area available for chemical reactions to take place. Increases in surface area lead to faster reaction rates and in turn more rapid pollutant degradation capacities. One of the greatest hurdles to overcome in nanoparticle research is to prevent attractive particle–particle electrostatic and magnetic interactions. These forces cause the particles to form micro-scaled fractional aggregates that are unfavourable for nanotechnological applications. Interactions between particles can be expressed as the sum of Van der Waals attraction and electrostatic double layer repulsion, as described by the DLVO theory (theory generated by Derjaguin, Landau, Verwey, and Overbeek). These forces are influenced by particle size, surface potential, the Hamaker constant, and the solution’s ionic strength (Verwey 1948; Phenrat et al. 2007).

Aggregated nanoparticles do not behave as nanoscale entities, as they have limited mobility in porous media (only up to a few centimetres) (Phenrat et al. 2007), resulting in a significant loss in reactivity due to reduced surface area. Aggregated nanoparticles have a tendency to settle at faster rates in aqueous media, limiting their exposure time with dissolved or floating pollutants, and can block pores in porous media limiting mobility of
both nanoparticles and groundwater flows. In order to achieve colloidal stability, researchers have been focusing their efforts on generating coatings and stabilizers.

In 2007, Huang and Ehrman generated bimetallic iron nanoparticles particles with palladium seeds via a chemical reduction approach. To prevent passivation and agglomeration, they incorporated a poly(acrylic acid) (PAA) solution into an iron salt solution (containing palladium chloride), which they reacted with sodium borohydride to form stable nanoparticles with particle–particle repulsive properties. Repulsive properties were greater when iron nanoparticles were generated without palladium salts.

### 10.6.3 Shape and Particle Uniformity

Nanoparticles of various shapes have been synthesized, that is, spheres, rods, tubes, triangles, pyramids, and cubes, just to name a few (Sajanlal et al. 2011). The shape of nanoparticles may be of importance depending on their application. For instance, nanotubes and wires have been implicated in the conduction of electricity and could benefit the electronics industry (Li et al. 2011a). However, spherical nanoparticles may have fewer edges, which will benefit movement through porous media in groundwater remediation and will have the largest surface area-to-volume ratio for their mass, leading to optimum reactivity (Alam and Haas 2015).

For site remediation, it is important that nanoparticles be delivered to the pollution source quickly and in sufficient concentrations. To ensure this is possible without adding excessive nanoparticles to a specific site, it is important that variations in particle size be minimized. The addition of excess particles to a particular site is unfavourable, as remediation costs are elevated, porous media can be saturated, and the pores can be blocked, preventing particle delivery. In addition, the potential for nanoparticulate induced toxicity for ecosystems is enhanced. Nanoparticle uniformity allows researchers to better predict reactivity and delivery rates in porous media. Carrying out preliminary simulations is important for assessing site clean-up costs and effectiveness of nanotechnology as a treatment option (Cameselle et al. 2013).

Jana et al. 2004 were able to control the size and shape of iron oxide particles by controlling the reactivity and concentration of the precursors. Simply, iron salts were dissolved in methanol and oleic acid. Sodium hydroxide was then added to form a brown precipitate. The brown precipitate was digested in hydrochloric acid and dissolved in 1-octadecane at 60–70°C. The aforementioned solution was mixed with octadecane and oleic acid and heated up to 300°C in anaerobic conditions. Increasing oleic acid concentrations increased the particle size and extended heating times led to the formation of cubic particles over spherical particles when specific precursor concentrations were used.
10.6.4 Aging/Storage Times/Stability/Passivation

Researchers aim to create nanoparticles for their enhanced reactive properties; however, due to their rapid tendency to react with oxygen and to some extent water, coatings are often generated that reduce their reactivity and extend their shelf life (Zhang 2003).

\[
\begin{align*}
1. \quad 2\text{Fe}^0 + 4\text{H}^+ + \text{O}_2 & \rightarrow 2\text{Fe}^{2+} + 2\text{H}_2\text{O} \\
2. \quad 2\text{Fe}^0 + 2\text{H}_2\text{O} & \rightarrow \text{Fe}^{2+} + \text{H}_2 + 2\text{OH}^- \\
\end{align*}
\]

Coatings have been generated to improve nanoparticle life in aerobic environments; however, nanoparticles have the capacity to catabolize pollutants in anaerobic environments and may also need to be deliverable to the pollution source before they start to react.

Iron nanoparticles, in particular Fe\(^0\), readily oxidize under aerobic conditions and will form an oxide shell made up of either hydroxides or oxyhydroxides. The core of these particles is shielded from the external environment and can therefore remain as metallic iron (Fe\(^0\)). Further weathering utilizes the core to generate more oxides, increasing the thickness of the outer layer, decreasing the percentage of metallic iron, and reducing the reactivity of particles (Grieger et al. 2010).

During contaminant degradation, particle reactivity may also have a role to play in Fe\(^0\) utilization and or oxide layer thickness. Kim et al. (2012) reported that iron nanoparticles generated with the borohydride reduction method (95% Fe\(^0\) content) generated particles where the oxide layer thickness remained the same but the Fe\(^0\) core shrunk during pollutant remediation. Liu and Lowry (2006) performed similar tests using iron nanoparticles generated by the reduction of iron oxides by H\(_2\) at high temperatures (48% Fe\(^0\) content). Their observations were different and showed that the thickness of the oxide shell layer increased while the Fe\(^0\) core decreased during pollutant remediation.

Kim et al. (2012) showed that a number of factors were responsible for the utilization of the Fe\(^0\) core of their borohydride generated nanoparticles when used to remediate nitrate in aqueous solutions. The following reactions were responsible for degeneration of the iron core: (i) nitrate reduction (39.1%), (ii) initial shell modifications (25%), (iii) hydrogen production (possibly 5%), (iv) oxidation of Fe\(^0\) by dissolved oxygen (1.2%), and (v) acid flushing for nZVI regeneration (possibly 9%). The remaining Fe\(^0\) expected to remain in their experimental vessels was approximately 20.7% of the original concentration. O’Hara et al. 2006 generated emulsified nZVI nanoparticles by adding the particles to a vegetable oil solution mixed with surfactants. These particles have properties that prevent their interaction with atmospheric oxygen and groundwater constituents that may pacify the nanoparticles (Figure 10.3). Emulsified nZVI particles were used to treat trichloroethylene...
(TCE) contamination in groundwater with degradation rates of between 60%–100% at all depths targeted.

10.7 Synthesis of Iron Nanoparticles

There are many different methods for producing nZVI. A number of the most utilized techniques include co-precipitation, reduction of aqueous iron salts, hydrothermal synthesis, thermal deposition, microwave, microemulsion, ball milling, sonochemical and sol-gel, and forced hydrolysis techniques (Figure 10.4).

10.7.1 Ball Milling

Ball milling is a physical approach to the generation of nano-scaled materials, including crystalline structures, nanoparticles, and nanocomposites using mechanical energy. Starting materials (i.e., iron oxides) are powdered and then put into a milling chamber containing balls (made from silicon, steel, iron, carbide, or tungsten) (Li et al. 2009). The milling chamber rotates, and the powdered iron/iron oxide is crushed against the wall of the vessel. The balls aid in crushing the iron particles, leading to smaller particle sizes. Pressures experienced with the crushing of the parent material lead to compositional changes, including hardening, fracturing, and rewelding of powdered particles. Milling mixtures of different materials can lead to chemical reactions and subsequent formation of new materials (Andrews et al. 2010). This method of nanoparticle synthesis is simple and cheap. Surfactants or stabilizers can be added to the freshly ground nanoparticles to prevent agglomeration and passivation (Poudyal 2008). Nanoparticles with monodispersed attributes are difficult to achieve via this method. However, some degree of particle sizing can be controlled by increasing temperatures and milling time (Safarik and Safarikova 2012).
FIGURE 10.4
The complexity involved in the generation of iron nanoparticles for environmental remediation purposes.
10.7.2 Co-Precipitation

Co-precipitation is perhaps the simplest method of generating iron oxide nanoparticles (mainly used to generate Fe₃O₄, γ-Fe₂O₃, or FeOOH species). Simply, nanoparticles are generated by aging stoichiometric mixture of ferric (Fe³⁺) and ferrous (Fe²⁺) salts in a 1:2 M ratio, respectively, under highly aqueous alkaline medium.

As magnetite particles are particularly susceptible to oxidation, this process is usually performed under anaerobic conditions using an inert gas, that is, nitrogen. It is very difficult to generate monodispersed particles using this method. However, in an attempt to control and or alter the size, shape, and mono-dispersity of the particles, a number of different approaches have been taken.

Factors such as differing iron salts in different ratios, changes in reaction temperatures, optimization of pH conditions, mixing speed of solution, speed of reducing agent addition to iron salt solution, and ionic properties of the media have been all considered and optimized (Wu et al. 2008, Wu et al. 2015). To further maintain and control particle physical attributes, stabilizers and coatings are often incorporated to reduce passivation, agglomeration, size, and uniformity.

10.7.3 Reduction of Aqueous Iron Salts

The most common method for the generation of nZVI for environmental purposes is the reduction of iron salts using sodium borohydride under anaerobic conditions. The borohydride reduction of iron salts can proceed via the following ways:

1. \[ \text{Fe(H}_2\text{O)}_6^{3+} + 3\text{BH}_4^- + 3\text{H}_2\text{O} \rightarrow \text{Fe}^0 + 3\text{B(OH)}_3 + 10.5\text{H}_2 \]
2. \[ 4\text{Fe}^{3+} (\text{aq}) + 3\text{BH}_4^{-}(\text{aq}) + 9\text{H}_2\text{O(l)} \rightarrow 4\text{Fe}^0 (\text{s}) + 3\text{H}_2\text{BO}_3^{-}(\text{aq}) + 12\text{H}^+ (\text{aq}) + 6\text{H}_2(\text{g}) \]
3. \[ 2\text{Fe}^{2+} (\text{aq}) + 8\text{H}_4\text{a}(\text{aq}) + 3\text{H}_2\text{O(l)} \rightarrow 2\text{Fe}^0 (\text{s}) + \text{H}_2\text{BO}_3^{-}(\text{aq}) + 4\text{H}^+ (\text{aq}) + 2\text{H}_2(\text{g}) \]

Although this method is fast and simple, sodium borohydride is a toxic compound that is likely to contaminate the environment. Ideally, it should therefore be substituted for more environmental alternatives (Crane and Scott 2012). However, in medical applications where smaller nanoparticle quantities are required, this synthesis technique may be appropriate. Sodium borohydride-generated particles are susceptible to spontaneous combustion in air and accelerated weathering in aerobic solutions. For these reasons, coatings are often generated to prevent these occurrences (Yan et al. 2013).

One such alternative is the use of polyphenols from plant leaves or fruits. Not only are the antioxidative extracts from plant biomass more environmentally friendly than sodium borohydride, but they also provide a protective capping for the nanoparticles. This capping reduces the initial passivation rate by shielding the particles from atmospheric and dissolved oxygen. When using plant-derived polyphenols for nanoparticle synthesis, the end product may not be nZVI due to the incomplete reduction of iron and therefore thorough x-ray diffraction (XRD) analysis should be used to confirm the composition of generated particles (Dorofeev et al. 2012).

Green synthesis is cheap, as costly chemicals are not necessary, and the process does not require increased temperatures, pressures, or additional energy inputs (Stefaniuka et al. 2016). Different plant extracts can lead to differing size, shape, particle uniformity, and agglomeration characteristics. As plant biomass weight is not directly related to its polyphenol-containing concentration, measurements of the reducing capacity of extracts may be required between preparations to ensure reproducible results.
10.7.4 Hydrothermal/Hydrosolvo Synthesis

Hydrothermal synthesis, as the name suggests, utilizes supercritical water to generate nanoparticles. Hydrosolvo synthesis uses the same technology; however, solvents other than water are often used to generate species with specific attributes. Reactants are placed into aqueous reactors or autoclaves and subjected to temperatures that can exceed 300°C and pressures over 2000 psi. During these conditions, the water/solvent hydrolyzes and dehydrates metal salts. In the autoclave, iron salts and stabilizers can be dissolved in concentrations that are not soluble at ambient conditions. There are also regions within the vessel with lower temperatures that allow for seeding and particle growth to occur (Hayashi and Hakuta 2010; Safarik and Safarikova 2012).

Nanoparticles generated via this method possess ordered structures with a high degree of crystallization. Size and shape characteristics can be modified by altering the reaction time temperature, pressure, and solvent (Wu et al. 2015).

10.7.5 Thermal Decomposition

As previously mentioned, in order to obtain nanoparticles with high degrees of crystallinity, they must be subjected to high temperatures in many cases. Thermal decomposition is a method of generating nanoparticles via the thermal decomposition of organometallic precursors, often organometallic complexes. Iron precursors may be thermally decomposed via hot-injection methods, or prepared reaction mixtures can be heated in a reaction vessel at temperatures that may exceed 300°C (Tartaj et al. 2003; Frey et al. 2009). Due to the tendency of iron and certain iron oxides (i.e., Fe⁰ and magnetite, respectively) to rapidly oxidize in aerobic conditions, the generation of particles via thermal decomposition is often conducted with coatings/stabilizers including hot organic surfactants. Nucleation/seeding can be separated from growth phases via this synthesis method, unlike co-precipitation methodologies. This aids in the generation of monodispersed particles and the control of size parameters by optimizing temperature, decomposition time, and ferric salts used (Wu et al. 2015). This technology is regarded as simple to use and low in cost, and has the ability to generate high-quality nanoparticles on large scales (Safarik and Safarikova 2012).

10.7.6 Microwave-Assisted Synthesis

It has been shown that nanoparticle synthesis methods incorporating high temperatures can aid in improving or instilling crystallinity in nanoparticles. In microwave-assisted synthesis of nanoparticles, two heating mechanisms are utilized. First, dipolar polarization, in which microwave radiation forces molecules to align their dipoles within the electric field. The oscillating field reorients the molecules, and the dipoles attempt to realign, causing friction and subsequent heat generation and loss.

Ionic conduction is the second heating method and is a result of charged ions vibrating due to their excitement from the microwave field. This vibration leads to collisions with molecules or atoms, resulting in heat generation (Baghbanzadeh et al. 2011, Wu et al. 2015, Kalyani et al. 2015).

Due to significant and rapid heating, crystalline nanoparticles can be generated in less than half an hour. This NP synthesis method also provides control over particle size distribution, enhanced physiochemical properties, and generation of crystalline particles. It is seen as being faster, more environmentally friendly, and cheaper than conventional NP synthesis methods (Safarik and Safarikova 2012, Singh and Nakate 2013).
10.7.7 Microemulsion

Microemulsion-mediated iron nanoparticle synthesis consists of a nonpolar oil phase, a surfactant phase (containing both hydrophobic and hydrophilic groups), and an aqueous polar phase (Wu et al. 2015). The hydrophobic ends of the surfactant dissolve into the oil phase, and the hydrophilic ends dissolve into the aqueous phase. A cosurfactant may also be used to lower the interfacial tension (Mohapatra and Anand 2010).

The aqueous phase generally contains metal salts, and the oil phase may contain complex mixtures of hydrocarbons, olefins or other nonpolar entities. Small droplets of the immiscible phase become dispersed into the continuous phase (this can be either nanosized aqueous phase droplets dispersed in an oil phase stabilized by surfactants or vice versa) (Mohapatra and Anand 2010). In these nanocavities, self-assembled nanoparticles can form. The size of the nanoparticles is generally limited to the dimensions of the formed nanocavities and therefore monodispersed nanoparticles with spherical shapes are often generated. Size parameters can be controlled by altering the concentrations of the dispersed and surfactant phases. Due to the low temperatures experienced via this synthesis method, the nanoparticles generated often have poor crystallinity (Hasany et al. 2012).

10.7.8 Sonochemical

In sonochemical synthesis of nanoparticles, water and other secondary organic compounds are subjected to ultrasonic radiation (20 kHz–10 MHz), where acoustic waves create bubbles (cavities) that vibrate and accumulate ultrasonic energy. When a bubble grows too large due to diffusion of the solute vapor, it collapses and releases the stored energy resulting in an implosion with temperatures over 4500°C experienced (temperatures that are rapidly quenched) and pressures up to 1800 atm. This process generates H• and OH• radicals (the addition of organic additives can produce other radical species), which act as reductants. These radical species reduce iron salts to form iron oxide nanoparticles (Bang and Suslick 2010, Mousavi and Ghasemi 2010). Nanoparticle synthesis using this approach has little control over shape and mono-dispersity of particles. Furthermore, particles with amorphous structures are often formed. This can be beneficial for enhanced reactivity in some cases (Tables 10.1 and 10.2). Furthermore, particles generated by this method have been shown to remain suspended in solution without settling for over 12 months (Teo et al. 2009, Wu et al. 2015).
<table>
<thead>
<tr>
<th>Method of Synthesis</th>
<th>Nanoparticle Generated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ball milling</td>
<td>nZVI, ( \gamma )-Fe(_2)O(_3), ( \alpha )-Fe(_2)O(_3)</td>
<td>Li et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lemine et al. (2009)</td>
</tr>
<tr>
<td>Reduction (sodium borohydride)</td>
<td>nZVI, FeOOH, Fe(_3)O(_4), ( \alpha )-Fe(_2)O(_3)</td>
<td>Agarwal et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Farahmandjou and Soflaee 2014</td>
</tr>
<tr>
<td>Reduction (plant extracts)</td>
<td>Fe(_3)O(_4), FeOOH, ( \alpha )-Fe(_2)O(_3), amorphous NP’s</td>
<td>Ahmmad et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Herlekar et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shahwan et al. (2011)</td>
</tr>
<tr>
<td>Co-precipitation</td>
<td>Fe(_3)O(_4), ( \gamma )-Fe(_2)O(_3) or FeOOH species, amorphous NP’s</td>
<td>Mohapatra and Anand (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wu et al. (2008)</td>
</tr>
<tr>
<td>Hydrothermal/hydrosolvo synthesis</td>
<td>( \alpha )-Fe(_2)O(_3), FeOOH, Fe(_3)O(_4)</td>
<td>Mohapatra and Anand (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wu et al. (2008)</td>
</tr>
<tr>
<td>Thermal decomposition</td>
<td>Fe(_3)O(_4), ( \alpha )-Fe(_2)O(_3), ( \gamma )-Fe(_2)O(_3)</td>
<td>Wu et al. (2008)</td>
</tr>
<tr>
<td>Microwave-assisted synthesis</td>
<td>Fe(_3)O(_4), ( \gamma )-Fe(_2)O(_3), ( \alpha )-Fe(_2)O(_3), FeOOH, amorphous NP’s</td>
<td>Mohapatra and Anand (2010)</td>
</tr>
<tr>
<td>Microemulsion</td>
<td>Fe(_3)O(_4), ( \alpha )-FeOOH, ( \gamma )-Fe(_2)O(_3), ( \alpha )-Fe(_2)O(_3), amorphous NP’s</td>
<td>Mohapatra and Anand (2010)</td>
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<tr>
<td></td>
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<td>Petrova et al. (2006)</td>
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<td></td>
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<td>Wu et al. (2008)</td>
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<td></td>
<td></td>
<td>Avanaki and Hassanzadeh (2013)</td>
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<td></td>
<td></td>
<td>Cheng et al. (2011)</td>
</tr>
<tr>
<td>Sonochemical</td>
<td>nZVI, Fe(_3)O(_4), Fe(_2)O(_3), amorphous NP’s</td>
<td>Mohapatra and Anand (2010)</td>
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<tr>
<td></td>
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<td>Wu et al. (2008)</td>
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</tbody>
</table>
## TABLE 10.2
Surface Modifications for Iron Nanoparticles, Their Properties, and Advantages for Their Use

<table>
<thead>
<tr>
<th>Surface Coating</th>
<th>Properties</th>
<th>Advantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural polymers, that is, starch</td>
<td>• Organic polymer</td>
<td>• Easy to source</td>
<td>• He and Zhao (2009)</td>
</tr>
<tr>
<td></td>
<td>• Various different coatings with different properties (dependent on polymer used)</td>
<td>• Capping agent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Nontoxic</td>
<td>• Reduce agglomeration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Cost effective</td>
<td>• Advantageous for negatively charged metal ion remediation</td>
<td></td>
</tr>
<tr>
<td>Carboxymethyl cellulose (CMC)</td>
<td>• Linear</td>
<td>• Nontoxica</td>
<td>• Liang et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>• Anionic</td>
<td>• Cost effective</td>
<td>• Kim et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>• Nongelling polymer</td>
<td>• Environmentally friendly</td>
<td>• Sakulchaicharoen et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>• Negatively charged</td>
<td>• High density of charged functional groups</td>
<td>• He et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>• Polyelectrolyte</td>
<td>• Enhances remediation due to absorption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• High molecular weight</td>
<td>• Strong binding to nanoparticle</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Advantageous for TCE degradation (with increasing chain length)</td>
<td></td>
</tr>
<tr>
<td>Polyvinyl alcohol (PVA)</td>
<td>• Metal-ion-polymer-complex formed</td>
<td>• Three coatings, each providing beneficial properties</td>
<td>• Bepari et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>• Hydrophilic</td>
<td>• Block 1—capping agent for protection from oxidation</td>
<td>• Kundu et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>• Can transform into polymer gel</td>
<td>• Block 2—targeting of NAPL pollutants</td>
<td>• Liu et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Block 3—particle–particle electrosteric repulsion</td>
<td></td>
</tr>
<tr>
<td>PMAA-PMMA-PSS triblock</td>
<td>• Triblock coating</td>
<td>• Structure can be modified, that is, cross-linked, chemically modified</td>
<td>• Sirk et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>• Block 1—poly methacrylic acid (carboxylic acid)</td>
<td>• Reduces agglomeration</td>
<td>• Phenrat and Lowry (2014)</td>
</tr>
<tr>
<td></td>
<td>• Block 2—poly methyl methacrylate (low polarity)</td>
<td>• Stable dispersions</td>
<td>• Krajangpan et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>• Block 3—polystyrenesulfonate (strong electrolyte)</td>
<td>• Nontoxic</td>
<td>• Saleh et al. (2005)</td>
</tr>
<tr>
<td>Mesoporous carbon/silica coating</td>
<td>• Inert</td>
<td>• Large surface area</td>
<td>• Petała et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>• May act as an absorbent</td>
<td>• Pore size can be altered</td>
<td>• Tang et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Limited particle–particle attraction</td>
<td>• Knežević (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• High thermal stability</td>
<td>• Zamani et al. (2009)</td>
</tr>
<tr>
<td>Emulsified nZVI</td>
<td>• Viscous</td>
<td>• Potential to absorb and then degrade pollutants</td>
<td>• O’Hara et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>• Biodegradable</td>
<td>• Interacts with NAPL pollutants by dissolving to expose iron</td>
<td>• Müller and Nowack (2010)</td>
</tr>
<tr>
<td></td>
<td>• Hydrophobic</td>
<td>• Protects nanoparticles from oxidation and hydrolysis</td>
<td>• Yanga and Chang (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• May promote bioremediation</td>
<td>• Rajan (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Enhances reactivity of nanoparticle</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
TABLE 10.2 (Continued)
Surface Modifications for Iron Nanoparticles, Their Properties, and Advantages for Their Use

<table>
<thead>
<tr>
<th>Surface Coating</th>
<th>Properties</th>
<th>Advantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological capping using plant- or algal-based polyphenols</td>
<td>• Water soluble</td>
<td>• Environmental friendly</td>
<td>Machado et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>• Various different coatings with different properties (dependent on plant extract used)</td>
<td>• May promote bioremediation</td>
<td>Shahwan et al. (2011)</td>
</tr>
<tr>
<td>Immobilization</td>
<td>• Fixed nanoparticles</td>
<td>• Easy to apply</td>
<td>Pattanayak et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>• Loaded within matrix or on surfaces of different materials</td>
<td>• Prenanoparticle synthesis capping from oxidation</td>
<td>Mahdavi et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cost effective</td>
<td>Pattanayak and Nayak (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Decreases aggregation potential</td>
<td>Machado et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Removable from system, reducing environmental impact</td>
<td>Petala et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Can be used for surface water remediation</td>
<td>Fu et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Optimum colloidal properties</td>
<td>Tang et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Capacity to incorporate catalysts that would otherwise be toxic to the environment for enhanced remediation</td>
<td>Luo et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Potential pollutant-absorptive properties</td>
<td>Abbassi et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Arancibia-Miranda et al. (2016)</td>
</tr>
</tbody>
</table>

10.7.9 Optimization

Iron nanoparticles for environmental remediation purposes are a current and successful technology; however, they are far from perfect. Different contaminated environments require different attributes for optimum environmental regeneration. For example, if using nanoparticles for groundwater remediation, particles should possess coatings to facilitate movement through the underground matrices. However, in surface water, nanoparticles may need to be affixed onto solid supports, so they can be retrieved and prevent the release of excessive nanoparticles into the environment (Figure 10.5).

There is a vast plethora of different attributes that nanoparticle researchers are aiming to optimize. Some issues that are in focus relate to desired shape, crystallinity, passivation reduction, increased reactivity, increased mobility, agglomeration reduction, coatings that target pollutants, solid support matrix materials, and reduction in synthesis costs.

10.8 Different Methods of Generating Coatings

Currently, there is significant research in the area of generating stabilizers and coatings for nZVI particles. Bare or naked iron nanoparticles may be highly reactive; however, they cannot be used for in situ remediation projects due to a number of inherent pitfalls.
Particle agglomeration reduces the surface area available for reactions to occur on nanoparticle surfaces and in turn reduces remediation efficiency. Also, agglomerated nanoparticles are unable to travel significant distances through the underground matrix for groundwater remediation.

**FIGURE 10.5**

Comparison between the use of free-iron nanoparticles and immobilized iron nanoparticles for surface water remediation.

Coatings may be applied to nanoparticles to prevent the attractive interactions they have with one another (Figure 10.4). Coatings are also added to particles to help them overcome attractive forces of groundwater constituents while targeting pollution sources. Passivation of particles may also be counteracted via surface coatings. These coatings will greatly increase shelf lives and prevent rapid oxidation. Oxidized particles have lower intrinsic reactivity and may be rendered useless for application.

Coatings for nanoparticles are generally applied pre-synthesis, post-synthesis, or a combination of both. Pre-synthesis methods usually involve the addition of the coating to the precursors, that is, iron salt solution and reductant addition, while post-synthesis methods, as the name suggests, involve the coating of the nanoparticle bulk material. Generally, iron nanoparticles with post-synthesis coating application show reduced reactivity, whereas pre-synthesis methods may increase reactivity and aid in controlling particle size, shape, and uniformity.

Although commercially used nanoparticles are currently used for remediation purposes, they still have shortcomings regarding reactivity, transport in underground matrices, and overall pollution catabolism (Mueller et al. 2012).

Some of the commonplace and emerging coating technologies are described below:

**10.8.1 Natural Polymers—Starch**

Polysaccharides, including starch and cellulose, are highly abundant and easy-to-source plant biomass components that have been shown to be effective stabilizers for nanoparticles. These entities prevent oxidation and particle–particle attractive forces. Starch is a linear, neutral, and gelling polymer. In some circumstances, that
is, the absorption of negatively charged metal ions, the use of starch stabilizers may be beneficial over carboxymethyl cellulose (CMC)-stabilized particles (He and Zhao, 2009).

10.8.2 Polyelectrolyte-Carboxymethyl Cellulose

Carboxymethyl cellulose is a linear, anionic, and nongelling polymer (Liang et al. 2013). Negatively charged polymers are seen as beneficial for in situ remediation, as they repel attractions from negatively charged species, that is, soil particles or oxidized humic acids. Polysaccharide coatings for nanoparticles can be applied via both pre- and post-synthesis routes. Kim et al. (2009) showed increases in mobility through porous media with CMC modified with a posttreatment method of nZVIs, and Sakulchaicharoen et al. (2010) showed enhanced stability and de-chlorination of bimetallic Fe/Pd nanoparticles using a pre-synthesis approach. CMC has a stronger affinity to iron nanoparticles compared to that of starch, as it stabilizes particles via electrostatic repulsions as opposed to steric hindrance (Liang et al. 2013). It has been shown that CMCs with increasing chain length have a greater capacity to degrade TCE (He et al. 2009, Fan et al. 2015).

10.8.3 Polyvinyl Alcohol

Polyvinyl alcohol (PVA) generation/coating of iron nanoparticles is a simple procedure whereby PVA aqueous solution is used to precipitate iron salts. PVA’s OH groups form a metal-ion-polymer complex, and the resulting nanoparticles are hydrophilic and form electrostatic and steric repulsions, leading to stable dispersions of relatively monodispersed particles (Kundu et al. 2011, Bepari et al. 2014). One drawback of using PVA alone is that it instantly dissolves in water and therefore is short lived for many environmental applications (Liu et al. 2013). Modified PVA coatings (cross-linked PVA or chemically modified structures) have provided additional adsorption onto nanoparticles and reduced solubility in aqueous solutions. However, their use as a host matrix for block addition of further coatings has also been reported (Gao and Seliskar 1998). This polymer is a biodegradable synthetic polymer and therefore its use within in situ environments is possible.

10.8.4 Poly(Methacrylic Acid)-Block-(Methyl Methacrylate)-Block-(Styrenesulfonate) Triblock

Poly(methacrylic acid)-block-(methyl methacrylate)-block-(styrenesulfonate), or the PMAAPMMA-PSS triblock coating, has been specifically engineered to combat a number of pitfalls associated with bare nanoparticles. Poly methacrylic acid is a carboxylic acid that has a strong affinity to adsorb to iron nanoparticle surfaces. This block acts as a capping agent that reduces rapid passivation of the particle while presenting functional groups that will interact with the poly methyl methacrylate coating. The PMMA block not only creates a low polarity region that aids in thermodynamically targeting non-aqueous phase liquids (NAPLs) pollutants, but also prevents water from reaching the particle. This layer prevents passivation and collapses upon interacting with NAPL, allowing the encapsulated nanoparticle to react and catabolize the pollutant. The third block, the polystyrenesulfonate coat, consists of a strong electrolyte that generates electrosteric repulsions between particles so that they remain independent and do not agglomerate. Furthermore, the negative charge on this coat also minimizes particles interactions with negatively charged groundwater species. In summary, PMAA-PMMA-PSS-coated nanoparticles are electrosterically independent particles that target NAPL pollutants and possess extended shelf lives compared to uncoated nanoparticles. Due to electrosteric particle–particle repulsions and subsequent reduction in agglomeration potential, their capacity to travel through groundwater matrices is superior to uncoated nanoparticles. This coating is applied after synthesis of nanoparticles (Saleh et al. 2005, Sirk et al. 2009, Krajangpan et al. 2012, Phenrat and Lowry 2014).
10.8.5 Mesoporous Carbon/Silica Coated

Iron nanoparticles containing mesoporous carbon or silica coatings have had a large focus in the medical nanotechnology field, as drugs or ligands can be added in larger concentrations than bare nanoparticles. This is due to the large number of pores that increase the surface area and the fact that carbon and silica are inert and therefore will not cause secondary reactions within the body (Zamani et al. 2009).

Little research has been conducted with mesoporous silica/carbon nanoparticles in terms of remediation with NAPL pollutants; however, a significant focus has been directed toward the absorbance of toxic metal ions (Petala et al. 2013). These particles possess large specific surface area, pore size that can be altered, hydrophobicity, high thermal stability, limited particle–particle attractions, and increased magnetism (Tang et al. 2014). Both mesoporous silica and carbon possess absorbent properties that can assist in retrieving metal ions from solutions and in turn speed up the rate of remediation. Addition of these coatings is conducted using post-synthesis techniques. There are several mesoporous silica coatings (i.e., SBA-15, KIT-6, MMSN) and mesoporous carbon coatings (i.e., CMK-3, CNC, OMC) being used and researched (Alam et al. 2009, Knežević 2014).

10.8.6 Emulsified Nanoscale Zero-Valent Iron

Iron nanoparticles need to be in contact with water to achieve reductive dehalogenation. However, NAPL pollutants are insoluble in water and therefore surface areas available for reaction and/or transport to the oil/water interface may be limited.

Emulsified iron nanoparticles are a technology where nanoparticles are suspended in an aqueous solution, that is, a water droplet, which is then further encapsulated by a surfactant and biodegradable oil (usually a vegetable oil). This technology aids in both transport of particles to the non-edge areas of NAPL pollutants and to thin out NAPL to increase the surface area available for degradation (O’Hara et al. 2006, Müller and Nowack 2010). Reactive nanoparticles are protected against reactive groundwater constituents, passivation is decreased, and breakdown constituents of large NAPL pollution molecules can dissolve in the oil, reducing their prevalence in the groundwater’s aqueous phase. Due to pollutants dissolving through the oil layer, a concentration gradient is achieved so that nanoparticles are not overwhelmed by reactants and steady degradation rates can be achieved (Rajan 2011, Yanga and Chang 2011).

Due to the increased viscosity of nanoparticle solution because of the oil, emulsified nanoparticles are only useful in sandy aquifers or for direct injection to the polluted zone, as low permeable soils will not facilitate transport. However, in optimum geographical conditions, their effectiveness against chlorinated solvents has been shown (Yanga and Chang 2011).

The majority of research conducted with emulsified nZVI is directed toward remediation of NAPL pollutants within groundwater scenarios. This is due to the hydrophobic/oil layer being beneficial for dissolving and interacting with these pollutants and its role in facilitating transport through the underground matrix. Quinn et al. (2006) utilized emulsified nZVI for a different approach. Sea water is particularly corrosive toward iron and therefore the use of iron nanoparticles for ocean remediation is not a highly researched area in the field. However, Quinn et al. (2006) were able to demonstrate that emulsified nZVI was able to withstand rapid weathering from sea water salts and also remove over 90% of lead and copper, with initial concentrations of these metal pollutants being at 5 mg/L (Quinn et al. 2006).
**10.8.7 Biological Capping Agents-Plant/Algal-Based Polyphenols**

Reducing iron salts with plant-based polyphenol solutions is an environmentally friendly and simple method for capping and generating iron nanoparticles. Simply, plant biomass is heated in aqueous solutions below 100°C and then filtered to remove solids. The resulting solution is reacted with iron salts, reducing them into nanoparticles, and a plant-derived coating is generated, preventing them from oxidation.

A number of plant extracts have been used to generate capping properties for iron nanoparticles. Machado et al. (2015) compared extracts from 26 different tree species to determine differences in particle size, shape, reactivity, and agglomeration tendency. Findings from this study showed that different extracts generated particles with different properties; however, the vine and medlar extracts were considered best for site remediation (Machado et al. 2015).

In the vast majority of literature published, plant extracts for nanoparticle synthesis and coating resulted in agglomerated particles (Machado et al. 2015 [various plant extracts], Shahwan et al. 2011 [green tea], Pattanayak et al. 2013 [clove], Mahdavi et al. 2013 [seaweed], Pattanayak and Nayak 2013 [neem tree]). However, exceptions do exist (e.g., Machado et al. 2014 [mandarin]).

**10.8.8 Immobilized Nanoparticles on Solid Supports**

Iron nanoparticles for the remediation of pollutants in surface waters should be used in conjunction with either permeable reactive barriers or loaded onto immobilized solid supports. Due to their small size, retrieval of free particles is virtually impossible in uncontrolled natural environments. In addition, free iron nanoparticles have been shown to adversely affect fish and their embryos (Chen et al. 2013), which can be detrimental for aquatic health (Figure 10.5).

Considerable research is being conducted in this area with nanoparticles being immobilized onto clays, mesoporous silica, rectorite, resins, alginate, multiwalled carbon nanotubes, and bentonite, and by using two solvent-reduction methods, just to name a few recent examples. Solid-support immobilization of iron nanoparticles allows particles to be added to surface waters and then removed when reactions are complete, or nanoparticles are pacified. Nanoparticles may either be synthesized and then added to immobile supports or generated directly onto solid supports. The latter has been shown to reduce large variations in size fluctuations and decrease aggregation potential (Petala et al. 2013). Additionally, some coatings (i.e., zeolites and SBA-15) can increase the catalytic and sorptive properties of iron nanoparticles.

A study conducted by Fu et al. (2013) used ion exchange resins as a solid support matrix for nZVI nanoparticles, as they have been implicated in the removal of either cations or anions from metals in solution. Upon stabilization of nZVI to the resin, the resin/nZVI support was able to remove both anions and cations. At optimal conditions, >84% of Cr(VI) was removed from solution when the initial Cr(VI) concentration was 20 mg/L.

Tang et al. (2015) used a dual-solvent method for impregnating SBA-15 (mesoporous silica) with nZVIs. SBA-15 was mixed with n-pentane and then a ferrous solution was added dropwise and allowed to dry. Under anaerobic conditions and in the presence of n-pentane, the supports were exposed to sodium borohydride, forming
immobilized nZVI onto SBA-15 supports. These supports were used to degrade p-nitrophenol, a nitro aromatic compound. Following 30 days of aerobic exposure, >80% of p-nitrophenol was degraded.

Immobilized iron nanoparticles on clay supports have been the focus for a number of researchers (Abbassi et al. 2013, Luo et al. 2013, Arancibia-Miranda et al. 2016) due to their pollutant absorption capacity, low cost, and optimal colloidal properties. Luo et al. (2013) generated nanoscale zero valent iron-rectorite (nZVI-R) composites for the removal of orange II dye. Very small nZVI nanoparticles (10 nm) were immobilized between the interlayer regions of the rectorite clay. This ability of this composite to degrade Orange II dye (70mg/ L) was compared to commercial nZVI and proved to be superior, achieving complete removal of the parent compound within 10 minutes.

10.8.9 Bimetallic Particles

For contaminant remediation, iron nanoparticles (mainly nZVI) have been considered optimal when compared to other monometallic nanoparticles due to their cost effectiveness, high reactivity, and relatively low toxicity. However, it has been shown that the addition of a metal catalyst can increase reactivity. As iron nanoparticles have a negative redox potential, noble metals with a positive redox potential (i.e., Cu, Ag, Pd, Pt, and Ni) are combined with iron to form bimetallic nanoparticles. With these particles, iron can either be a surface for pollutant absorption/adsorption or act as an electron donor for interface reductive degradation of pollutants (Liu et al. 2014a,b).

To date, there are a number of commercially available iron nanoparticles available for environmental site remediation. Bimetallic particles are less prevalent in the marketplace despite their exceptional degradation efficiencies due to the uncertainties related to their environmental toxicity of the noble catalyst (O’Carroll et al. 2013). Furthermore, bimetallic nanoparticles are subject to elevated corrosion rates and therefore optimization of Fe to catalytic metal and the application of coatings to increase shelf lives may be required (Schrick et al. 2002).

An exception to the rules can be seen with iron/palladium bimetallic particles. They are sold commercially, have shown reduced corrosion compared to their Fe⁰ counterparts (Liu et al. 2005), and their usage has, in the degradation of trichloroethane, resulted in a reduction in up to two orders of magnitude of TCE compared to iron nanoparticles alone. The addition of palladium to iron nanoparticles has been shown to be environmentally benign in groundwater conditions, as it is likely to remain insoluble and therefore will not be bioavailable (Cook 2009).

10.9 Characterization Techniques

10.9.1 Scanning Electron Microscopy

Scanning electron microscopy (SEM) is routinely used within nanoparticle research. SEM’s primary function is to provide information pertaining to the surfaces of specific entities. Samples to be imaged using SEM must have a surface that is electrically conductive. For dried metallic nanoparticle imaging, no further sample preparation will be required. However, if nanoparticles possess a thick organic surface coating or are immobilized onto a surface without electrical properties, they may require further preparation. Biological samples may first require critical
point drying followed by sputter coating of electrically conductive material (often gold or platinum). Nonorganic samples may only require sputter coating before imaging can proceed.

Once a sample is loaded onto the stage, it is irradiated with a high-energy electron beam that, in most circumstances, scans across the sample. There are a number of signals produced when the electron beam hits a sample. These include secondary electrons, backscattered electrons, Auger electrons, characteristic x-rays, and photons of various energies. Although some information relating to composition and crystallinity may be derived from Auger electrons, characteristic x-rays, and photons, the data will not be quantitative. Secondary and backscatter electrons are mainly used for sample imaging. Secondary electrons are mainly responsible for mapping out the surface topography and morphology of samples, while backscattered electrons illustrate the contrast in composition for multiphase samples (Goldstein et al. 2017).

Scanning electron microscopy is a particularly powerful tool for visualizing milli- to nanoparticulate matter; however, in many cases SEM does not have the necessary resolution to clearly image nanoparticles between 1–100 nm in size. If detailed surface imaging is not necessary or possible, the use of a transmission electron microscope (TEM) is used for particle imaging.

10.9.2 Transmission Electron Microscopy

When obtaining visual images for individual particle shape, size, oxide layer, and agglomeration tendency, TEM is the one-stop tool for the job. Like SEM, TEM uses electrons to generate images of miniature-sized samples. In TEM, the electrons interact with the sample as they are transmitted through it (Chekli et al. 2015). The transmitted electrons form an image on a fluorescent screen that can be imaged using a charge-coupled device (CCD) camera. Specimens imaged by TEM need to be thin enough in at least one dimension for electrons to pass through them (<50–100 nm). The interactions between the electrons and the specimen’s internal matter aid in generating images that can give information about internal components of entities. For example, in a polymer surface-coated iron oxide nanoparticle, there will be differences in contrast between the surface coating, the oxide layer, and the metallic iron core.

When determining the crystallinity of a sample by means of crystal lattice structure, electron scattering, and diffraction are important parameters to test. When the electron beam hits the sample in a TEM, the electrons are not truly transmitted but scattered in a forward direction. With diffraction patterns, the majority of the intensity is found within the direct beam resulting from un-scattered electrons. Scattered diffracted waves create diffraction spots. These diffraction waves are then recombined to generate an image. The use of electromagnetic lenses allows diffracted electrons to be focused into a regular arrangement of diffracted spots, which is known as a diffraction pattern (Bendersky and Gayle 2001). These diffraction patterns are characteristic of specific entities depending on the purity and crystallinity of the sample being analysed.

10.9.3 X-Ray Diffraction

Each crystalline material has a group of atoms ordered with a specific arrangement. This is called a unit cell, and it is repeated continuously in three dimensions to make up a crystal lattice. The number of times the unit cell repeats is unrestricted; however, the repeating unit is characteristic for a specific entity.
X-ray diffraction (XRD) is a non-destructive technique used to determine the crystalline phases and their orientation within a particular solid. In order to do this, an x-ray beam is generated and passed through a substance. The atomic planes of a crystal will cause these x-rays to diffract in different directions. Characteristic scattering patterns are designated to a specific entity (diffraction patterns). The Scherrer equation is often used to determination of size of particles of crystals in the form of powder.

The Joint Committee on Powder Diffraction Standards (JCPDS) has a database of powder diffraction patterns of more than 50,000 entries. Once a signature diffraction pattern has been generated from XRD analysis, the resulting spectra are compared against species in this database to confirm the crystal makeup of the analysed sample. (Massa 2004).

Not all iron nanoparticles will possess a XRD pattern, and this is dependent on the degree of order in the nanoparticle structure itself. Different synthesis methods can lead to disordered structures; that is, the sonochemical method for preparing iron nanoparticles involves a step whereby extreme high temperatures are experienced followed by rapid cooling. This process is not beneficial for crystal structure formation.

**10.9.4 Energy-Dispersive X-Ray Spectroscopy**

Energy dispersive x-ray spectroscopy (EDS) is a technique used in conjunction with SEM or TEM to determine the elemental composition of a sample. An electron beam is focused or scanned across a sample, and this excites electrons within a sample.

Electrons from an inner shell of atoms move to a higher energy level, and there is an unstable gap that needs to be filled. An electron from a higher energy level fills this void, and a signature x-ray emission is created and released. This x-ray is representative of the atomic number of the element it is derived from and is the difference in energy between the high and low energy shells. Elemental composition is measured using an energy-dispersive spectrophotometer (Shindo and Okawa T. 2002; Rahman et al. 2011).

**10.9.5 Fourier Transform Infrared**

Fourier transform infrared (FTIR) spectroscopy is a technique used to identify signature vibrational frequencies related to specific chemical functional groups located near the surface of molecules. Infrared absorbance spectroscopy measures the loss of IR radiation transmitted through a sample. An uncoated Fe⁰ nanoparticle is expected to have a specific vibrational frequency upon being exposed to IR radiation, as all molecules are made up of the same pure Fe⁰ element (Devi and Gayathri 2010). Coatings deposited to the outside of Fe⁰ nanoparticles will disrupt this frequency and alter the signature resonance due to absorption of radiation (Bellisola and Sorio 2011).

Simply, IR radiation is directed toward the sample, and this radiation either transmits through or is reflected off its surface. Different frequencies of IR radiation are used, and the variances in transmitted frequency can be due to bond stretching/bond deformations, asymmetric bending, twisting, wagging, rocking, and scissoring motions (Bellisola and Sorio 2011). The aforementioned bond characteristics alter the absorbance potential of the differing IR radiation wavelengths. The absorption bands of specific functional groups are characteristic for each entity, and once a vibrational band assignment is identified, the measured signal is transferred into an IR spectrum. The IR spectrum shows the absorption intensity as percentage transmission vs. wave numbers (cm⁻¹) and is
comparable against databases containing spectra for specific entities for species identification (Devi and Gayathri 2010, Baraton 2015).

This tool is used in nanoparticle research when organic polymer coatings need to be identified or plant-based capping agents are used (Heera and Shanmugam 2015).

10.9.6 Dynamic Light Scattering & Zeta Potential

Dynamic light scattering (DLS) is commonplace for determining the size of nanoparticles among researchers. Unlike SEM and TEM, it is capable of analysing large numbers of nanoparticle sizes, permitting more representative findings for non-agglomerated monodispersed samples. However, intensities generated from agglomerated particles overlay those of smaller particles, providing skewed results (Lim et al. 2013, Fissan et al. 2014).

DLS measures the Brownian motion of suspended particles and calculates their size based on their velocity or translational diffusion coefficient. Larger particles move more slowly through the liquid, and this alters the frequency of measurable scattered light (Lim et al. 2013, Neoh et al. 2015).

Not only can DLS estimate particle size, it can also determine particles zeta potential (surface charge) by electrophoretic light scattering measurements. An electric field is applied to the particles and those with positive charge will migrate to the negatively charged terminal and vice-versa. A laser beam is used to determine the migration speed because of particle charge. As the particles are mobile the scattered light has different frequency than the original laser and the frequency shift is proportional to the speed of the particles. Particles colloidal stability can be measured with this approach with zeta potential values of ± 0–10 mV, ± 10–20 mV and ± 20–30 mV and > ± 30 mV as highly unstable, relatively stable, moderately stable and highly stable (Lewicka and Colvin 2013).

10.9.7 X-Ray Absorption Spectroscopy

X-ray absorption spectroscopy (XAS) is particularly useful for determining the atomic structure of nanoparticles. Unlike XRD, XAS can give structural information (oxidation state, bond length, coordination number, and electronic configuration) of both crystalline and amorphous materials alike (Chekli et al. 2015). Furthermore, samples can be analysed under varying environmental conditions. For nZVI particles, which have a tendency to rapidly oxidize in aerobic conditions, their properties can be measured in aerobic conditions (Chekli et al. 2015).

Upon a sample being irradiated with synchrotron radiation, an absorption edge is witnessed if the radiation is sufficient to photo-ionize and eject the core electrons from the nanoparticle sample. Higher energies are representative of higher oxidation states, and in turn valences can be identified (Sun et al. 2006, Barcaro et al. 2013a).

10.9.8 X-Ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) is a quantitative method for analysing surface composition (depth less than 10 nm) and chemical states (Sun et al. 2006). Samples are subjected to monochromatic x-rays of known wavelength, and as a result, electrons are ejected from atomic core levels. Kinetic energy is released and recognized by a detector that translates this information to binding energy (Chekli et al. 2015).
This is particularly useful for iron nanoparticles, as surface composition and rapid oxidation are prevalent. X-ray photoelectron spectroscopy can identify all elements involved in nanocomplexes and can also differentiate between each element’s oxidation state (Barcaro et al. 2013b). Furthermore, XPS can also be used to determine the catalytic potential of different nanoparticles. If nanoparticles readily oxidize in oxygen environments, the samples must be kept in anoxic conditions until just before use.

10.10 Application of Iron Nanoparticles for Pollution Remediation

Although iron nanoparticles and a limited number of bimetallic nanoparticles are being used commercially for in situ site remediation, there are still hesitations and concerns regarding their use due to nanoparticles being a relatively new technology for groundwater research. The first field-scale test of nZVI for groundwater remediation was in Trenton, New Jersey, in 2008 (Zhang and Elliott 2006).

Well-established site remediation methods are likely to be selected over new technologies such as nanoremediation until there is irrefutable evidence the new technology is effective and does not contribute to considerable environmental harm itself. With further research enabling cheaper methods of generating nanoparticles and with more companies competing for a market share, their cost will decrease, and subsequent use is set to increase. With this increase, data on their effectiveness as a remediation strategy will be sufficient for contractors to consider them as a viable technology. Currently companies in the United States and Europe are using iron nanoparticles for remediation of groundwater. However, other countries around the world have not yet adopted them for the aforementioned reasons (Waltlington 2005).

10.10.1 Comparison of Nanoparticles with Other Technologies

Granular zero-valent iron particles (>100 nm in diameter) are currently utilized as a treatment material used to detoxify a wide range of environmental contaminants in groundwater, including viruses, heavy metals, chlorinated hydrocarbons, nutrients (nitrates and phosphates), arsenite (As [III]), herbicides, phenolic compounds, chelating agents, dyes, and pesticides (Nicole et al. 2011, Wijesekara et al. 2014, Lee et al. 2014). With such a broad spectrum of pollutant detoxification potential, zero-valent iron particles appear to be a “miracle treatment method” at first glance.

However, current approaches require granular zero-valent iron particles to be used in conjunction with permeable reactive barriers (PRBs) due to their size and affinity to agglomerate (particle charges create electrostatic attractions). This reduces the site-specific surface area on particles able to react with contaminants and also hampers their ability to be injected directly into the groundwater contaminant zone, as larger and bonded granular iron particles (>100 nm in diameter) cannot follow groundwater flows through the underground matrix (Nicole et al. 2011).

PRBs may be successful in some applications but can only treat pollutants that flow through the barrier. This means pollutants are not treated at the source of contamination. Pollutants that have trouble percolating through the soil matrix may not come into contact with the barriers, and remediation times for polluted areas where groundwater flows are slow may be lengthy. Furthermore, costs associated with excavating land to install and replace PRBs are high, and PRBs can only be installed in shallow unconsolidated aquifers (Grieger et al. 2010).
Since the year 2000, attention has shifted from millimetric zero-valent iron research to nanoscale zero-valent iron research. The key factors favouring nZVIs include large surface area-to-weight ratio, favorable quantum size properties, low standard reduction potential, higher in situ reactivity, and potential increase in transport efficiency through groundwater’s underground matrix (Kim et al. 2009, 2012, Nurmi et al. 2005). nZVIs have shown degradation rates of up to four orders of magnitude higher than their millimetric counterpart. However, for groundwater remediation, issues relating to agglomeration and nZVI binding to groundwater matrix constituents (i.e., clay soils, humic acids, and other naturally occurring groundwater elements) still generate problems for particle mobility and targeted contaminant reduction (Grieger et al. 2010, O’Carroll et al. 2013).

nZVIs have shown a great deal of promise for pollution catabolism, but the pitfalls preventing adequate groundwater remediation need to be addressed before they can be utilized and recognized as a viable technology. In order combat these pitfalls, modifications to the nZVI particles are suggested to enable them to become efficient remediation tools for groundwater.

10.11 Types of Pollutants Iron Nanoparticles Catabolize

Iron nanoparticles have shown tremendous capacity to mineralize a number of different pollutant classes either via direct or indirect means (Figure 10.4). Direct avenues may involve the reduction of pollutants, while indirect means can involve their status as a catalyst for the generation of reactive free radical species through processes such as the Fenton reaction.

Pollutants shown to be degraded in the presence of iron nanoparticles include but are not limited to the following:

- Chlorinated methanes—Dichloromethane (CH₂Cl₂), Chloroform (CHCl₃)
- Chlorinated benzenes—Hexachlorobenzene (C₆Cl₆), Trichlorobenzenes (C₆H₃Cl₃), Chlorobenzene (C₆H₅Cl)
- Chlorinated ethenes—Vinyl chloride (C₂H₃Cl), Trichloroethene (C₂HCl₃), trans-Dichloroethene (C₂H₂Cl₂)
- Polychlorinated and nonchlorinated hydrocarbons—PCBs, Pentachlorophenol (C₆H₅Cl₂O)
- Azo and anthroquinone dyes—Orange II (C₁₆H₁₁N₂NaO₄S), Remazol Brilliant Blue-R
- Pesticides—Lindane (C₁₄H₁₀Cl₉), DDT (C₁₄H₉Cl₅)
- Nitroaromatics—N-nitrosodimethylamine (NDMA) (C₄H₁₀N₂O), RDX C₃H₆N₆O₆
- Heavy metals—Mercury (Hg²⁺), Silver (Ag⁺)
- Other organic contaminants—(TNT)
- Inorganic anions—(NO₃⁻, AsO₄³⁻)
- Bromonated compounds—Decabromodiphenyl ether (DBDE) (Zhang 2003)

10.11.1 Chlorinated Solvents

Iron nanoparticles (nZVIs) have been utilized for the remediation of a number of different chlorinated compounds. Reductive dechlorination is the main driving force for their resulting in partial dechlorination of the parent compound or, ideally, complete dechlorination and conversion to ethene and chloride.

A simplified representation of the degradation process can be explained via the following reaction:

\[ \text{Fe}^0 + \text{RCl} + \text{H}_2\text{O} + \text{H}^+ \rightarrow \text{Fe}^{2+} + \text{RH} + \text{Cl}^- + \text{H}_2\text{O} \]
However, a number of different reductive steps may be at play (particularly for chlorinated methanes, ethanes, and ethenes). These include:

**Hydrogenolysis**—Substitution of a chlorine atom with hydrogen (reaction requires both an electron and a hydrogen donor to proceed).

**Beta Elimination**—Release of chlorine atoms and subsequent saturation of a carbon-carbon bond (dominant reductive dechlorination mechanism with nZVI) without the addition of a hydrogen atom.

**Alpha Elimination**—Removal of two chlorine atoms from a single carbon within the compound, saturating the bond. The reaction proceeds without the addition of hydrogen atoms.

**Hydrogenation and hydrolysis**—Breakdown of triple bonds to double bonds or double bonds to single carbon–carbon bonds via the addition of hydrogen (in the presence of water for hydrolysis) (O’Carroll et al. 2013).

Rajajayavel and Ghoshal (2015) showed that not only can nZVI degrade TCE, the process can be enhanced up to 40fold by the addition of sulfide to the surface of the particles. Sulfide is not used in the reaction and therefore degradation is mainly due to β-elimination degradation mechanism from nZVIs.

### 10.11.2 Dyes

Iron nanoparticles (predominantly nZVI) have been implicated in azo and anthroquinone dye degradation (Costa et al. 2012). The predominant mechanism for this is reduction. Absorption of dye molecules may also remove dye from solution; however, under passivation, the bound dye molecules are likely to dislodge and re-enter the system (Li et al. 2011b, Truskewycz et al. 2016).

The reduction mechanism for degradation is a consequence of iron nanoparticles reacting with oxygen and water to form unstable transitional compounds (free radicals), which are very reactive toward organic molecules. Of these, the hydroxyl radical is particularly efficient at cleaving azo bonds. The Fenton reaction (reaction of iron and hydrogen peroxide under acidic conditions) has been implicated in pollutant catabolism with iron nanoparticles (Pereira and Freire 2006, Bokare et al. 2007, Nidheesh et al. 2013) and will be discussed in further detail elsewhere in this chapter (see Fenton/Fenton like reactions).

In acidic media, it has been postulated that iron can donate two electrons to H⁺ and transform them to atoms. These atoms can interact with azo dye molecules, break their structure, and convert them into colourless amines (Rahman et al. 2014).

Nam and Tratnyek (2000) have shown that zero-valent iron was able to directly reduce 9 azo dyes (each containing SO₃⁻ groups) in acidic conditions. One of these reactions was with Orange II, and the resulting products from reductive cleavage of the azo bond were sulfanilic acid and 1-amino-2-naphthol. This can be seen in the following reaction:

\[
C_{16}H_{11}N_{2}NaO_{4}S + 5H^+ + Fe^{0} \rightarrow C_{6}H_{7}NO_{3}S + C_{10}H_{9}NO + Fe^{2+}
\]
10.11.3 Pesticides

Pesticides are persistent organic pollutants that can be found in surface and groundwater alike. They are made up of diverse structures and may possess specific or nonspecific toxicity to pests and the surrounding environment.

Due to the plethora of different organic pesticide structures, it is impossible to show a specific route for iron nanoparticle degradation of these compounds. In fact, nZVIs may follow degradation routes experienced with a number of different pollutants.

Chlorinated pesticides are likely to be degraded via reductive chlorination (Bezbaruah et al. 2009); nitrogen containing pesticides can be degraded via reductive de-nitration. Furthermore, reactions between iron nanoparticles and oxygen/water can lead to reactive oxygen species that can attack complex organic pollutant chemical structures (Choe et al. 2000).

Elliott et al. (2009) used nZVIs to degrade the chlorinated pesticide lindane. Following adsorption of lindane to the nZVI particles, two subsequent hydrogenolysis steps occurred, as shown by the following reaction:

\[ \text{C}_6\text{H}_6\text{Cl}_6 + \text{Fe}_0 \rightarrow \text{C}_6\text{H}_6\text{Cl}_4 + \text{Fe}^{2+} + 2\text{Cl}^- \]

Following the reduction of lindane to \( \gamma \)-3,4,5,6-tetrachlorocyclohexene, subsequent reduction reactions led to the complete removal of chlorine from degradation intermediates forming benzene as an end product. To further support this finding, Poursaberi et al. (2012) demonstrated that nZVI reductively dechlorinated the pesticide DDT → DDD → DDMS → DPE in a step-by-step process.

The non-chlorinated herbicide molinate was degraded in the presence of nZVI. The mechanism behind this was the generation of free radicals because of the interaction between iron and O\(_2\). Disproportionation of the radicals can lead to the formation of hydrogen peroxide, which further reacts with Fe\(^{0}\) to produce the hydroxyl radical. This reactive species is known to attack the chemical structures of complex organic pollutants (Joo et al. 2004).

10.11.4 Nitrogen

Zero-valent iron has an affinity to remove nitrogen from nitro-aromatic compounds and also interact with nitrate to form stable products, that is, nitrogen gas, nitrite, or ammonium, depending on the reaction conditions.

Najan et al. (2008) demonstrated that the use of nZVI on hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) led to degradation via two routes. First was the removal of an NO\(_2^-\) group to form an unstable intermediate. This species broke down in water to form MEDINA (methylenedinitramine), which was then further transformed to N\(_2\) and NH\(_4^+\).

The second route involved an initial de-nitration of RDX and the subsequent de-nitration or reduction of hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) to eventually form hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). TNX de-nitrozation occurs in the presence of iron nanoparticles to eventually break the aromatic structure. Further reactions lead to the formation of N\(_2\) and NH\(_4^+\). nZVIs can also interact with nitrate to form nitrogen gas, nitrite, and ammonium via the following reactions:
1. \( 5Fe^0 + 2 NO_3^- + 6H_2O \rightarrow 5Fe^{2+} + N_2 + OH^- \)
2. \( Fe^0 + NO_3^- + 2H^+ \rightarrow H_2O + NO_2^- \)
3. \( NO_3^- + 6H_2O + 8e^- \rightarrow NH_3 + 9OH^- \)

10.11.5 Metals

Heavy metals are entities that may be naturally occurring; however, due to their high concentrations and wide distributions as a result of industrial, domestic, agricultural, medical, and technological practices, these metals have become serious environmental pollutants. Some heavy metals have high toxicity (e.g., arsenic, cadmium, chromium, lead, and mercury). They are classified as cancer-causing or possible cancer-causing entities that can induce organ damage at low concentrations (Tchounwou et al. 2012).

Iron nanoparticles have a number of mechanisms by which they can be used to treat heavy metal entities in the environment. The process the iron nanoparticle will utilize to remove toxic heavy metals depends on the standard redox potential \( (E^0) \) of the metal contaminant compared to that of iron (-0.44 V). Metals with lower \( E^0 \) than irons will be absorbed to iron’s surface (i.e., Zn, Cd). If the toxic metal species has an \( E^0 \) significantly higher than irons (i.e., Cr, As, Cu), it will in most cases undergo reduction, and subsequent precipitation. Co-precipitation and oxidation reactions may also occur in some cases, depending on the environmental conditions experienced (O’Carroll et al. 2013).

Absorption of Zn onto nZVI containing an FeOOH surface layer was observed by Liang et al. (2014). Zinc ion concentrations of 100 mg/L were subjected to nZVI concentrations between 0.1–2.0 g/L. At concentrations of 0.8 g/L or above, the nZVI was able to remove 99% of zinc ions present in solution. Dissolved oxygen increased the removal capacity due to the enhanced absorption efficiency of FeOOH compared to that of nZVI.

10.11.6 Fenton/Fenton-Like Reactions

Many types of iron nanoparticles have a high intrinsic reactivity attributed to them, that is, nZVI; however, this reactivity may also be a downfall due to reactions with oxygen and water leading to passivation. One process that can utilize iron nanoparticles with or without oxidation is the Fenton reaction. The Fenton reaction has the capability to destroy several recalcitrant organic compound structures, including environmental contaminants by the generation of free radicals. This reaction can be coupled with light (photo-Fenton) to further enhance the free radical-generating capacity and lead to elevated pollution degradation rates.

In the Fenton/photo-Fenton reaction, iron itself may only slightly contribute to the overall pollutant degradation outcome (Barbusiński 2009). Iron in the Fenton reaction isn’t used up but serves as a catalyst (Nidheesh et al. 2013) for the following cascade of reactions between hydrogen peroxide and iron in acidic environments:

1. \( Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^* \)
2. \( OH^* + H_2O_2 \rightarrow HO_2^- + H_2O \)
3. \( Fe^{3+} + HO_2^- \rightarrow Fe^{2+} + H^+ + O_2 \)
4. \( Fe^{2+} + HO_2^- \rightarrow Fe^{3+} + HO_2^- \)
5. \( Fe^{2+} + OH^- \rightarrow Fe^{3+} + OH^- \)

There is potential for iron nanoparticles and the \( HO_2^- \) radical to participate in pollutant degradation reactions (Chatterjee and Mahata 2004). However, their contribution is likely to be insignificant compared to that of the \( OH^* \) radical. Conclusions for pollutant degradation:
nZVIs exhibit high reactivity, are able to effectively reduce organic environmental contaminants, and can interact with heavy metals.

Iron oxide nanoparticles tend to have low reactivity with organic pollutants and have a higher capacity to interact with heavy metals (compared to nZVIs).

Both iron oxides and nZVI will undergo Fenton reactions when in the presence of H₂O₂.

10.12 In Situ Remediation Using Iron Nanoparticles

10.12.1 Groundwater Remediation

Groundwater is an important component of the hydrological cycle, with groundwater and surface waters being an interconnected and interchangeable resource (Evans 2007). Groundwater contamination is often overlooked when compared to that of land-based water systems, as environmental damage is difficult to identify and costly to monitor in the underground matrix.

Groundwater is the source of drinking water for an estimated 2 billion people worldwide and an irrigation resource used to support 40% of the world’s agricultural food production. In Australia, Victoria, New South Wales, and South Australia, irrigation is composed of approximately 60% groundwater, while in Western Australia, 72% of groundwater is utilized for urban and industrial processes (Thiruvenkatachari et al. 2007).

Despite groundwaters widespread applications, agricultural, mining, and other industrial processes have been identified as directly and indirectly contaminating these water sources. A vast array of contaminants including chlorinated hydrocarbons, nitrates, phosphates, pesticide, fuels, metals, and radioactive contaminants have been found in groundwater (Ball et al. 2001, Khan et al. 2004). In Europe, approximately 20,000 sites are polluted and require remediation, while in the United States, this figure rises to more than 235,000 sites (Nicole et al. 2011). In India’s groundwater, severe and widespread heavy metal concentrations are present, with much of the literature reporting on arsenic contamination. Chromium, nickel, cadmium, and mercury are also widespread in India’s groundwater, with reported findings in 43 districts of 14 states in India. In addition, fluoride is particularly prevalent, with approximately 20 of the 28 Indian states containing some degree of fluoride in their groundwater. More than 66 million people in India are suspected of suffering from the severe chronic condition fluorosis (Chakraborti et al. 2011, Chakraborti et al. 2016).

Traditional treatments for groundwater remediation have relied heavily on the transportation of groundwater to surface-dwelling plants. These technologies have been deemed to be inefficient within reasonable timeframes and are often costly due to the energy requirements required to pump water to the surface and the cost of infrastructure to facilitate remediation (Bayer and Finkel 2006).

Monitored natural attenuation and bioremediation are other remediation strategies employed to remediate contaminated aquifers. Although these technologies are cheap to implement, lengthy timeframes are required for remediation, and many pollutants may be recalcitrant to break down from these biological entities. Furthermore, introducing microorganisms to deep underground aquifers is troublesome, as the pressures experienced during field-scale groundwater injections may damage microbial cells (O’Carroll et al. 2013).
The complexities relating to groundwater remediation are vast, and each site will have differing characteristics that may be favourable or unfavourable for the use of nanoparticles (mainly nZVI) as a treatment option. Groundwater flow rates; geological strata; connection with surface waters; use of water for future irrigation or drinking; aerobic/anaerobic conditions; nontargeted species that will react with nanoparticles, that is, clays, humic acids, minerals, and so on; and changes in pH are just a few of the issues one should consider before commencing site remediation.

To combat and bypass some of the aforementioned obstacles in relation to groundwater remediation, researchers have been focusing their efforts on optimizing nanoparticle characteristics and coatings. Ideally, iron nanoparticles should be reactive, uniform in size, not too large to be immobile and not too small to be used up before reaching the contaminant, resistant to passivation, and able to target pollutant sources without interacting with groundwater constituents and should possess particle–particle repulsive forces. With these factors addressed, nanoparticles that can travel through the underground matrix, target pollutants (mainly NAPL pollutants), and rapidly degrade these pollutants can be generated.

### 10.12.2 Migration and Monitoring

*In vitro* proof-of-concept studies assess the migration of nanoparticles through columns packed with sand soil, clays, and other geological entities often found within the underground groundwater matrices. Although these studies are vital for proof of concept, they cannot simulate real-life scenarios (Figure 10.2). Changes in mineral and salt content of groundwater, differences in geological strata, pH variations, differences in oxidation/reduction potential, organic and inorganic microaggregate species that may react with particles, groundwater flows that may spread out or bottleneck in certain areas and simulating real-life underground injection of particles are just a few parameters that are irreproducible in lab-scale tests. Furthermore, determining the microbial, invertebrate, plant, and animal species likely to be exposed during an on-site application of nanoparticle remediation is difficult, and it is even harder to determine the potential impacts of these particles on their well-being.

Assessing the migration of nanoparticles through the groundwater matrices following site remediation trials is often conducted by indirect measurements such as pH, total iron content, and oxidation-reduction potential by potentiometry. In many cases, when trying to determine the extent of nanoparticle movement, the samples taken are overly dilute with respect to nanoparticle concentration. Aggregation, deposition, reactions with groundwater constituents, and filtration are common causes for this. When samples are taken close to or directly from the injection zone, the presence of iron nanoparticles is visible with the naked eye due to their signature black color. In concentrations as high as these (>10 mg/L), nanoparticle measurements are possible via TEM and inductively coupled plasma mass spectrometry (ICPMS) (Shi et al. 2015).

Recently, work has been conducted to attach fluorescent tags to nanoparticles in order to determine the extent of their movement. Bhomkar et al. (2014) have successfully attached green fluorescent protein with a poly-lysine tag (His-GFP-LYS) to gold, iron oxide, cerium oxide, and zinc oxide nanoparticles, which have then been stabilized with poly-acrylic acid coating. A portable spectrophotometer was capable of distinguishing turbidity changes representative of the presence of nanoparticles. Although this technology is only limited to samples containing low levels of organic matter in surface waters and can only detect particles with a negative charge, its concept is one that should be optimized for groundwater samples.

In the medical field, tagging or labelling nanoparticles for drug delivery is commonplace. Nanoparticles may be labelled with dye fluorophores giving amplified optical signals. These particles are unlikely to be useful for
environmental remediation purposes, as the dye fluorophores are expensive and considered persistent organic pollutants (Miranda et al. 2015). For environmental applications, tagged/labelled nanoparticles should be able to withstand the sheer forces attributed to particle injection and percolation through the porous groundwater matrix. Additional research with respect to adherence of tags/labels during the sheer forces experienced with site application and use of tags that are environmentally benign is important.

10.12.3 Surface Waters

Unlike groundwater, surface water health is easier to monitor, as sampling doesn’t require drilling and often visual cues can represent tarnished waterways, that is, visual pollution, deceased animals, and plants. Ecosystem dynamics are completely different in surface waters, also. Higher-order animals are found in rivers and streams, that is, fish, frogs, and freshwater crayfish. Photosynthetic algae and water plants exist within these water bodies, microorganisms are largely anaerobes or facultative anaerobes (unless considering deep sediments), and other animals rely on the water for a direct drinking source.

Beside the living organisms reliant on surface waters, there are also a number of other important differences between surface waters and groundwater that help to paint a picture as to the vastly different dynamics they possess. Some factors impacting surface waters but not groundwaters are as follows; presence of sunlight, flow rate of water, freely flowing water, differing turbidity, differences in gravitational effects, largely fluctuating temperatures, evaporation, recharge, dissolved oxygen, and salt and mineral concentrations. With such varying dynamics, it can be expected that the treatment of surface waters will vary from that of groundwater.

Delivery of nanoparticles to natural surface waters should not be done without appropriate means of recovering the particles post-remediation (Figure 10.5). Several studies assessing the toxicity of iron nanoparticles on fish have shown considerable health implications indicating that fish and other aquatic organisms may be particularly sensitive to these entities (Chen et al. 2013, Remya et al. 2015, Saravanan et al. 2015).

Due to nanoparticles’ properties, they are likely to remain suspended in aquatic solutions for extended periods of time and subsequently will have direct contact with organisms throughout the whole water column. Fish gills may become saturated with particles, and the permeable skin of frogs may accumulate particles, leading to reduced oxygen uptake capacity. In addition, some iron nanoparticles have the capacity to generate reactive radical species that may also harm biological entities (Pereira and Freire 2006, Remya et al. 2015). Additionally, excess nanoparticles within the water column may restrict light from penetrating the water, leading to plant death.

To rectify these problems, iron nanoparticles impregnated into permeable reactive barriers or adsorbed onto solid supports can be used for surface water remediation. Permeable reactive barriers are constructs that allow water or solutions to flow through a reactive matrix. Iron nanoparticles impregnated into these filtration constructs can detoxify contaminants as they pass through.

Solid supports loaded with nanoparticles allow reactions to occur at the surface of the nanoparticles. Nanoparticles are adsorbed onto the support matrices so that the maximum surface area is available for reaction to occur. Furthermore, the addition of metal catalysts or other adsorbent materials can be incorporated into the design to enhance reactivity and adsorptive potential of the pollutants (Rajabi et al. 2013). In these technologies, nanoparticles are not released into the system and in turn are not likely to pose significant environmental harm. Also, metal catalyst-doped structures can often be recharged, leading to their reuse and subsequent reduction in remediation costs (Fan and Gao 2006).
10.12.4 Terrestrial Remediation

Contamination of terrestrial sites has historically been addressed by removing the contaminated soil to landfill (costly), degradation of contaminants via chemical methods (potentially toxic), and bioremediation (slow process). Although the vast majority of research using iron nanoparticles deals with the remediation of water, particularly groundwater, studies have shown successful results for degrading contaminants in soils also.

Naja et al. (2009) showed that CMC coated nZVs were capable of degrading 98% of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) under anaerobic conditions in soil in less than a day. Furthermore, in soil packed columns, 95% of RDX (60mg/kg⁻¹) was degraded using a CMC-nZVI solution.

Ibrahim et al. (2012) showed that trichloroethylene could be degraded by surfactant modified nZVI particles. Soil was doped with TCE and subjected to nanoparticle treatment. Results showed that this method was 23 orders of magnitude slower than studies conducted in aqueous phases; however, after a week, complete removal was achieved.

Varying reports on effectiveness have been shown above for in vitro studies. Upon transferring this technology to site remediation, other factors should also be considered. Nanoparticulate dust has been implicated in several health problems via inhalation (Donaldson et al. 2005).

If the technology is to be used around populated areas, proper control measures should be in place to ensure dust is not airborne (nanoparticle slurries are used without chance of drying out). Furthermore, mixed findings for iron nanoparticle impacts on microorganisms and plant germination have been reported. Some microorganisms are killed under iron nanoparticle exposure, while others thrive (Borcherding et al. 2014, Shahzeidi and Amiri 2015). Also, some plant seeds germinate faster in the presence of iron nanoparticles, while at high concentrations, iron nanoparticles cause toxicity (Alam et al. 2015, Chichiriccò and Poma 2015). All of the aforementioned factors should be considered before utilizing this technology for terrestrial systems, and this will be dependent on the site itself.

10.12.5 Cost of Iron Nanoparticle Remediation/Feasibility

Nanoparticles have been hailed for their high reactivity, elevated pollution degradation rates, and seemingly environmentally friendly nature however, concerns about their use as a viable on-site technology are often raised. From a landholder’s perspective, the cheapest and fastest option for site remediation is preferable. Bioremediation may be the cheapest option; however, the timeframes required to clean up sites are lengthy and limited persistent pollutants are bioavailable for degradation from these organisms. In many cases, contaminated sites are excavated and sent to landfill. This is particularly expensive and environmentally unsound (He et al. 2009). With iron nanoparticle technology still in its infancy in regard to site remediation, many landholders and contractors may prefer to choose well-established technologies over ones with question marks surrounding their efficiency, potential delivery to the source, and ecotoxicity.

Site remediation using iron nanoparticles has been estimated to be competitive in respect to price for a number of priority pollutants, including cadmium, chromium, zinc, lead, arsenic, xylene, toluene, TCE, lindane, chloramphenicol, atenolol, and gemfibrozil. While the price of remediating metals depends on the metal and extent of pollution present, sludge formation is minimized when utilizing nZVI for treatment. In addition, if
remediation of pollutants utilizing iron nanoparticles is not capable of completely remediating a site, the remaining pollution may be cost-effectively managed (Adeleye et al. 2016).

It is a common belief in the environmental nanotechnology field that iron nanoparticles can provide comparable or significantly higher pollution degradation rates at a much cheaper price than conventional methods (Grieger et al. 2015). While reactivities and pollutant degradation capabilities are not questioned, the cost associated with producing them is.

Currently, the most popular method for generating nZVI particles is through the sodium borohydride reduction of aqueous salts method (Adeleye et al. 2016). These particles also require capping and/or further coatings to prevent oxidation and enhance remediation. The cost of using sodium borohydride to manufacture particles has been deemed too expensive to give iron nanoparticles the edge they need to be widely implemented in site remediation. Costs of nZVI particles in 2011 were between $50 and $200/kg, and the price has not shifted much to today, when prices are between $50 and $100/kg. It has been forecasted that to be considered viable, nZVI costs need to be reduced by at least 20% (Elliott et al. 2009, Crane and Scott. 2012, Adeleye et al. 2016). Depending on the scale and complexity of the remediation project, the cost to remediate one cubic meter of chlorinated hydrocarbon contaminated soil with nZVI is between $89.50 and $218.66 (Cook 2009).

10.13 Fate of Fe NP's in the Environment and Ecotoxicity Concerns

When a contaminated environment is remediated, the aim is to generate technologies that are low-cost, effective, easy to implement, environmentally benign, and do not have health implications for people who come into contact with them. It is therefore important that when assessing the potential for nanoparticles to be used as a viable technology, we must first determine if the nanoparticles themselves could become environmental contaminants or toxic to humans.

Although iron nanoparticles are currently being used for site remediation (Lowry et al. 2012, Su et al. 2012) there are mixed opinions about their toxicity. Contractors for site remediation have their reservations about using this relatively young technology due to fear of potential environmental damage from the nanoparticles themselves being apparent in the future.

Nanoparticle toxicity is dependent on a number of functional parameters such as particle size, shape, surface charge, chemistry, and composition. The cytotoxicity of nanoparticles is thought to be due to their ability to generate oxidative stresses and activate proinflammatory genes (Yildirimer et al. 2011). Many studies have been conducted in relation to iron nanoparticle toxicity within microorganisms, human cell lines, mice, fish, and plants (Figure 10.4).

10.13.1 Microorganisms

Microorganisms are very sensitive indicators of change in an environment. Population and diversity changes may be a result of simple natural changes in temperature or may result from more serious events like significant environmental damage caused by large-scale anthropogenic contamination (Dion 2008).
Changes in temperature are unlikely to kill microbes (unless severe increases in temperature are experienced). However, they may instil a competitive advantage for some organisms, reducing the prevalence of those originally present. During a severe contamination event, the majority of microorganisms may have already died as a result of pollution-induced toxicity and inability to access food, nutrient, or water sources trapped by the pollutant.

However, links between iron nanoparticles and microbial toxicity have been shown, although iron nanoparticles are seen as less environmentally damaging compared to others metallic nanoparticles, that is, copper, aluminum, and silver, to name a few. Shahzeidi and Amiri (2015) used nutrient agar plates impregnated with Fe₃O₄ nanoparticles to assess the growth kinetics of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. Results indicated that nanoparticle concentrations as low as 1.56 mg/ml were sufficient to slightly inhibit microbial growth. Increasing nanoparticle concentrations did not have a marked effect on increased microbial toxicity except with *P. aeruginosa* at 25 mg/ml nanoparticle concentration, where microbial growth was considerably inhibited.

Conversely, Arakha et al. (2015) showed that iron oxide nanoparticles (n-Fe₃O₄) only exhibited significant antibacterial activity against *Bacillus subtilis* and *E. coli* at high nanoparticle concentrations (>50 µM). However, the addition of a chitosan coating reversed the surface potential of the nanoparticles from negative to positive (mainly due to –OH groups on the chitosan molecule), and bacterial toxicity was significantly increased. Findings from this study indicated that the positive surface potential of the nanoparticles led to the production of reactive oxygen species (ROS), which is known to inactivate microbial cells (Arakha et al. 2015). Regardless of whether the nanoparticles themselves exhibit toxicity, the end product that is to be used should be tested to ensure that changing nanoparticle properties and/or coatings themselves are not toxic.

Borcherding et al. (2014) linked the size of iron oxide nanoparticles with bacterial proliferation. A decrease in nanoparticle size induced growth and increased the viability of *P. aeruginosa* (PA01) and subsequent biofilm generation. In contrast, small iron oxide nanoparticles have been shown to inhibit 5′ adenosine monophosphate activated protein kinase (AMP) activity and deregulate the host’s innate immunity. With a weakened immunity and iron oxide’s ability to induce the growth of potential pathogenic bacteria, human health may be at risk from nanoparticles at the smaller side of the nano-range. However, nanoparticles that are too small are not beneficial for environmental restoration, as passivation transforms them into iron oxides before they can reach the pollution source (Bardos et al. 2011).

In cases where the natural microflora has been significantly altered or killed off, the use of nanoparticles for site remediation may be seen as a viable technology. If particles revert to an inert form and/or pose no long-term damage following degradation, there are increases in micro-floral diversity and populations are likely to return to the site. Following severe ecosystem-altering events, it is rare for the original microflora to return to the site with the same diversity and populations (Nurulita et al. 2015).

Iron nanoparticles have been hypothesized as being relatively safe for environmental use by many researchers. Iron is the fourth most abundant element found within the earth’s crust and exists mainly as metal oxides. Iron nanoparticles initially have a high degree of reactivity, but upon being used in detoxification reactions, their reactivity decreases as passivation increases. The resulting products are iron oxides in most cases, thereby reverting back to natural end products following use. Furthermore, the use of many coatings for iron nanoparticles are composed of organic polymers, which, when broken down, may increase the carbon balance of the soil and in turn stimulate microbial growth (He et al. 2009).
Due to the increase in nanoparticle research and the multitude of different modifications (size, shape, coatings, porosity, crystallinity, stability, functional groups attached, etc.) comparing the degree of toxicity of one type of nanoparticle against another is virtually impossible unless standardized tests are created. These tests should be tailored to the field they are suited for; for example, iron nanoparticles for use in the medical industry may require toxicity testing on animal cells, whereas environmental applications may require testing on microbial, plant, and animal cells (Soenen et al. 2012).

10.13.2 Plants

When it comes to plants, there is such a thing as too much of a good thing. Excessive sunlight can burn leaves, too much water can rot roots, and too much fertilizer also deteriorates plant health. In many cases, this trend can also be seen with the effect of iron oxide nanoparticles on plants.

Sheykhbaglou et al. (2010) showed that small concentrations of iron nanoparticles (0.75 g/ L) increased the dry weights of soybean leaf and pod weights. Also, the highest yields of soybean were experienced when soybeans were supplemented with 0.50 g/ L iron oxide nanoparticles. Similar increases in tomato, watermelon, and wheat growth have been shown with low concentrations of iron oxide nanoparticles (Alam et al. 2015, Shankramma et al. 2015, Wang et al. 2015). Iron deficiency can lead to a reduction in chlorophyll, reducing photosynthesis, and can induce peroxidation, leading to leaking of intracellular electrolytes. Iron is an essential plant nutrient in the correct quantities. Iron nanoparticles may also increase the water uptake by seeds by penetrating their surface and in turn increase seed germination. Increased seed germination has been seen with peanut plants and wheat (Alam et al. 2015, Li et al. 2015).

When levels of iron are too high (including from the presence of iron nanoparticles), plant growth is adversely affected. Wang et al. (2015) showed elevated watermelon growth with up to 20 mg/L iron nanoparticles; however, at 100 mg/L, protein denaturation in plant tissue was apparent, leading to cellular aging. Small ferrofluid concentrations (10–50 µL/ L) may initially increase nucleic acid and chlorophyll-α concentrations; however, at higher concentrations (100–250 µL/ L) chlorophyll a and b ratios are significantly disrupted (decreased by approximately 35% each), significantly affecting photosynthesis (Chichiriccò and Poma. 2015). In addition, if nanoparticle concentrations are excessively high around the rhizosphere of plants, they may interfere with the microbial populations that colonize the area. Changes in microbial diversity and population dynamics around the plant’s root/soil interface may hamper stimulatory plant growth interactions.

Iron nanoparticles for environmental restoration, particularly in groundwater systems, are unlikely to impact plant growth dynamics dramatically. The use of iron nanoparticles for soil restoration is not commonplace, and the dynamics of the site, including the potential damage to flora, should be taken into consideration before attempting such a venture.

10.13.3 Fish

As there is potential for nanoparticles to enter surface water upon remediation, their toxicity on aquatic life forms is important to determine. The predominant routes of exposure for fish are likely to be through absorption through eggs, via the gills, or through ingestion.

Chen et al. (2013) studied the effect of CMC-stabilized-nZVI, nFe₃O₄ and Fe²⁺(aq). Their findings showed that CMC stabilized nZVI and Fe²⁺ (aq) induced acute mortality, developmental toxicity, and oxidative stress responses in
**Oryzias latipes** embryos and hatchlings. \( \text{Fe}_2\text{O}_4 \) showed minimal acute mortality on embryos, as the embryo containing solutions were void of \( \text{Fe}^{2+} \) and ROS.

Saravanan et al. (2015) showed that at concentrations of 1 and 25 mg/L, \( \text{Fe}_2\text{O}_3 \) nanoparticles showed toxicity responses in *Labeo rohita* over a short exposure period of 96 hours. Increases in plasma glucose concentrations were observed, a stress response that may be a result of respiratory disturbances. Plasma proteins were significantly decreased for both 1 and 25 mg/L nanoparticle exposure concentrations. Decreases in plasma protein can be an indicator of direct nanoparticle toxicity to cells or the production of ROS, which can damage cells. Additionally, gill \( \text{Na}^+ / \text{K}^+ \)-ATPase concentrations were significantly reduced, indicating that nanoparticles are directly impacting the function of the fish’s gills.

Fish and their embryos tend to show an elevated susceptibility to iron nanoparticles compared to mammalian counterparts. This may be due a number of factors, including permeable eggs that allow transfer of nanoparticles to developing embryos; ease of nanoparticles to be in contact with the gills, leading to a depletion in oxygen exchange and/or allowing NPs to enter the bloodstream; and direct and nonselective ingestion of nanoparticles (Remya et al. 2015).

It is suggested that the use of nanoparticles within surface waters should be in conjunction with permeable reactive barriers or loading into immobilized supports. In this way, the release of nanoparticles that can impact aquatic organisms can be minimized, particles can be retrieved, and environmental harm can be minimized (Figure 10.5). Furthermore, application of nanoparticles to soils or injection of nanoparticles into subsurface environments should include risk assessments as to the potential of iron nanoparticles to reach surface waters.

### 10.13.4 Mammals

Although iron is an essential trace element for mammals, excessive concentrations have resulted in several different disorders in humans. Iron concentrations between 20 to 60 mg/kg body weight have been considered potentially serious, inducing GI toxicity, and concentrations over 60 mg/kg body weight have been considered potentially lethal, inducing systemic toxicity. High concentrations of iron entering the body via ingestion can lead to pathological changes in the GI tract, liver, and cardiovascular system (Singh et al. 2013). People with hemochromatosis are also considered to be at higher risk from high iron concentrations in drinking water.

The size of nanoparticles is one of their key attributes, instilling increased surface area for faster reactions to occur. However, it may be regarded as a detrimental feature with regards to toxicology. Nanoparticles that are smaller than 100 nm are absorbed by intestinal cells and lymphatic tissues and can enter the circulatory system. The particles’ surface and surface chemistry also impact their toxicity. Small particles will absorb into the lining layer of the lungs due to their low surface tension. Smaller particles have been shown to be more damaging to lungs due to increased surface area, greater ability to conjugate, and ability to sustain energy (Ai et al. 2011). Furthermore, particles with sizes of less than 70 nm are not phagocyted by macrophages in the alveolar region efficiently, and this could lead to accumulation (Bergeron and Archambault 2005).

The major concern in relation to nanoparticle toxicity is their ability to generate ROS, which can lead to DNA damage and cancer. Apopa et al. (2009) showed that the presence of iron oxide nanoparticles (\( \text{Fe}_2\text{O}_3 \)) led to the increased permeability of microvascular endothelial cells through their generation of ROS. These cells are largely implicated in carcinogenesis and tumour growth.
Inhalation studies of Fe$_3$O$_4$ dust in Wistar rats were conducted by Pauluhn (2011). Rats were exposed to nanoparticle concentrations up to 52.1 mg m$^{-3}$ for 6 hours per day, 5 days a week, for 13 consecutive weeks. Results indicated that no mortality, consistent changes in body weight, changes in eating habits, or systemic toxicity were observed. Only some sub-chronic symptoms were experienced in rats with poorly soluble particles. Furthermore, no cytotoxicity was observed when healthy Sprague Dawley rats were made to inhale up to 90 µg m$^{-3}$ Fe$_2$O$_3$ iron nanoparticles (average size = 72 nm) for 6 hrs/day for 3 days (Zhou et al. 2003).

With such extensive exposures leading to relatively minor health implications, rats and potentially other mammalian systems have great capacity to withstand iron nanoparticle induced toxicity. For environmental nanoparticle applications, aerosol nanoparticle concentrations are estimated to be low and minimal inhalation exposure is expected. The majority of nanoparticle-based remediation is conducted with nanoparticle slurries, preventing the occurrence of volatile and aerosol nanoparticles.

### 10.13.5 Ecotoxicity Extrapolations

Although research into the ecotoxicity dynamics of iron nanoparticles is still in its infancy, trends can be extrapolated from the current scientific research. In environments where organic and metallic contaminants pose a risk to environmental harm (Ball and Truskewycz 2013), several considerations should be addressed before selecting the use of iron nanoparticles as a treatment option. Briefly, nanoparticles themselves should not be tested for toxicity alone, as surface coatings can change the charge of nanoparticles themselves, or coatings may have inherent toxicity. Iron nanoparticles have shown both positive and negative growth dynamics for microorganisms and plants. In subsurface groundwater injections of iron nanoparticles, the impact on the environment is not likely to be excessively damaging. This is due to the particles pacifying over time, and their transport through the underground matrices is likely to be limited. Organic coatings on particles may also stimulate microbial growth due to the increased carbon balance of the environment.

In terrestrial environments or in surface waters, the introduction of iron nanoparticles may be troublesome. High concentrations of nanoparticles induced protein denaturation and cellular aging in plants and altered chlorophyll $a$ and chlorophyll $b$ ratio dynamics by lowering them affecting usual photosynthesis functioning. Plant/microbe interactions may also be altered in the rhizosphere. Fish and their embryos have been shown to be particularly sensitive to iron nanoparticles, inducing mortality, oxidative damage, and altered gill functions. Nanoparticles are therefore recommended to be used in conjunction with PRBs or loaded onto surface supports for surface water remediation.

Additionally, standardized ecotoxicological tests should be in place so that nanoparticle toxicity can be compared. With this testing, bare nanoparticles and coated nanoparticles alike should be tested to determine the end product toxicity. Ecotoxicity on a range of model organisms should be conducted to ensure the impact on all environments can be assessed. Parameters to be assessed are likely to include but should not be limited to surface characteristics and coatings, shape, size, physical composition and chemical reactivity, and passivation potential.

### 10.14 Conclusions

The use of iron nanoparticles for environmental remediation offers great promise for fast, effective, and targeted pollution remediation. However, each specific site will require research as to which particles to use.
• Surface waters are likely to require particles to be loaded onto solid matrices or within PRBs to prevent toxicity to aquatic life forms and to ensure nanoparticles are removable from the system.
• Application of iron nanoparticles to terrestrial soils may be site specific depending on the type and abundance of threatened/endangered flora and fauna in the region. Furthermore, minimization of airborne nanoparticles is important, as their effects on humans are not completely understood.
• Groundwater remediation needs to factor in the geological strata, flow rate of groundwater, aquifer characteristics, pollutant type, closeness to surface waters, and future use of groundwater, just to name a few.

Like any new and emerging remediation technology, there are concerns regarding safety and effectiveness. One of the major drawbacks with providing community peace of mind with regards to this is that there are no widely accepted ecotoxilogical standards to compare particle toxicity. Furthermore, every new alteration made to nanoparticles, whether it is a new coating, different size characteristic, addition of a catalyst for enhanced remediation, or another change, has the potential to significantly alter particle behaviour. Without ecotoxilogical standards, concerns and hesitations are likely to restrict their usage until sufficient in situ pilot-scale tests have returned positive outcomes without adverse effects.

Great advances have been made in regard to iron nanoparticle properties with surface functionalization strategies used to combat agglomeration; reduce rapid passivation; instil pollutant targeting; and control size, shape and particle uniformity. There is still plenty of scope to enhance nanoparticle properties. Increased reaction rates, reduction in production costs, isolation of environmentally friendly coatings, catalysts, and generation of novel solid support matrices and further optimization of particle size, shape agglomeration, and pollutant targeting are of importance.

Despite the benefits of iron nanoparticles over competing technologies (increased pollutant degradation speeds, ability to treat groundwater pollutants on site, little persistent environmental toxicity for groundwater remediation, etc.), their cost is too high for many contractors to consider them as an option. Millimetric iron particles cost between $0.36 and $1.08/kg, while iron nanoparticles are on the order of $50–$100.00/kg (Elliott et al. 2009, Bardos et al. 2011, Crane and Scott. 2012, Adeleye et al. 2016). The use of sodium borohydride for generation of nZVIs is expensive, and waste products from this process are toxic. Alternative routes for the generation of nZVIs may hold significant commercial promise if iron nanoparticle production costs can be reduced.

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References


Part 2 – Research and Conclusions

Forward

The overarching aim of this study was: to synthesize nanoparticles with plant derived phytochemicals and to use these nanoparticles for pollution remediation applications.

Current approaches for synthesis of iron nanoparticles use toxic and costly chemical reducing agents (e.g. sodium borohydride (NaBH₄) and hydrazine hydrate (N₂H₄)) which limit their environmental suitability and generate large amounts of toxic waste products. In addition, these chemicals rarely result in the synthesis of nanoparticles with optimum morphological traits due to their fast and hard to control reduction reactions resulting in particles with uneven size distributions. In addition, iron nanoparticles synthesized with these reducing agents rapidly react with oxygen and atmospheric moisture limiting their reactivity and storage times. Surface functionalization of nanoparticles is beneficial for protecting them from oxidation and to prevent particle-particle attractions which result in agglomerated particles with reduced surface areas. The use of organic molecules (some derived from plants) have been shown to provide a protective capping barrier from the elements and may lead to the generation of small mono-disperse nanoparticles which are optimal for pollution remediation.

Current synthesis approaches of nanoparticles may involve heavy mechanical grinding, thermal decomposition of larger particles, sonochemical methods, co-precipitation and chemical reduction techniques. These approaches suffer from high operating costs and the addition of additional chemical species that may be toxic. Reduction of metal salts using bottom up synthesis approaches is the most economically feasible but finding environmentally friendly reducing agents with the capacity to generate nanoparticles with optimum morphologies is challenging. Plant derived phytochemicals and polymers have been used for the reduction of iron salts and subsequent capping of iron nanoparticles. Further investigations on the use of plant derived compounds for nanoparticle capping without reducing their pollution remediative efficiency is thought to be of great importance. Concerns pertaining to nanoparticle suitability for site remediation have been raised as little research has been conducted on their environmental toxicity. Nanoparticles must be removable from the environment following remediative action unless they are environmentally benign and can remove the pollutant from the contaminated site.

The primary aim for this research was to generate nanoparticles by substituting the use of toxic and bio-incompatible chemical reducing agents with plant-derived compounds. However, the utilization of waste organic material also increases cost effectiveness and likely to improve bio-compatibility which is paramount for the generation of a practical end-product.
The aim of the first research chapter of this thesis (Chapter 2) was to synthesize iron nanoparticles capable of degrading complex dye mixtures by replacing toxic chemical reducing agents with ‘green’ plant derived alternatives. The work described utilises aqueous green tea extract for the reduction of iron salts resulting in the formation of iron nanoparticles. Numerous studies present the synthesis of zero-valent iron nanoparticles (nZVI) from the reduction of iron salts with plant extracts. Zero-valent iron is highly reactive and nanoparticulate nZVI offers higher surface area and faster reaction rates compared to millimetric sized particles. Investigation into the speciation of the nanoparticles showed that the particles were not nZVI but rather iron oxides/iron oxy-hydroxides and therefore the research focus shifted to using these particles as Fenton-like catalysts for concentrated dye degradation. This chapter set out to investigate the optimal parameters for green tea generated iron oxide nanoparticles to act as Fenton catalysts for remediative purposes. The effect of pH, temperature, iron nanoparticle concentration and hydrogen peroxide (required for Fenton-like reactions) were assessed to define optimum conditions for dye degradation. In depth characterisation of the nanoparticles was conducted by means of transmission electron microscopy (TEM), scanning electron microscopy coupled with energy dispersive X-Ray spectroscopy (SEM-EDS), Fourier-transform infrared spectroscopy (FTIR), X-Ray Diffraction (XRD) and zeta potential.

The aim of the second research chapter (Chapter 3) was to assess how different plants phytochemical profiles impact iron nanoparticle formation and how different nanoparticle morphologies aid in the removal of hexavalent chromium from water. The work described here investigated the mechanisms underlying the impact of the plant extracts on the properties of the nanoparticles formed and their efficiency for adsorbing hexavalent chromium from water. Aqueous extracts of four different plants (Pittosporum undulatum, Melia azedarach, Schinus molle, and Syzygium paniculatum (var. australe)) with different phytochemical profiles were used to synthesize iron oxide nanoparticles. This study aimed at linking various properties of plant extracts, such as reducing powers capacity, antioxidants concentration, phenolic concentration, reducing sugars concentration and iron-chelating capacity with nanoparticle morphology. In addition, nanoparticle morphology was linked with their capacity to adsorb hexavalent chromium from aqueous solutions.

Chapter four deviated away from the use of iron nanoparticles due to the inability to recover these particles following remediation and the acidic conditions often required for application. The aim of research chapter five was to determine if waste plant-based materials can be used to enhance silicon nanoparticle properties for concentrated anionic dye adsorption. Silicon based sorbents have been heavily studied due to their biocompatibility and high adsorption capacities. However, their negative charge only makes them useful for the removal of anionic pollutants. This chapter utilised 3-aminopropyl-triethoxysilane (APTES) as a silicon precursor (as opposed to negatively charged silicon dioxide (SiO$_2$)) as it is commonly used to prepare positively charged silicon surfaces for immunohistochemical and in situ hybridization procedures. 3-aminopropyl-triethoxysilane was
subjected to hydrothermal treatment in the presence of waste *Helium annuus* husk extract and was assessed for its potential to increase silicon nanoparticles affinity for cationic mixed dye adsorption. This study not only examined enhancing silicon-based nanoparticles properties using plant extracts but also assessed their toxicity towards *Vibrio fischeri* (model microorganism routinely used to study the toxicity).

In summary the specific aims of the work described in the research chapters were:

- Synthesize iron nanoparticles capable of degrading complex dye mixtures by replacing toxic chemical reducing agents with ‘green’ plant derived alternatives (Chapter 2 and Chapter 3).

- Assess how different plants phytochemical profiles impact iron nanoparticle formation and how different nanoparticle morphologies aid in the removal of hexavalent chromium from water (Chapter 3).

- Determine if waste plant-based materials can be used to enhance silicon nanoparticle properties for concentrated anionic dye adsorption (Chapter 4).
Chapter 2

Iron Nanoparticles synthesized using Green Tea Extracts for the Fenton-Like Degradation of Concentrated Dye Mixtures at Elevated Temperatures

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Adam Truskewycz (candidate) planned the experimental design, performed experiments, carried out all research, analysed and interpreted data. Truskewycz also reviewed the literature, executed the writing and was corresponding author for Chapter 2.

Ravi Shukla contributed to co-supervision of research and manuscript evaluation in Chapter 2.

Andrew S Ball contributed to the planning of experimental design, supervision of research, manuscript preparation and evaluation Chapter 2.

All the co-authors give full consent to Adam Truskewycz to present these above papers for examination towards the Degree of Doctor of Philosophy.

Adam Truskewycz (candidate)
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Iron nanoparticles synthesized using green tea extracts for the fenton-like degradation of concentrated dye mixtures at elevated temperatures

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\section*{A B S T R A C T}

Environmental harm caused by the release of textile dye contaminated wastewater from industry is an issue that requires immediate attention, particularly in developing countries. We herein demonstrate that green tea derived amorphous iron nanoparticles, in conjunction with Fenton like chemical processes, catabolise anthraquinone and azo dye mixtures that constitute a significant proportion of industrial dye waste. Iron nanoparticles, synthesized following a green tea mediated greener synthesis approach were able to degrade concentrated dye mixtures with over 90% decolourisation in only 20 min with 0.53 ppm nanoparticle concentration at a temperature of 70 °C. Detailed experimental degradation efficiencies were significantly pH and temperature dependant favouring lower pH values at temperatures between 70 and 90 °C, conditions which are commonly found in textile wastewaters. This research has shown the capacity for green tea synthesized nanoparticles to be used as a promoter for Fenton like dye degradation reactions. This rapid treatment approach may gain interest in the textile industries for dye waste remediation.

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\section*{1. Introduction}

Environmental contamination with dye pollutants resulting from various textile staining activities is a concern but an all too common practise. Pollution may stem from a variety of activities including but not limited to; out-dated waste management processes, accidental spills and cost cutting activities [1]. Furthermore, in developing countries, legal and standards to address the release of pollution into the environment are less stringent and policed [2], leading to increased levels of pollution. In particular the impact of dyes in wastewaters is significant.

The applications for commercial dyes are vast and include the colorization of textiles, paper, leather, paper and plastics. Dyes are also used in food, drug, cosmetic and photochemical productions [3].

\begin{strip}
\textsuperscript{Abbreviations: NP, nanoparticle; RBB-R, Remazol Brilliant Blue Dye; DB80, Direct Red 80 Dye.}
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The two most common methods used to colour textiles are exhaust and continuous dying. Exhaust dying has two distinct phases: an absorption phase and a diffusion phase. Diluted soluble or partially soluble dyes are brought into contact with the fabric and are initially adsorbed onto the surface of the fibres. The heat of the dyeing vessel may then be increased to over 130 °C to facilitate the diffusion of dyes into the fibres. In continuous dying, concentrated dye solutions are applied to textiles and then subsequently fixed chemically or by thermal means in one simultaneous operation [4]. High temperatures therefore play a significant part of the dyeing process resulting in wastewaters that are also chemically but also thermally polluting.

Dye molecules are made up of colour bearing groups (e.g. an aromatic structure absorbing visible light) called a chromophore, and auxochromes which can aid in water solubility and strength for bonding to fibres [5]. Dyes can also be classified based on their mode of application to textiles such as: acid, basic, direct, dispersive, reactive, mordant, sulphur and vat dyes [6].

Azo dyes are the largest class of dyes with making up around 70% of all dyes produced (e.g. Direct Red 80 (DB80)). Anthraquinone dyes fall under the second largest produced dyestuff category (e.g. Remazol Brilliant Blue (RBB-R)) [7]. The difference between
Azo and anthraquinone dyes lies within its structure. Azo dyes possess one or more azo chromophore (N=N) and bond between two or more aromatic rings whilst anthraquinone dyes possess the chromophore groups (−C=O & −C−C−) which form the anthraquinone complex [8].

Dye is a valuable commodity used to stain specific objects particular colours for aesthetic purposes. Nearly 40,000 dyes and pigments are listed making up over 100,000 different types of commercially available dyes. These dyes are produced in quantities of over 7 x 10^3 t per year with 10–15% of this entering the environment through industrial effluents [5].

One key attribute is that the dyes maintain their structure under harsh conditions and are therefore very resistant to biotic and abiotic degradation [5]. This makes it ideal for use within the textile industry. However, once these chemicals are released into the environment this same characteristic which makes it such a useful commodity becomes an environmental threat turning the dyes into persistent organic pollutants (POP’s) [10].

Traditional treatments for water remediation have relied heavily on the transportation of water to remediation plants. These technologies have been deemed to be inefficient within reasonable timeframes and are often costly due to the energy requirements to pump water to the plant and the cost of infrastructure to facilitate remediation [11].

Monitored natural attenuation and bioremediation are other strategies employed to remediate contaminated sites. Although these technologies are cheap to implement, lengthy timeframes are required for remediation and many pollutants may be recalcitrant to breakdown from these biological entities [12].

Taha et al. have shown biosorption as a promising method for removing dyes from wastewater. In 12 h 81.14% of dye (100 ppm) could be removed from wastewater using inactivated T. indicacae-seudateica at 55 °C [9].

The use of iron nanoparticles to catabolize pollutants and remediate contaminated sites is another technology which has been shown to be promising with research displaying degradation of a vast array of different dye classifications [13]. Although iron millimetric particles have been extensively studied and used, the shift from millimetric to nanoscale particles is the next frontier. With increased surface areas, NP’s have the capacity to degrade pollutants orders of magnitude faster than their millimetric counterpart [14,10]. Preliminary work has shown that iron NP’s remediate dyes to over 98% in 1 h at 30 °C. Furthermore, increases in temperature have been shown to accelerate the rate of dye degradation using iron NP’s [10].

The existing methods of iron nanoparticles synthesis are limited in their scope; as the reaction of an iron salt (e.g., iron (III) chloride) with sodium borohydride produces iron NP’s but there are complications with this approach. Firstly, these particles quickly oxidise in the presence of air or oxygenated water reducing their stability and subsequent reactivity. Furthermore, these particles tend to agglomerate. In addition, sodium borohydride is an expensive and toxic compound which is therefore likely to contaminate the environment upon application. Therefore, extensive washing of the particles is required leaving behind sodium borohydride waste which must be disposed [15].

Commercially available nanoscaled zero valent iron particles (nZVI) from Toda Kogyo Corp are generated by reducing goethite and hematite using a thermal method in conjunction with hydrogen gas which not only requires large amounts of energy but also produces crystalline particles which have reduced reactivity compared to their amorphous counterpart. These particles are costly to manufacture due to high energy requirements and also have a tendency to agglomerate [13].

The use of green tea polyphenols to generate nanoparticle has been utilised for medical applications [16], but is also a promising approach for environmental pollution remediation because nanoparticles can be generated which possess the desired size, shape, and crystallinity and the process can be considered an environmentally friendly, sustainable remediation approach. Polyphenols from the green tea reduce the iron salts to form iron nanoparticles and also cap the metal protecting it from oxidation (Table 1). Furthermore, green tea polyphenols are considered to be non-toxic and are thereby safer for the environment than sodium borohydride generated particles [17]. It is important to note that the use of iron salts for homogeneous Fenton processes has already been reported for pollutant remediation purposes. However, the main drawbacks of using salts include a small workable pH range (2.5–4.0), the formation of ferric hydroxide sludge at pH over 4 which can contain high concentrations of toxic pollutant degradation intermediates, and difficulty in catalysis recovery of iron [18]. Furthermore, since the hydroxyl radicals that are responsible for catalytic degradation process have a half-life of microseconds, a constant addition of iron salt solution is required to remediate pollutants which may be reintroduced into the system [19].

<table>
<thead>
<tr>
<th>Component</th>
<th>Example of Chemical Constituents</th>
<th>Percentage Make-up (%)</th>
<th>Ferric Reduction Capacity?</th>
<th>Iron Binding Capacity?</th>
<th>Polyphenol?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechins</td>
<td>Gallocatechin, Catechin, Epicatechin, Epicatechin Gallate, Epigallocatechin</td>
<td>22–45.4%</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>[43,44]</td>
</tr>
<tr>
<td>Flavonoids (other than catechin)</td>
<td>Gallate, Epigallocatechin, Epigallocatechin Gallate, Flavonols</td>
<td>7–14%</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>[45,46]</td>
</tr>
<tr>
<td>Methylxanthines</td>
<td>Caffeine</td>
<td>7–9%</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>[47]</td>
</tr>
<tr>
<td>Organic Acids</td>
<td>Gallic acid, Ascorbic acid, Quinic acid</td>
<td>3–4%</td>
<td>Yes</td>
<td>Unknown</td>
<td>Yes</td>
<td>[48,49]</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>Aspartic Acid, Glutamic Acid, GABA, Alanine, Ammonia, Threonine, Threonine, Phenylalanine, Tryptophan, Ure, Methionine, Isocitric, Lactate, Valine</td>
<td>7–9.5%</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td>[50,51]</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Reducing sugars, lignin, Pectin</td>
<td>8–12.0%</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>[52,53]</td>
</tr>
<tr>
<td>Minerals and Trace Elements</td>
<td>Ca, Mg, Cr, Mn, Fe, Cu, Zn, Mo, Se, Na, P, Co, Sr, Ni, K, F, and Al</td>
<td>10–15%</td>
<td>Reducing sugars = Yes, Lignin and pectin = No</td>
<td></td>
<td></td>
<td>[54]</td>
</tr>
<tr>
<td>Volatiles</td>
<td>1-Pentene-3-ol, 1,4-Benzened, 1,4-Dimethoxybenzene, Benzoic acid</td>
<td>0.02%</td>
<td>Unknown</td>
<td>No</td>
<td>No</td>
<td>[56,57]</td>
</tr>
</tbody>
</table>
Within this present study a concentrated mixture of both azo and anthraquinone dyes was subjected to an iron NP combined with hydrogen peroxide treatment under differing pH and temperature conditions and differing nanoparticle concentrations. We demonstrate that not only has pH been a determining factor in iron NP dye catalysis but the presence of hydrogen peroxide is vital for rapid reaction times via Fenton like processes [20]. To the best of our knowledge, RBB-R and DR80 dye mixture catalysis efficiencies have not been investigated with green tea-generated NPs. Optimal hydrogen peroxide concentrations were determined for enhanced degradation kinetics and NPs were characterized via transmission electron microscopy (TEM) and X-ray diffraction (XRD) analysis. This study aims at developing a rapid and sustainable remediation technology for dye waste within industrial textile industries.

2. Methods

2.1. Preparation of iron NPs

The synthesis of iron NPs using iron chloride combined with aqueous green tea leaf extract was carried out as described by Huang et al. [21]. Briefly: 60 g of commercially sourced green tea (Lipton’s) was removed from teabags and made up to 1 L with a 50% ethanol solution. The resulting mixture was heated to 80 °C for 1 h in a water bath, allowed to cool and then vacuum filtered through a 0.2 μm filter paper. Filtered green tea extract was then mixed at top speed using a magnetic stirrer and 0.1 M FeCl₃·6H₂O solution was slowly introduced using a burette ensuring a 2:1 ratio v/v of green tea extract to FeCl₃, respectively. The colour of the solution changed to black indicating the synthesis of NPs.

The resulting solution was centrifuged at 20,000 g for 20 min; the supernatant was discarded and the pellet was washed 3 times with deionised water to remove unreacted salts and tea phytochemicals on colloidal surfaces. Following final wash, the iron particles were centrifuged at 20,000 g, the supernatant was discarded and particles were freeze dried. Freeze dried particles were manually ground using a mortar and pestle.

2.2. Characterization

2.2.1. TEM

TEM characterization was undertaken for nanoparticles prior to and after the dye degradation reaction. The samples for transmission electron microscopy (TEM) were prepared by drop coating the samples on a carbon coated copper grid. The grid was left to dry overnight in a dust free environment and the morphological characteristics of iron nanoparticles were analysed by a JEOL1010 TEM operated at 100 kV (Thermoelectron cathode).

2.2.2. SEM and EDS

Scanning electron microscope (SEM) images of green tea nanoparticles were performed using a Philips XL30 SEM at an operating voltage of 25 kV and a spot size of 5. Particles were uncoated to facilitate EDS analysis using Oxford X-MaxN 20 EDXS Detector (2014) and AztecEnergy analysis software.

2.2.3. FTIR

FTIR spectra of the green tea extract generated iron nanoparticles were determined by a Fourier transform infrared spectroscope (Perkin Elmer Frontier). Nanoparticles were oven dried at 40 °C, added directly without further preparation to the spectroscope and the spectra was recorded. An average of 9 scans was collected for each measurement with a resolution of 1 cm⁻¹ in the range of 4000–400 cm⁻¹.

2.2.4. X-ray diffraction

An X-ray diffractometer (Bruker D8 Advance) was used for powder analysis. XRD analysis was conducted using a Bruker AXS D8 Discover with General Area Detector Diffraction System (GADDS) micro-diffraction instrument attached. Both instruments were equipped with a CuKα radiation source. All X-ray data was obtained in the 2θ-2θ locked-couple mode over a 2θ interval of 10–60°. The various component minerals were assigned through auto-fitting in the instrument software (Bruker Evaluation).

2.2.5. Zeta potential

To determine the extent of the electrostatic or charge repulsion/attraction between particles zeta potential measurements were performed in absolute ethanol (pH 7) using a Malvern 2000 Zetasizer with 1 ml nanoparticle solutions. The nanoparticles were appropriately diluted, sonicated and placed carefully in the sample cuvette (DTS 1060C, Malvern) avoiding any air bubble. The cuvettes were placed into zetasizer and data was acquired at 25 °C.

2.3. Dye decolourisation

2.3.1. Analytical methods

Decolourization activity was expressed in terms of decolourization efficiency (%) and was calculated as follows:

\[
\text{Decolourization efficiency (\%) = 100 - \left( \frac{C_f}{C_0} \right) \times 100}
\]

2.3.2. Where C₀ is the OD of the dye at time 0 and Cₜ is the OD of dye at time t.

2.3.3. Batch decolourisation

C. Remazol Brilliant Blue R (RBB-R, C.I. No. 61200, C₉H₁₃N₅O₇S₂, M.W. = 626.54 g mol⁻¹, 50% dye content) and Direct Red 80 (DR80, C.I. No. 35780, C₁₃H₁₉N₉O₆Na₂O₃S₄, M.W. = 1373.07 g mol⁻¹, 25% dye content) (Fig. 1) were obtained from Sigma-Aldrich and were used as received. H₂O₂ (30% purity) and FeCl₃·6H₂O (>96% purity) were also purchased from Sigma-Aldrich.

2.3.3. Influence of pH on dye degradation

RBB-R dye was made to 150 ppm and DR80 dye was made to 25 ppm with a 0.75% H₂O₂ solution. Preliminary studies showed that these concentrations were optimal for lambda max (λ max) measurements without requiring dilution of the samples. Aliquots (100 ml) of each different dye solution were put into separate flasks and the pH was adjusted to: 3, 5, 7 and 9 using 0.05 M NaOH.

![Image](image.png)

Fig. 1. TEM images of iron NP’s used in this study at 25,000× magnification.

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and 0.05 M HCl. The flasks were stored overnight in a refrigerator at 4°C and 8 h later the pHs were re-checked and re-adjusted. Small vials were wrapped in aluminium foil to prevent light affecting the reaction. Dye (15 mL adjusted to different pH values) was added to each vial. Each pH range had 3 samples and 3 controls. All experiments were conducted at room temperature (23 ± 2°C).

Absorbance scan of dyes was performed to determine lambda max via optical density (OD) measurements for each dye and dye mixture. Lambda max was determined via a wavelength spectral scan between 220 nm and 1000 nm using BMG POLARstarOmega, BMG Labtech, Germany, in conjunction with flat bottom COSTAR 96 well plates. An aliquot (200 μL) of sample was removed for each sampling point and absorbance readings were taken.

Initial spectrometry analysis was conducted at 594 nm for RBB-R (150 ppm) and 536 nm for DR80 (25 ppm). An aliquot (70 μL) of the 2 day aged NP solution was added to each vial and samples were withdrawn periodically during the Fenton like oxidation for spectrometric analysis.

2.3.4. Influence of temperature on dye degradation
Stock RBB-R and DR80 blend was prepared by mixing equal volumes of 250 ppm of each dye solution together. This dye cocktail possessed a final H2O2 concentration of 3% and pH of 5. Five different temperatures were chosen to assess dye degradation kinetics (4, 21, 50, 70 & 90°C). Temperature regulation was maintained via a heating block for 50, 70 & 90°C. Ice was used to maintain 4°C and 23 ± 2°C was the ambient room temperature.

Scanning of absorbance of dyes was performed to determine peak absorbance (OD) for the dye cocktail. Maximum absorbance was determined via a wavelength scan using BMG POLARstarOmega, BMG Labtech, Germany, in conjunction with flat bottom COSTAR 96 well plates. An aliquot (50 μL) of sample was removed for each sampling point and mixed with 50 μL of distilled water. Absorbance readings were then taken to assess time 0 dye OD’s.

Initial spectrometry was conducted at 544 nm. Two day aged green iron nanoparticles (20 μL in solution) were added to each vial and samples were withdrawn periodically during the Fenton like oxidation for spectrometric analysis.

The aforementioned methodology was repeated for a dye cocktail containing 500 ppm RBB-R and 500 ppm DR80. For spectrometry measurements of this dye 25 μL of dye was mixed with 75 μL of distilled water to ensure maximum absorbance reading limit of the spectrophotometer was not reached.

2.3.5. Effect of nanoparticle concentration on dye degradation
Dye cocktail (250 ppm RBB-R & 250 ppm DR80) was prepared as previously described and temperature was kept at 70°C using a heating block. Five different NP concentrations were selected to assess dye degradation efficiencies using serial dilutions of 26.67–3.33 ppm with no NPs added to the negative control. Spectrometric analysis was conducted as previously described.

3. Results and discussion

3.1. Iron nanoparticles from green tea

Chelation of iron salts by aqueous green tea extract is likely to proceed due to the action of numerous different chemical constituents. Table 1 outlines the main components of dried green tea leaves and shows that many compounds have iron reducing properties. Of these compounds, catechins make up between 32 and 45% of the total chemical composition and are therefore likely to be dominant drivers in this process.

TEM analysis of fresh iron nanoparticles confirmed the successful preparation of iron nanoparticles from green tea, showing that the majority of particles were between 20 and 50 nm in size, possessed a circular morphology and were uniform in shape (Fig. 1). These properties are seen as beneficial as if particles are too small they may become oxidised readily or react with other organic compounds in the environment before they reach the pollution source [22]. Particles with sizes over 100 nm are not considered nanoscale and will have reduced pollution catalysis efficiency due to the reduction in surface area able to be in contact with pollution source or hydrogen peroxide. However, as the particles are agglomerated and forming a lattice in this study, a reduction in their potential maximum surface area and in turn reactivity is expected.

XRD analysis confirmed that no defined peaks were present on the spectra (Fig. 2). This indicated that the particles were not crystalline in nature and were amorphous. Amorphous nanoparticles have shown enhanced dye degradation rates compared to their zero valent iron counterparts but have also been shown to oxidise faster, reducing storage time of particles and in turn reduces reactivity [13,14].

To further confirm findings, Scanning electron microscopy (SEM) coupled with EDX was conducted to map the elements present in the nanoparticle sample (Fig. 3). Elemental analysis of
the sample showed that 67.1% of the samples surface was composed of carbon. This is evidence that organic matter derived from the aqueous green tea extract has attached to the surface of the particles. Although some of this value may be due to the backscattering of electrons from the carbon tape the sample was loaded on, green tea extract generated nanoparticles synthesized by Kuang et al. [23] have shown surface carbon concentrations exceeding 50%. Iron concentration appears low in the elemental analysis (17.4%) however, as SEM is used for surface characterization, the iron residing under the carbon and oxygen is not measured. This concentration is similar to that reported elsewhere [23,24].

Iron nanoparticles are prone to rapid oxidation. These particles are likely to be iron oxides or iron oxy-hydroxides and not zero valent iron due to the lack of crystallinity found with XRD analysis (Fig. 2) and the presence of oxygen (13.8%) on the surface of the particles. Chlorine present in the sample is very low (0.7%) and this can be explained as residue from the iron chloride salt solution used to synthesize the particles. Aluminum is also present in small quantities (0.5%) and this is likely due to backscattering of electrons from the aluminium sample holder.

To further confirm findings, FTIR analysis was conducted to determine green tea chemical components which were responsible for carbon signature found with EDX (Fig. 4). Results showed peaks of 3376, 1609, 1446, and 1141 cm⁻¹ which are representative of epigallocatechin gallate (EGCG) [25], a major polyphenol of green tea, which presumably due to its strong antioxidant properties leads the reaction and subsequently gets adsorbed on the surface on iron nanoparticles. This provides a capping effect preserving their shelf life by reducing their exposure to atmospheric oxygen (Fig. 4) [25,26]. Peaks and corresponding bond identities were identified as follows: 3376 cm⁻¹ is indicative of O–H stretching vibrations [27], 1609 cm⁻¹ shows C–C aromatic ring stretching vibrations [27], 1516 cm⁻¹ relates to N–O asymmetric stretch [28], 1446 cm⁻¹ corresponds to aliphatic C–H bend [28], 1200 cm⁻¹ is a marker for phenolic C–O stretching [29], 1141 cm⁻¹ shows C–OH stretching and 1015 cm⁻¹ describes C–O stretching for the OH substituent of a pyran ring [28,30].

The peak located at 1015 cm⁻¹ is evidence for the presence of either (+) catechin, (−) epicatechin and/or (−) epigallocatechin. This finding coupled with the aforementioned signature markers of ECGC indicates that more than one catechin is capping the green tea extract generated nanoparticles.

Nadagouda et al. have reported that green tea polyphenols are able to surround the iron particles and provide a protecting capping effect resulting in particles not being readily oxidised [31]. This is one benefit of synthesizing iron nanoparticles with aqueous plant extracts over the popular sodium borohydride reduction of iron salts which requires anaerobic conditions for particle synthesis. Furthermore, sodium borohydride is seen as a toxic compound which is likely to contribute to environmental harm upon application [17,20,32]. The zeta potential, a measure of the extent of the electrostatic or charge repulsion/attraction between particles was shown to be 12.3 (data not shown) indicating that the particles had a strong electrostatic attraction and therefore appeared agglomerated (Fig. 1) and did not exist as individual homogeneous entities. This presumably may be due to the hydrogen bonding and other electrostatic interactions between polyphenolic moieties from green tea that are present on the surface of nanoparticles (Fig. 5).

3.2. Dye degradation

Dye contaminated wastewaters exhibit a large degree of variation in pH depending on their source and the type of dye used [1]. It is therefore important to determine if pH will influence the rate of dye catalysis reactions so that pre-treatments or alternative remediation strategies may be implemented if conditions are unfavourable. If dyes can be treated on site before being released into the environment, more manageable and rapid treatment approaches can be applied, legal ramifications relating to environmental harm can be avoided and savings in site restoration can be achieved [1,33].

Wavelength scans for determining the maximum absorbance parameters of both RBB-R and DR80 indicated that maximum absorbance was found to be 594 nm for RBB-R’s at an OD of 1.6 and 536 nm at an OD of 2.0 for DR80’s (data not shown). Similar
maximum absorbance wavelengths for RBB-R have been reported in the literature by Elkhattabi et al. [34] whilst absorbance values for DR80 have been measured at 540 nm by Kim et al. [35].

The maximum OD(394) value for RBB-R (150 ppm) at time 0 was 1.25 for pH dependent microcosms. DR80 (25 ppm) showed maximum OD(394) value of 1.62.

Dye cocktails comprising of 250 ppm RBB-R and 250 ppm DR80 had maximum absorbance wavelengths of 544 nm and 50% dilutions produced OD(394) of approximately 2.0.

3.2.1. Influence of pH on dye degradation

Within this study we demonstrated the degradation efficiencies of nanoparticles on an AZO dye (DR80 25 ppm) and an anthraquinone dye (RBB-R 150 ppm) in the presence of 0.75% H₂O₂ at different pH’s.

Dye degradation microcosms exhibited a pH dependant degradation trend favouring acidic conditions which is consistent with Fenton processes. There were statistically significant differences in dye degradation between pH’s 3, 5, 7 and 9 at 60 min for NP’s by means of one way ANOVA and Tukey post hoc tests.

At 60 min, RBB-R showed 92.2% decolourization efficiency at pH 3, compared with 46.3% for pH 5, 8.6% for pH 7 and 1.0% for pH 9. At the completion of the experiment at 18 h, dye decolourization efficiencies were shown to be 98.3%, 95.0%, 50.4% and 2.5% respectively (Fig. 5a).

Degradation of DR80 (25 ppm), showed the same trend as that of RBB-R. At 64 min dye decolourization efficiencies were as follows: 68.7% for pH 3, 41.5% for pH 5, 3.9% for pH 7 and 1.0 for pH 9. Upon completion of the experiment at 18 h dye decolourization efficiencies were shown to be 94.0%, 93.2%, 11.4% and 2.5% respectively (Fig. 5b).

There are two possible factors which may explain why iron NP’s are more efficient at breaking down dyes in acidic conditions. Firstly, in alkaline conditions it was shown that NP’s turned from black to a rusty brown colour which precipitated out of solution.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>1446.5</td>
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<tr>
<td>6</td>
<td>1141.0</td>
</tr>
<tr>
<td>7</td>
<td>1015.8</td>
</tr>
</tbody>
</table>

Fig. 5. Influence of pH on dye decolourization efficiencies of (a) RBB-R and (b) DR80 when subjected to iron nanoparticle treatment with 0.75% hydrogen. Experiments were conducted in triplicate and error bars correspond to the standard deviation of the mean.
This indicates that charged iron molecules were interacting with OH\textsuperscript{-} species and forming stable and unreactive Fe(OH)\textsubscript{2} entities. Secondly, Fenton processes are more efficient in acidic conditions leading to move OH\textsuperscript{-} and in turn faster dye degradation rates [36]. A simple adjustment of acidic dye degradation conditions to alkaline conditions will aid in the precipitation and subsequent removal of added iron to the reaction vessel. This will reduce the concentration of iron released into the environment following industrial dye degradation processes.

In neutral conditions Fenton processes favour the generation of the ferryl ion (Fe(IV)O\textsuperscript{2+}) over the hydroxide ion. It is therefore assumed that the ferryl ion is a less efficient oxidant than OH\textsuperscript{-} [37]. Degradation rates of the AZO dye DR80 showed slower initial rates of degradation than RBB-R; however at pH 3 and 5 significant catalysis of the parent pollutant was apparent.

In order for rapid dye decolourisation to proceed, acidic conditions coupled with the presence of both iron and hydrogen peroxide was required. This is consistent with the requirements for the Fenton reaction [38,39]. As ferrous iron is substituted with iron oxide nanoparticles, the reaction is termed Fenton-like. In order for hydrogen peroxide to decompose and form hydroxyl radicals it must first be adsorbed to the surface of the iron oxide. The rate of catalytic capacity of the nanoparticles has not been determined, however its mechanism has been described elsewhere [36,39].

3.2.2. Influence of temperature on dye degradation

In textile dyeing industries, a number of different dyes are usually mixed and applied to products to achieve desired colour combinations. It is therefore rare to find final industrial waste products containing singular dye entities. Furthermore, temperatures exceeding 100 °C are often experienced during the dye process which may render treatments such as bioremediation ineffective until sufficient cooling has been achieved [4]. Treatment of dyes on site may be beneficial as waste products do not need to be delivered to treatment plants and increased temperatures experienced in the textile industry may facilitate remediation using Fenton like reactions as identified in this study.

Concentrations of DR80 and RBB-R in the dye mixture were 10 times and 1.6 times of that found in pH dye degradation microcosms respectively. Due to this increase in concentration, degradation of dye at room temperature or below was negligible. However, a rise in temperature favoured rapid and significant dye degradation. At 90 °C, 79.5% degradation was observed after only 20 min, 96.2% dye discoloration was shown at 76 min and 99.1% degradation was shown in just over 3 h. At 70 °C a similar trend was observed with 84.3% dye discoloration observed at 76 min and over 94% decolourisation in just over 3 h (Fig. 6).

High temperatures also aided dye decolourisation with controls of 90, 70 and 50 °C showing marked decolourization without NPs. However, significantly elevated degradation was observed with their treatment counterparts (Fig. 6). A repeated experiment assessing the decolourization of a dye cocktail with 500 ppm RBB-R and 500 ppm DR80 showed the same trend with slightly slower dye degradation rates (Supplementary Fig. 1).

3.2.3. Influence of iron NP concentration on dye degradation

It has been shown that NP concentration significantly affects dye degradation efficiencies [10,40]. In this research, significantly increased dye decolourisation (mixed) was observed with increasing iron NP concentration at 20 min with 0.53, 0.27, 0.13, 0.07 and 0 ppm NP concentrations showing 89.8, 81.4, 74.0, 66.1 and 15.5% decolourisation respectively. However, at 80 min all NP amended treatments were able to achieve more than 85% dye decolourization efficiencies with >95% colour removal efficiency seen at 0.53 ppm. At 140 min all treatments (excluding the negative control) were able to provide between 93 and 98% dye decolourisation. The negative control (0 ppm NP concentration) showed nearly 37% decolourization efficiency and this is attributed to increased temperatures breaking bonds of the dyes chemical structure.

All treatments were able to provide significant decolourization efficiencies at 70 °C whilst NP concentration only slightly impacted dye degradation efficiency. If treatment times can be extended slightly, smaller concentrations of NPs may be used reducing remediation costs for industry (Fig. 7).

The use of iron salt precursors in the presence of hydrogen peroxide will generate hydroxyl radicals in acidic conditions and will destroy parent dye molecules. However as the hydroxyl radical has a very short lifespan, the ability to control reaction rates is limited with the use of salts. Nanoparticles will provide a sustained

![Fig. 6. Influence of temperature on dye degradation efficiencies when RBB-R (250 ppm) mixed with DR80 (250 ppm) subjected to iron nanoparticle treatment with 3.0% hydrogen peroxide at pH 5. Experiments were conducted in triplicate and error bars correspond to the standard deviation of the mean. Control represents the dye solutions without nanoparticles added. Inset shows the extent of decolourization at 90 °C in absence (control) and presence of nanoparticles.](image-url)

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Fig. 7. Influence of nanoparticle concentrations on dye degradation of RBB-R (250 mg/L) mixed with D880 (250 ppm) and subjected to iron nanoparticle treatment (0-0.53 ppm) with 3% hydrogen peroxide at pH 5 at 70°C. Experiments were conducted in triplicate and error bars correspond to the standard deviation of the mean.

reaction speed over time until the hydrogen peroxide is used or conditions change (i.e. pH changes). Iron nanoparticles are currently used for groundwater remediation. However for surface waters, further research on particle coatings and the immobilization of nanoparticles onto solid supports is required for particle recoverability and reduced environmental impact. Iron salts have little scope for optimization compared to their iron nanoparticle counterparts [41].

Currently, the remediation of dyes in industrial settings can be accomplished by a number of different avenues, all possessing inherent faults. Physical methods tend are only effective on low concentrations of dyes and do not destroy the dye molecule but rather change its phase. Adsorption of pollutants onto activated carbon is overly expensive and reclaiming of carbon is required. Chemical methods are either expensive, pre-treatment is required or sludge containing toxic chemicals remain following remediation. Biological remediation is sensitive to temperature, pH, increased dye concentration and is slow compared to other treatment methods [42].

Vijayaraghavan concludes that biosorption is the most environmentally benign and an optimum method to remove dye pollution [42]. Biosorption may remove pollutants from the wastewater, however, the dye itself has not been broken down and the sorption biomass has now accumulated concentrated dye pollution that needs to be treated before being disposed of.

Findings from this current study show that iron NPs were able to remove concentrated dye mixtures (250 ppm RBB-R mixed with 250 ppm D880) at temperatures up to 90°C and achieve just under 90% decolourisation in 20 min with 0.53 ppm nanoparticle concentration at a temperature of 70°C. Not only were decolourization times more than 36 orders of magnitude faster than that reported by Taha et al. [9] but no additional toxic chemicals were used and the resulting treated dye solution had been remediated (as opposed to adsorbed) and therefore additional post treatment may not be necessary. If dafughter dye products are present they are likely to be more susceptible to bioremediation due to reduced chemical structure size.

Optimum conditions for the degradation of concentrated RBB-R and D880 mixtures were acidic with 3% H2O2 concentration at temperatures of between 70 and 90°C. Increasing NP concentration sped up dye catabolism reaction times; however high degrees of dye decolourisation were shown at the lowest tested NP concentration of 0.07 ppm in less than 2.5 h.

4. Conclusions

Temperature had a major impact on the rate of concentrated Azo (D880) and Anthraquinone (RBB-R) dye mixture degradation with just under 90% decolourisation in 20 min with 0.53 ppm nanoparticle concentration at a temperature of 70°C. Over 99% of concentrated dye mixture was achieved at 90°C after 191 min.

The ease of application, reduced cost compared to that of other chemical methods, rapid remediation times compared to bioremediation, and the increase in dye degradation rates at temperatures known to deactivate biological treatment methods explain why green tea synthesized nanoparticles are a promising candidate for industrial dye wastewater remediation.

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2. Victorian X-Ray Structural Determination and Materials Characterisation Facility – Mr Frank Antolasic and Dr Rahul Ram.
3. Centre for Environment, Sustainability and Remediation (EnSure) – Prof Andrew Ball.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jece.2016.10.008.

References

Dye decolourization efficiencies of RBB-R (500 mg/L) mixed with DR80 (500 mg/L) when subjected to iron nanoparticle treatment with 3.0% hydrogen peroxide at pH 5 at different temperatures. Experiments were conducted in triplicate and error bars correspond to the standard deviation of the mean.
Phytofabrication of Iron Nanoparticles for Hexavalent Chromium Remediation

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Statement of authorship

Adam Truskewycz (candidate) planned the experimental design, performed experiments, carried out all research, analysed and interpreted data. Truskewycz also reviewed the literature, executed the writing and was corresponding author for Chapter 3.

Ravi Shukla contributed to co-supervision of research and manuscript evaluation in Chapter 3.

Andrew S Ball contributed to the planning of experimental design, supervision of research, manuscript preparation and evaluation of Chapter 3.

All the co-authors give full consent to Adam Truskewycz to present these above papers for examination towards the Degree of Doctor of Philosophy.

Adam Truskewycz (candidate)
Ravi Shukla
Andrew S Ball
Phytoturbation of Iron Nanoparticles for Hexavalent Chromium Remediation

Adam Truskewycz, Ravi Shukla, and Andrew S. Ball

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ABSTRACT: Hexavalent chromium is a genotoxic and carcinogenic byproduct of a number of industrial processes, which is discharged into the environment in excessive and toxic concentrations worldwide. In this paper, the synthesis of green iron oxide nanoparticles using extracts of four novel plant species [Pithoragorum undulatum, Melia azedarach, Schinus molle, and Syzygium paniculatum (var. australe)] using a "bottom-up approach" has been implemented for hexavalent chromium remediation. Nanoparticle characterizations show that different plant extracts lead to the formation of nanoparticles with different sizes, agglomeration tendencies, and shapes but similar amorphous nature and elemental makeup. Hexavalent chromium removal is linked with the particle size and monodispersity. Nanoparticles with sizes between 5 and 15 nm from M. azedarach and P. undulatum showed enhanced chromium removal capacities (84.1–96.2%, respectively) when compared to the agglomerated particles of S. molle and S. paniculatum with sizes between 30 and 100 nm (43.7–85.7%, respectively) in over 9 h. This study has shown that the reduction of iron salts with plant extracts is unlikely to generate vast quantities of stable zero-valent iron nanoparticles but rather favor the formation of iron oxide nanoparticles. In addition, plant extracts with higher antioxidant concentrations may not produce nanoparticles with morphologies optimal for pollutant remediation.

INTRODUCTION

Chromium is a valuable resource in a number of different industries, having essential roles in refractories, alloys, electroplating, pigments, catalyst generation, and leather tanning, to name a few applications. It is most commonly found in the environment as the trivalent (Cr(III)) cation Cr\(^{3+}\) or the hexavalent (Cr(VI)) oxonium, that is, HCrO\(_4^−\) and CrO\(_4^{2−}\). Trivalent chromium is an essential macronutrient, which aids in the regulation of lipid, carbohydrate, and protein metabolism; however, overabundance may lead to erythrocyte membrane disturbances and cause skin irritation. Hexavalent chromium is considerably more toxic than its trivalent counterpart. Unlike Cr(III), it can penetrate the skin and kill cells and/or damage DNA through the generation of reactive oxygen species. It is genotoxic and recognized as a human carcinogen (group 1) by the World Health Organization. In addition, Cr(VI) is considerably mobile in soils and waterways, increasing its potential for human and widespread ecosystem exposure.

More than 1700 industrial facilities have been listed by the USEPA's "Toxic Release Inventory-2003" for releasing chromium into the environment with a total discharge of approximately 52,600 metric tons. For example, in India, it is estimated that greater than 2000 tons of chromium is released into the environment annually. Wastewater from some of these regions contains up to 5000 ppm of chromium, far exceeding the discharge limit of 2 ppm.

As a consequence of the amounts of Cr being released into the environment and the potential environmental impact, a number of different techniques have been implemented for the removal of Cr(VI) species from the environment. The environmental remediation of chromium is faced with a plethora of different factors and conditions, which must be considered, that is, pH, Cr(VI) concentration, temperature, interfering species in aqueous environments, absence of oxygen in groundwater, likelihood of contamination to pose a health risk, time-sensitive remediation occurrences, and so forth. Considering this, different remediation technologies may be required in different situations. The benefits and disadvantages of each technology are compared in Table 1.

Recently, the use of nanoparticles for the remediation of hexavalent chromium species has been investigated. Zhou et
Table 1. Current Hexavalent Chromium Removal Strategies

<table>
<thead>
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<th>technique</th>
<th>approach</th>
<th>advantages</th>
<th>disadvantages</th>
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</thead>
<tbody>
<tr>
<td>adsorption</td>
<td>activated carbon</td>
<td>high absorption capacity, can remove both Cr(VI) and Cr(III) species</td>
<td>expensive, nonselective, competing agents often needed for optimal performance</td>
</tr>
<tr>
<td>filtration</td>
<td>polymeric membranes</td>
<td>low space requirements</td>
<td>extreme chemical and thermal environments may compromise stability, membrane fouling</td>
</tr>
<tr>
<td>membrane technology</td>
<td>reverse osmosis</td>
<td>high level of selectivity</td>
<td>costly and requires regular monitoring/maintenance</td>
</tr>
<tr>
<td>ion exchange</td>
<td>anion resin to remove Cr(VI)</td>
<td>requires little maintenance, very effective, suitable for large- or small-scale applications</td>
<td>pretreatment necessary, removal efficiency is affected by other water-soluble ions, fouling</td>
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</tbody>
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*References 8 and 9.

Table 2. Plant Extracts with Their Corresponding Phytochemical Profiles

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<th>plant extract</th>
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<th>antioxidant (mM Tiron (equiv)/g)</th>
<th>phenolics (mg CA (equiv)/g)</th>
<th>reducing sugars (mg glucose (equiv)/g)</th>
<th>iron-chelating capacity (mg EDTA (equiv)/g)</th>
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<td>1.9 ± 0.2</td>
<td>42.9 ± 9.9</td>
<td>90.9 ± 16.4</td>
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<td>M. azedarach</td>
<td>6.0 ± 0.1</td>
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<td>S. paniculatum</td>
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</table>

*Different superscript letters (a, b, and c) denote significant differences between samples.

K. Wang et al.10 used Fe/Ni bimetallic particles to remove Cr(VI) from aqueous solution in an ultrasound-assisted system across pH 3–9. Albadarin and U1.11 used hydros cerium oxide nanoparticles for Cr(VI) adsorption at pH 2; and Valle et al.12 showed Cr(VI) phytoextraction to K3MnO4 nanoparticles at pH 2.

Generating iron nanoparticles through the reduction of iron salt precursors with various crude plant extracts has been widely demonstrated and has been hailed for its environmentally friendly nature and relatively low cost when compared to alternative methods. However, the reproducibility of results in terms of nanoparticle properties (i.e., size, shape, reactivity, monodispersity, oxidation potential, crystallinity, etc.) presents a challenge. Many different bioactive molecules found within plant cells (e.g., polyphenols, proteins, reducing sugars, and lipids) are likely to interact with iron precursors to influence nanoparticle formation and properties. The application of iron and bimetallic nanoparticles is vast; however, their use in the environmental remediation of organic and inorganic pollutants has shown encouraging results.

Wang et al.13 synthesized iron nanoparticles with green tea and eucalyptus leaves, which were able to remove 39.7 and 41.4% of nitrate, respectively, from an initial concentration of 20 mg/L. Luo et al.14 showed that grape leaf-mediated synthesis of Fe/Pd bimetallic nanoparticles removed 81.7% of orange II dye from solution with an initial concentration of 90 mg/L in 12 h. Martinez-Cabanas et al.15 used eucalyptus-generated Fe nanoparticles encapsulated in a chitosan matrix for the removal of As(V) using column flow-through experiments, reporting that almost 100% of As(V) from a 100 μg/L solution was captured or transformed in over 30 h at a flow rate of 2 mL/min.16

Current studies utilizing plant extracts for the synthesis of iron nanoparticles tend to select plant candidates based on high antioxidant concentrations,17-20 however, this may not be beneficial for optimal iron nanoparticle morphologies as iron is highly reactive and is likely to interact with various phytochemicals in plant extracts in different manners. To test this, plants were selected based on their differing antioxidant profiles and interesting phytochemical makeup, all of which have been linked to antimicrobial and/or medical applications.21-22

The aim of this study was to obtain water-soluble phytochemical extracts from the leaves of four different plant species found within Australia (Pittosporum undulatum, Medin azeedarachi, Schinus molle, and Syzygium paniculatum [var. australis]) and use these extracts to reduce iron salt precursors into iron nanoparticles. All plant species used in this study are novel in terms of iron nanoparticle synthesis and have diverse and varying phytochemical profiles. Furthermore, their use for antimicrobials and therapeutics indicate that they have interesting phytochemical compositions, which may lead to novel interactions between biological and inorganic species.23-25 The differences in nanoparticle morphologies and phytochemical properties, along with their capacity to remove Cr(VI) species from solution, were investigated.

### RESULTS AND DISCUSSION

**Characterization of Plant Extracts.** In the search for plant species capable of generating "green" nanoparticles with optimal morphologies/properties, much of the selection process has focused on species containing high plant antioxidant concentrations. Although increasing antioxidant concentrations are often trended with increasing reducing powers, their ability to instill optimum morphological nanoparticle features has not been ascertained. From our results, the antioxidant concentrations for P. undulatum, M. azeedarachi, S. molle, and S. paniculatum were 12.6, 9.9, 7.2, and 48.1 mM Trolox (equiv)/g, respectively, with only S. paniculatum showing statistically higher concentration. Reducing powers of the same plant extracts were 5.6, 6.0, 4.7, and 7.5 mg ascorbic acid (AA) (equiv)/g, respectively, indicating that the species with the highest and lowest antioxidant concentrations also possessed the highest and lowest reducing powers (Table 2). Antioxidative effects have been linked with the generation of free radicals, which are free radical chain...
reaction terminators. Therefore, antioxidant activity has, in some cases, a relationship with the reductive capacity.\textsuperscript{25} Phenols are known antioxidants, and therefore, it is no surprise that phenolic content followed the same trend as antioxidant concentration. \textit{P. undulatum}, \textit{M. azederach}, \textit{S. molle}, and \textit{S. paniculatum} possessed phenolic concentrations of 1.9, 1.9, 2.3, and 4.0 mg gallic acid (GA) (equiv)/g with only \textit{S. paniculatum} showing statistically higher concentration (Table 2). Reducing sugars, as their name suggests are reducing agents with the capacity to transform iron salts to nanoparticles.\textsuperscript{26} \textit{P. undulatum} and \textit{S. molle} possessed the lowest reducing sugar concentrations (42.9 and 64.5 mg glucose (equiv)/g, respectively) and were not significantly different from one another. \textit{M. azederach} possessed significantly higher reducing sugars than \textit{P. undulatum} but was not statistically different from \textit{S. molle}. \textit{S. paniculatum} possessed considerably higher concentrations of reducing sugars than all other plant extracts with 18.4 mg glucose (equiv)/g (\textgreater{} 1.5 times that of \textit{M. azederach}, which contained 9.0 mg glucose (equiv)/g). It is interesting to note that although \textit{S. paniculatum} contained higher reducing sugar and antioxidant concentrations when compared to the other species, its reducing powers was not significantly greater than those of \textit{P. undulatum} and \textit{M. azederach} (Table 2).

Iron chelation relates to the ability of plant extract constituents to bind to iron ions and form complex ring structures called chelates. Low and high pH compromise the stability of the chelating agents.\textsuperscript{27} Although the formation of nanoparticles resulted in highly acidic solutions following mixing of precursors, the original pH of the plant extract may also alter the chelation capacity. The pH of plant extracts of \textit{P. undulatum}, \textit{M. azederach}, \textit{S. molle}, and \textit{S. paniculatum} were 5.95, 5.11, 6.10, and 5.87, respectively (Table 2). The iron-chelating capacity for the aforementioned plant extracts was 2.6, 2.1, 5.8, and 0.3 mg ethylenediaminetetraacetaciddiacetate (EDTA) (equiv)/g, which shows a pH-dependent trend, with lower pH resulting in lower chelation capacity. Saito-Garcia et al.\textsuperscript{28} showed that the anthocyanin antioxidants, delphinidin and malvidin, were unable to bind to copper ions under acidic conditions. \textit{S. paniculatum} contains the glycosidases of malvidin and delphinidin (i.e., malvidin and delphinidin 3,5-di-glucoside),\textsuperscript{29} and its extract is also acidic, and this may explain its low metal chelation capacity. In addition, the type of phytochemicals present within the plant extract may react differently to pH or other competing influences. Interestingly, \textit{S. molle} possessed the lowest antioxidant concentration but more than twice the iron-chelating capacity of any other extract. Freeze-dried samples of this extract yielded a fakirike substance, which may not possess high antioxidative properties but may still bind to the particles. \textit{S. molle} was the only sample to possess a sap/resin following the hyposalinisation process.

**Synthesis of Iron Nanoparticles.** The introduction of iron chloride solution to the plant extract resulted in a color change from a yellow/brown extract to a jet-black nanoparticle solution instantaneously. This indicated that iron nanoparticles had been formed. The pH of the nanoparticle solutions dropped to 1.5 in all cases; however, nanoparticles were separated from their solution with the aid of dialysis centrifuge tubes (molecular-weight cutoff 30,000 Da) and freeze-dried prior to being used in Cr(VI) removal studies. The nanoparticles were not magnetic and did not settle out of solution under atmospheric conditions.

Current studies on plant extracts for the synthesis of nanoparticles tend to select plant candidates based on high antioxidant concentrations\textsuperscript{20-22} however, this may not be beneficial for optimal nanoparticle morphologies. In the current study, antioxidant concentrations along with phenolic content and reducing power capacity have shown little to no statistical differences between \textit{P. undulatum}, \textit{S. molle}, and \textit{M. azederach}. However, the morphologies of the nanoparticle are markedly different (Figure 1). \textit{P. undulatum} showed a mixed nanoparticle morphology with the majority of particles between 5 and 10 nm in size. However, a few larger particles with size ranges between 20 and 70 nm are infrequently present (Figure 1). In addition, there are flat plates that fold into rods with nanometer dimensions. The presence of numerous phytoactive compounds acting on the iron chloride precursors in different ways may be responsible for the numerous different nanoparticle morphologies present. \textit{M. azederach} possessed particles between 5 and 15 nm in size with little variation in the nanoparticle morphology. \textit{S. molle} formed chainlike structures with particles often failing outside of the nanoparticle size range (\textgreater{} 10 nm in all particle dimensions). Nanoparticle structures generated from \textit{S. paniculatum} were\textsuperscript{23} also chainlike in appearance. However, particle sizes were considerably smaller than that of \textit{S. molle} (sizes ranging between 30 and 60 nm) (Figures 1 and S1).

If the generation of maximum nanoparticle concentration is the only parameter of interest, then high antioxidant concentration and reducing powers may be beneficial. However, if specific nanoparticle properties are required, high antioxidant concentration may not be a major contributing factor in achieving this. Optimal nanoparticle properties for pollution remediation include particles that are small, monodisperse, possess no particle–particle attraction, and do not passivate readily under aerobic and aqueous conditions.\textsuperscript{30} Our findings, along with other studies that utilize

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{TEM images of iron nanoparticles generated from the reaction between plant extracts and iron chloride precursors. (A) \textit{P. undulatum} NP, (B) \textit{M. azederach} NP, (C) \textit{S. molle} NP, and (D) \textit{S. paniculatum} NP.}
\end{figure}

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antioxidant rich green tea extracts to generate iron nanoparticles, have shown that high antioxidant concentrations lead to particles that agglomerate and form chainlike structures, thereby reducing the nanoparticle surface area available for remitting interactions.

X-ray Photoelectron Spectroscopy (XPS) and Powder X-ray Diffraction (XRD). There is a common misconception that the production of black iron nanoparticles upon reaction of plant extracts with iron salt precursors confirms the presence of K3Fe(CN)6 (Fe6). Reactive iron is likely to interact with active phytochemicals within plant extracts making it difficult to form repeating lattice structures required for crystallinity. This is supported by the amorphous nature of nanoparticles generated in this study and several other studies producing iron nanoparticles with plant extracts. In addition to crystallinity, Fe6 nanoparticles are ferromagnetic in nature and show a representative XPS peak at 706 eV (Figure 2). XPS analysis of freshly synthesized iron nanoparticles with plant extracts and the C 1s, O 1s, and Fe 2p core levels revealed that the surface of the particles were composed of a mixture of carbon, oxygen, and iron species (Figure 2). Carbon peaks for the representative sample showed peaks at 284.6, 285.5, 286.6, and 288.6 eV corresponding to C=C, C=O, C–OH, and O=C=O bonds, respectively. These bonds show that plant bioactive compounds are bound to the surface of the iron oxide nanoparticles. Oxygen was also present on the surface of the particles, with peaks 353.1, 353.2, and 354.2 relating to oxygen in hydroxides, O=C=O, and C=OH/O=N, respectively (Figure 2). Iron signature peaks were numerous and , indicating the presence of mixed iron oxide species. However, no Fe2 was identified. The peaks identified around 710.4 and 711.9 relate to Fe3+ species representative of feric compounds such as FeO(OH), Fe3O4, and FeOOH. Peaks at 715.1, 728.5, and 718.7 are representative of Fe2O3, however the peak at 711.9 together with OH groups (peaks at 534.2 and 286.6 eV) point toward the presence of hydrolyzed iron oxides, such as FeOOH and/or Fe(OH)3.

Figure 3. FTIR spectra of iron nanoparticles generated with different plant extracts.

S. paniculatum also showed the existence of phenolic compounds by the presence of O–H stretching bonds between 3200 and 3400 cm⁻¹, C–H stretch between 3000 and 2850 cm⁻¹, and C–O stretching bonds between 1000 and 1100 cm⁻¹. Peaks located above 3000 cm⁻¹ are unsaturated and are willing to take part in hydrogen bonding. S. mole and S. paniculatum (and to a minor extent P. anulatum) possessed well-defined peaks in this region, and this may be responsible for the particles’ tendency to agglomerate.
All nanoparticle species contained peaks located in the regions of 1635–1545, 1430–1350, and 1000 cm⁻¹ (Figure 3), which are representative of the stretching of the carboxylate group (COO⁻), bending of O–H bonds from carboxylate groups, and C–O stretching bonds, respectively. Peaks relating to N–H bending are also found within the region of 1650–1500 cm⁻¹ and may be masked by the presence of the carboxylate signature.²⁹

Nanoparticles from P. undulatum and M. aceratach showed an absence of strong signatures above 3000 cm⁻¹ and possessed carboxylate groups, which have repulsive forces with other carboxylate groups.³⁰ This may explain the formation of monodispersed nanoparticle aggregates within these samples.

Hexavalent Chromium Reduction. Within this study, an equal weight of nanoparticles (5 mg/mL Cr⁶⁺ solution) was added to each treatment vessel as opposed to an equal volume of crude nanoparticle reaction solution. In this way, we were able to assess the efficiency of the nanoparticles on Cr⁶⁺ removal alone without the unreacted iron salt and unbound plant extracts interfering with the degradation kinetics. Mistry et al.²⁹ showed that pomegranate and clove plant extracts alone could remove approximately 25% Cr(VI) from solution over a 2-day period. This study also showed that clove extract possessed a low polyphenol content, however, it was still efficient at reducing Cr(VI). The authors mention that the type of polyphenols present within the plant may be a more important factor to focus on rather than the total polyphenol content of specific extracts.

In this current study, the plant extract of P. undulatum was the only plant extract, which had an impact on hexavalent chromium reduction with 100 μL of extract resulting in approximately 35% removal of Cr(VI) in the same concentration and volumes as the nanoparticle Cr(VI) removal assays (data not shown). However, as capping of the particles only ranged from 3.3 to 5.4% of the total weight of the particles (determined by differences in weight following the removal of organic matter in a blast furnace at 500 °C for 12 h), their influence is likely to have a negligible contribution to the overall Cr(VI) degradation within the nanoparticle treatment microcosms.

Although antioxidant concentration may show trends with increased reducing powers and hence in the formation of more concentrated nanoparticle solutions, when the ability of the resulting nanoparticles is assessed for Cr(VI) removal efficiency, they may not be the best performers.

This current study assessed the capacity of iron nanoparticle species generated from plant extracts with different antioxidant profiles to remove Cr(VI). Plant-generated nanoparticles (100 mg) with residual precursors removed were added to aqueous Cr(VI) solutions (20 mL of 50 mg/L solutions). Results showed that P. undulatum- and M. aceratach-generated nanoparticles had significant high Cr(VI) removal capacities with rates of 96.2 and 84.1%, respectively, in over 9 h (Figure 4). Nanoparticles generated from S. melle, which showed the lowest antioxidant concentration and reducing power capacity was only able to achieve 58.7% Cr(VI) removal capacity within 9 h. As all nanoparticles were made up of mixed oxides, Cr(VI) removal dynamics was expected to be similar. As Cr(VI) removal after the first 15 min was slow and steady, an absorption mechanism is suggested. This is supported by Jiang et al.³¹ who showed that absorption of Cr(VI) to maghemite nanoparticles was spontaneous and highly favorable.

Nanoparticle crystallinity, composition, surface bonds, and concentrations were similar (or the same), but their Cr(VI) removal capacities were different. This may be attributed to the size of the particles. It is widely documented that a decrease in the nanoparticle size greatly increases its surface area and in turn increases the available surface area for interactions to occur.²⁹⁻³⁰ The nanoparticles that showed the highest Cr(VI) removal capacities had the smallest nanoparticle sizes, with most particles falling within the 5–15 nm size range. In addition, these nanoparticles were also quite monodispersed, further maximizing the particle surface area. Conversely, the two worst performing nanoparticle types possessed chainlike structures. Although the individual particles were nanometric in size, the linking of particles reduces their surface area, and as a result, they behave like millimetric-sized particles with reduced Cr(VI) removal capacity. It should also be mentioned that the type of phytochemicals capping the nanoparticles may also slow down the interactions between the pollutant and the particle. To enhance the nanoparticle capacity for removing Cr(VI) from wastewater, functionalization of nanoparticles may aid in both monodispersity and selective binding to Cr(VI). S. melle and S. paniculatum both showed agglomeration tendencies, and therefore, additions to the synthesis solution of species, which are able to counteract particle–particle attractions may be of benefit. Palma et al. in 2015 employed the use of meso,2,3-dimercaptopropanionic acid and oleic acid to generate monodispersed iron nanoparticles via a thermal decomposition route.³¹ In addition to the particle size, particle binding affinity may be enhanced by functionalising nanoparticle-specific ligands. Eliche et al. in 2017 modified iron oxide nanoparticles with cetyltrimethylammonium bromide (CTAB) and showed enhanced Cr(VI) binding selectivity and removal efficiencies.³² This treatment required low pH and the use of CTAB, which has been shown to be toxic to microorganisms.³³ However, if nontoxic ligands can be applied to nanoparticles, this may aid in elevated remediation rates.

**CONCLUSIONS**

Reduction of iron salt precursors with different plant extracts can generate iron nanoparticles with different morphological features. However, the phytochemical fingerprints examined in this study were not comprehensive enough to link the compounds to morphology. All particles were composed of mixed iron oxide species and were amorphous in nature. Zero
valent iron was not identified and is unlikely to be present in nanoparticles because of the lack of representative XRD and XPS peaks along with an absence of magnetism. Nanoparticles possessed C=C–C, C–O–O, O=C=O, and C–OH bonds on their surface, which was confirmed with XPS and FTIR analysis, indicating that they were capped with plant-based photochemicals. Nanoparticles, which possessed strong O–H bonds peaks in the FTIR analysis above 3000 cm⁻¹, also showed agglomerated nanoparticles. The antioxidant concentration of plant extracts was linked with the reducing power capacity but not with optimum nanoparticle morphologies for chromium degradation. Plant extracts with the lowest and highest antioxidant concentrations showed the lowest Cr(VI) removal capacity for all nanoparticles tested. This was attributed to the nanoparticle-chained structures formed, which reduced the nanoparticle surface area and interaction with Cr(VI). *P. undulatum* and *M. azedarach* possessed the highest Cr(VI) removal capacity of 96.2 and 84.1%, respectively. Nanoparticles of these species were between 5 and 15 nm in size and were monodispersed. This research presents a green, cost-effective, and efficient technology for the removal of Cr(VI) from aqueous solutions. Further research into the fractionation of plant extracts is required to determine the specific phytochemicals responsible for instilling specific nanoparticle traits. In addition, modification of nanoparticles using ligands with a strong affinity for specific pollutants is expected to deliver elevated remediation capacities. The immobilization of nanoparticles onto a solid support may be of interest for removing pollutants and nanoparticles from the environment following remediation.

**MATERIALS AND METHODOLOGY**

**Dry Weight Determination.** The water content of the plant leaf was determined by drying 10 g of fresh plant leaf biomass at 40 °C for 5 h or until no further weight change was apparent. Percentage moisture content was determined by the following equation

\[
\text{% moisture content} = \left( \frac{\text{plant biomass following drying}}{\text{initial weight of fresh biomass}} \right) \times 100
\]

**Preparation of Plant Extracts.** Dried plant leaves of interest (10 g) were added to 100 mL of Milli-Q water and were put into a water bath at 80 °C for 1 h and then removed and allowed to cool at room temperature. The solution containing water-soluble phytochemicals was filtered through a 0.2 μm filter and stored at −20 °C until further use.

**Phytochemical Analysis of Extracts.** Trolley Equivalent Antioxidant Capacity (TEAC) Assay. The antioxidant capacity of the extract was determined using a modified method of that described by Stratil et al. and Re et al. *ABTS* (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) stock solution to 4.90 mM potassium persulfate solution (2.45 mM final concentration). This solution was left to stand for 15 h at 25 ± 2 °C in the dark to form the radical cation *ABTS*⁻.

To determine the TEAC, the *ABTS* solution was diluted with phosphate-buffered saline (PBS, pH 7.4) to get an absorbance reading (optical density OD) between 1.0 and 1.5 at 734 nm when 200 μL of the sample was measured on a 96-well plate reader (BMG POLARstarOmega).

A Trolley standard curve was created by making serial dilutions of an initial 1.5 mM Trolley solution in PBS. Ten microliters of the Trolley solutions was added to 190 μL of ABTS⁺ solution in a 96-well plate (COSTAR). Spectrophotometric measurements at 734 nm were taken exactly 6 min after initial mixing, and increased antioxidant concentration was determined by a decrease in the OD values.

The aforementioned procedure was repeated with 10 μL of plant extract. Dilutions of plant extract (diluted in PBS, pH 7.4) were made if values fell outside the measurable range, and OD values were multiplied by the dilution factor. Findings are expressed as Trolley equivalents/g plant biomass [mM Trolley (equi)/g].

PBS (pH 7.4) and Trolley mixed with each corresponding plant extract in volumes used (without ABTS⁺) served as controls and standards.

**Folin–Ciocalteau Assay.** The phenolic content of plant extracts was determined using the Folin–Ciocalteau method, as outlined by Meda et al. and Moen et al. Briefly, the plant extract (50 μL) was added to 450 μL of Milli-Q water and filtered through a Whatman no. 1 filter paper. An aliquot (50 μL) of this solution was then mixed with 250 μL of 0.2 M Folin–Ciocalteau reagent (Sigma-Aldrich Chemie, Steinheim, Germany) for 5 min, and 200 μL of 75 g/L sodium carbonate (Na₂CO₃) (Lab, Paris, France) was added. After incubation at room temperature for 2 h, the absorbance of 150 μL from the reaction mixture was measured at 760 nm against methanol blank in a 96-well plate reader. Phenolic content is expressed as GA equivalents/g plant biomass [mg GA (equi)/g].

The linear region of a standard curve, generated from the reaction of the above solution with GA (0–0.50 mg/mL), was used to determine the phenol content of plant extract samples (GA equivalents).

**Reducing Power Assay.** The Fe³⁺ reducing power (antioxidant capacity assay) of the extract was determined using the methods outlined by Berker et al. with modifications relating to the incubation method and the volumes of reagents. Briefly, 100 μL of Plant extracts were added to 20 μL of FeCl₃·6H₂O (0.2%) were combined to make a total of 180 μL solution.

The plant extract or the standard to be tested was made up to 20 μL with ethanol (96%). The volume of the sample added to ethanol needed to be optimized to fit within measurable parameters, and this differed between samples tested.

Both the aforementioned final solutions were mixed together to make a 200 μL solution, which was incubated at 50 °C within a thermocycler (Bio-Rad, T-100) for 20 min and then held at room temperature until further use. An aliquot (150 μL) of the resulting sample was transferred to a 96-well plate (COSTAR), and the absorbance was measured at 700 nm (A₇₀₀) against a reagent blank within 25 min.

AA solution (0.0–100.0 μg/mL) was used as the standard. Increased absorbance of the reaction mixture indicated increased reducing power. Findings are expressed as LAA equivalents/g plant biomass [mg AA (equi)/g]. All measurements were conducted in triplicate in a 96-well plate reader, BMG Labtech, Germany.

**3,5-Dinitrosalicylic Acid (DNS) Assay.** The reducing sugar content of plant extracts was determined using the DNS method, as outlined by King et al. Briefly, DNS working solution was prepared by combining 10 g of DNS with 403 g of KNa₄C₆H₃O₆·H₂O and then made up to 1 L in 0.4 M NaOH.
(heated at 50 °C till dissolved). The solution was then filtered through a 0.45 μm filter.

Sixty microliters of glucose standard (0.0-3.0 mg/mL) prepared in 0.1 M sodium acetate buffer pH 5.0 or filtered plant extract was added to 120 μL of DNS working solution in PCR tubes. PCR tubes were then placed into a PCR thermocycler (Bio-Rad, T-100), and the incubation cycle was set at 95 °C for 5 min, cooling to 48 °C for 1 min, and holding at 48 °C for 4 min. Fifty microliters of the completed DNS reaction product was then added to 150 μL of Milli-Q H2O in flat-bottom microplates (COSTAR), and the absorbance was measured at 540 nm. Findings are expressed as glucose equivalents/g plant biomass (mg glucose (equiv)/g).

Ferrous Ion-Chelating Activity.

The capacity of plant extracts to chelate Fe2+ ions was determined using the protocol described by Decker and Welchen19 with some minor modifications. Briefly, to a flat-bottom 96-well plate (COSTAR), the following were added: 67.5 μL of Milli-Q water, 50.0 μL of the standard or plant extract, and 2.5 μL of FeCl2·4H2O (2 mM). The reaction was initiated by addition and thorough mixing of 5.0 μL of ferrozine solution (5 mM), and the reaction was allowed to develop in the dark for 10 min. Absorbance values were measured using a 96-well plate reader (BMG POLARstar Omega, BMG Labtech, Germany) at 562 nm. The linear region of a standard curve, generated from the reaction of the above solution with EDTA (0-250 μg/mL), was used to determine the metal chelation capacity of the plant extract samples. Results are expressed as EDTA equivalents/g plant biomass (mg EDTA (equiv)/g).


The plant extract was mixed in a beaker at top speed using a magnetic stirrer, and 0.1 M FeCl2·4H2O solution was slowly introduced using a peristaltic pump with a flow rate of 2 mL/min, ensuring a 2:1 ratio of v/v of plant extract to FeCl2·4H2O, respectively. The color of the solution changed to black indicating the synthesis of nanoparticles. The resulting solution was centrifuged at 14,000g for 30 min within Amicon Ultra-0.5 Centrifugal Filter Devices with 10 kDa cutoff; the flowthrough was discarded and the nanoparticles pelleted on the filter were resuspended in Milli-Q water, frozen to ~80 °C, and lyophilized.

Characterization of Plant-Generated Nanoparticles.

Transmission Electron Microscopy (TEM), TEM characteristics were undertaken for nanoparticles prior to the dye degradation reaction. Plant extract-generated nanoparticle samples were prepared by drop-coating the samples onto a carbon-coated copper grid. The grid was left to dry overnight in a dust-free environment, and the morphological characteristics of iron nanoparticles were analyzed with a JEM1010 TEM operated at 100 kV (Thermoemission cathode).

FTIR Spectroscopy.

FTIR spectroscopy spectra of freeze-dried plant extracts, plant extract-generated iron nanoparticles, and plant-generated nanoparticles with organic material burnt off at 500 °C for 10 h in a muffle furnace were determined by a Fourier transform infrared spectroscopy (PerkinElmer Frontier). Nanoparticles were freeze-dried, washed in Milli-Q water, and centrifuged at 14,000g, and the nanoparticles were retained. Nanoparticles were dried at room temperature before being processed by a spectroscope. An average of 18 scans was collected for each measurement with a resolution of 0.5 cm⁻¹ in the range of 4000-400 cm⁻¹.

XRD. To determine the crystalline phases of plant-generated nanoparticle, XRD analysis was conducted using a Bruker AXS D8 Discover X-ray diffractometer instrument equipped with a Cu Kα radiation source (wavelength 1.5406 nm) operating at 40 kV and 35 mA. All X-ray data were obtained in the 2θ-2θ mode over a 2θ interval of 10 to 60. The identity of the diffraction peaks was assigned through auto-fitting in the instrument software (Bruker Evaluation).

XPS. Nanoparticle surface compositions were analyzed by XPS (Thermo K-Alpha XPS). The instrument was equipped with an Al Kα X-ray radiation source with a photon energy of 1486.6 eV. All measurements were conducted at room temperature under ultrahigh vacuum (10⁻⁷ Pa). The C 1s, O 1s, and Fe 2p core level spectra were recorded with an overall resolution of 0.1 eV. The spectra were handled with Avantage 4.88 Surface Chemical Analysis Software, Thermo Scientific. Hexavalent Chromium Removal. Aqueous solutions of Cr⁶⁺ (20 mL of 50 mg/L) were buffered to pH 3.5 in acetate buffer. The diphenylcarbazide (DPC) method was used to determine Cr⁶⁺ species in solution20 with some minor modifications. To each Cr⁶⁺ solution, 130 μL of DPC solution (200 mg of DPC/10 mL of acetone) was added. After 10 min, time 0 OD₅₂₀₅₅₄₉₉ measurements were taken using a BMG POLARstar Omega using COSTAR flat-bottom 96-well plates. Following this, 100 mg of plant-generated nanoparticles was added to vessels and periodic OD₅₂₀₅₅₄₉₉ measurements were taken for over 9 h. Hexavalent chromium removal efficiency (%) is calculated as follows:

Hexavalent chromium removal efficiency (%) = 100 − [(C / C₀) × 100]

where C₀ is the OD₅₂₀₅₅₄₉₉ of the hexavalent chromium/DPC complex at time 0 and C is the OD₅₂₀₅₅₄₉₉ of hexavalent chromium/DPC complex at time t.

Statistical Analysis. The concentrations of all bioactive components were compared within plant species and between plant species. Univariate ANOVA pairwise comparison coupled with Tukey and Duncan’s multiple comparison post hoc tests were used to identify correlations at a 0.05 level.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomeg- ga.8b00410.

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Notes

The authors declare no competing financial interest.

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REFERENCES


Supporting Information

Phytofabrication of Iron Nanoparticles for Hexavalent Chromium Remediation

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Figure S1: TEM of nanoparticles generated from differing plant extracts (A) *P. undulatum* NP (B) *M. azedarach* NP (C) *S. molle* NP (D) *S. paniculatum* NP.

Table S1: the surface atomic composition (%) of plant generated iron nanoparticles

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Carbon (%)</th>
<th>Oxygen (%)</th>
<th>Iron (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. undulatum</em></td>
<td>59.9</td>
<td>35.7</td>
<td>4.4</td>
</tr>
<tr>
<td><em>M. azedarach</em></td>
<td>58.7</td>
<td>37.7</td>
<td>4.4</td>
</tr>
<tr>
<td><em>S. molle</em></td>
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<td>36.9</td>
<td>4.8</td>
</tr>
<tr>
<td><em>S. paniculatum</em></td>
<td>58.2</td>
<td>36.9</td>
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</tbody>
</table>
Chapter 4

Amino-Silanol Nanoparticles Modified with Waste Aqueous Helianthus annuus Husk Extract for Increased Porosity and Rapid Adsorption of Concentrated Dye Mixtures

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Statement of authorship

Adam Truskewycz (candidate) planned the experimental design, performed experiments, carried out the vast majority of research, analysed and interpreted data. Truskewycz also reviewed the literature and executed the writing in Chapter 4.

Mohamed Taha contributed to dye adsorption experiments in Chapter 4.

Deshetti Jampaiah contributed to BET surface area analysis in Chapter 4.

Ivan Cole contributed to guidance of research and manuscript evaluation in Chapter 4.

Ravi Shukla contributed to co-supervision of research and manuscript evaluation in Chapter 4.

Andrew S Ball contributed to the planning of experimental design, supervision of research, manuscript preparation and evaluation of Chapter 4.

All the co-authors give full consent to Adam Truskewycz to present these above papers for examination towards the Degree of Doctor of Philosophy.

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Abstract

The capacity of an adsorbent to bind and remove dye from solution greatly depends on the type of functionalization present on the nanoparticles surface, and its interaction with the dye molecules. Within this study, novel amino silanol nanoparticles were hydrothermally synthesized resulting in the formation of rapid and highly efficient concentrated dye adsorbents. The addition of sunflower husk (\textit{Helianthus annuus}) extract further increased their dye removal capacities. Transmission electron microscopy (TEM) showed sub 1 nm pores on silanol nanoparticles amended with \textit{H. annuus} extract and Brunauer–Emmett–Teller (BET) surface area analysis showed a three-fold increase in surface area coupled with a 10-fold increase in pore volume compared to the positive control. The amorphous silanol nanoparticles showed a monolayer based mechanism of concentrated mixed dye adsorption. This treatment approach shows the highest reported concentrated mixed dye removal efficiency when compared to other silicon derived methodologies with removal capacities between 416.67 – 714.29 mg dye adsorption per g of adsorbent. Zeta potential measurements showed that dye removal is predominantly due to electrostatic attraction between the positively charged silanol nanoparticles (+8.8-9.2 mV) and the negatively charged dye molecules (-48.7 mV). The suitability of environmental application using these treatments is supported by bacterial viability assay showing >83% growth efficiency compared to the control.

Key Words: Porous; APTES; Nanoparticles; Adsorption; Sunflower; Silicon; Porosity; Phytochemical; Green Synthesis; Remediation

1. Introduction

Dyes are a valuable commodity, with global production of dyestuffs currently exceeding 7 X 10\textsuperscript{5} t annually \textsuperscript{1,2}. This figure is only set to rise with global population growth and increased consumer demand for textile goods. However, the environmental persistence and inherent toxicity of dyestuffs is of increasing concern. For example, hydrolysed remazol brilliant blue, a commonly used dye has been shown to possess high aqueous solubility, high toxicity along with an electrophilic vinyl-sulfone group capable of causing mutagenic effects. This dye has a half-life of 46 years at 25°C \textsuperscript{3-5}. 

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Among all dyes used within the textile industry, 10-15% are found in post-dye wastewater, with the potential to cause serious environmental and socio-economic damage. China, the world’s leading exporter of dyestuff passed legislation in 2003 requiring cleaner production and less environmentally damaging approaches to the manufacture of dyes (Law of the People’s Republic of China on Promotion of Cleaner Production). Similar legislation exists around the world prompting the need for the development of innovative methods for the minimization of dye-pollution and subsequent remediation strategies.

Currently, adsorption is the most widely used treatment option for dye remediation from wastewater as it is economically practical, fast, effective and removes dye molecules without breaking their structure into smaller toxic daughter products as commonly experienced with degradation routes. Functionalization of adsorbents is required for elevated dye removal capacity and identifying environmentally friendly, low cost options is seen as environmentally and economically necessary. A range of different technologies are routinely used (Table 1).

<table>
<thead>
<tr>
<th>Treatment Technology</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption</td>
<td>Cost effective (activated carbon is an exception), no generation of toxic break down products from degradation, fast, environmentally friendly, non-technical</td>
<td>Chemical modification on adsorbent is often required, pollution is not degraded</td>
<td>12</td>
</tr>
<tr>
<td>Ozonation</td>
<td>No residual chemicals left in the wastewater from treatment</td>
<td>High costs and deficient mass transfer rate of ozone leading to ineffective ozone utilization</td>
<td>13</td>
</tr>
<tr>
<td>Bioremediation</td>
<td>Environmentally friendly, low cost</td>
<td>Slow practice, bioremediation treatment type differs from site to site, nutrient addition often required which may lead to eutrophication</td>
<td>14</td>
</tr>
<tr>
<td>Membrane Filtration</td>
<td>Environmentally friendly, capacity to remove all dye types from solution</td>
<td>High pressures, high operational costs, sludge production and limitations on the volume of wastewater that can be treated due to membrane fouling</td>
<td>15,16</td>
</tr>
<tr>
<td>Sonolysis</td>
<td>No additional sludge production</td>
<td>High cost, requires a high concentration of dissolved oxygen</td>
<td>16,17</td>
</tr>
<tr>
<td>Advanced Oxidation</td>
<td>Complete catabolism of specific organic contaminants, minimal sludge production</td>
<td>High cost, often struggles with catabolising many different types of organic pollutants</td>
<td>16,18</td>
</tr>
</tbody>
</table>

Silicon dioxide (SiO₂) nanoparticles have gained increasing interest for a number of varying applications including catalysis, drug delivery, separation and bio-adsorption but as they possess a negative net charge due to Si-OH groups, surface functionalization is often required to enhance their ability to bind a range of differently charged entities e.g. anionic dyes. Alkysilane functionalization of oxide surfaces has recently been employed for binding nanoparticles to oxide surfaces and the attachment of organic ligands. As alkysilanes can self-assemble onto oxides, functional groups from these complexes are free to interact with biomolecules for bio-sensing, drug delivery and release, DNA separation and oil/water separation applications. 3-Aminopropyltriethoxysilane (APTES) is one such alkysilane which has shown both biocompatibility and readily undergoes hydrolysis in aqueous media indicating that its potential for environmental harm is minimal whilst the functionality of its hydrolysed form in regards to its binding to organic ligands seems promising.

The use of surfactants (i.e. oleic acid, polystyrene-b-poly(acrylic acid) (PS-b-PAA) and CTAB) to induce porosity within silicon nanoparticles has been demonstrated in a number of studies. Helianthus annuus husk is an unutilised material remaining following seed extraction. It is therefore a cheap resource which may contain phytochemicals capable of providing nanoparticles with optimum morphologies. It has been shown to contain oleic acid amongst many other phytochemicals (i.e. reducing sugars), which have been shown to interact with silicon-based materials to induce porosity.
Herein we design APTES derived porous amino silanol nanoparticulate wafers stabilised with *H. annuus* husk phytochemicals (P-Silanol-NP). The resulting silanol nanoparticles were employed as adsorbents to remove concentrated mixtures of azo, anthraquinone, acid and basic dyes from solution. Treatment of a concentrated mixed dye solution containing various different dye types (i.e. azo, anthraquinone, reactive and acidic dyes) was conducted as real-world dye contamination events are rarely composed of single pure dyes.

2. Methods

2.1. Preparation of *H. annuus* Extract

Dried *H. annuus* husks (10 g) were added to 60 ml of ultrapure water (18 MΩ) and placed in a water bath at 90°C for 2 h and then allowed to cool at room temperature. The remaining solution was filtered through a 0.2 µm filter and stored at -20°C.

2.2. *Helianthus annuus* Husk Phytochemical Analysis

2.2.1. Trolox® Equivalent Antioxidant Capacity (TEAC) Assay

Antioxidant capacity of the extract was determined using methods outlined by Stratil, et al. 34 and Re, et al. 35. ABTS⁺ cation stock solution was created by reacting 1:1 (v/v) of 7 mM ABTS (2,2’ azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) stock solution to 4.90 mM potassium persulfate solution. This solution was left to stand for 15 h at 25°C in darkness to form the radical cation ABTS++. To determine the Trolox® equivalent antioxidant capacity (TEAC), the ABTS⁺ solution was diluted with phosphate buffer solution (PBS, pH 7.4) to get an absorbance reading (OD) of between 1.0 - 1.5 at 734 nm (BMG POLARstarOmega).

A Trolox® standard curve was created by making serial dilutions of an initial 1.5 mM Trolox® solution in PBS. Ten microliters of the Trolox® solutions were added to 190 µL of ABTS •+ solution in a 96 well plate (COSTAR). Spectrophotometric measurements at 734 nm were taken exactly 6 minutes after initial mixing and increased antioxidant concentration was determined by a decrease in OD values.

The aforementioned procedure was repeated with 10 µL of *H. annuus* husk extract and findings are expressed as Trolox® equivalents/ ml *H. annuus* husk extract (mM Trolox® (eq)/ml)

2.2.2. Folin–Ciocalteu Assay

Total phenolic content of plant extracts was determined by the The Folin–Ciocalteu assay as outlined by Meda, et al. 36 and Moein, et al. 37. Briefly, plant extract (50 µL) was added to 250 µL of 0.2 M Folin–Ciocalteu reagent (Sigma–Aldrich Chemie, Steinheim, Germany) for 5 min and 200 µL of 75 g/L sodium carbonate (Na₂CO₃) (Labosi, Paris, France) was added. After incubation at room temperature for 2 h, the absorbance of 100 µL from the reaction mixture was measured at 760 nm against a methanol blank in a 96 well plate reader. Phenolic content is expressed as gallic acid equivalents/ ml *H. annuus* husk extract (mg GA (eq)/mL).
2.2.3. Bradford Assay

Total protein concentration of our husk extract was determined via a Bradford assay 38.

Bovine serum albumin (BSA) protein standards (0.0-100.0 mg/ml) and *H. annuus* husk extract (5 µl) were mixed with 250 µL of a Bradford Reagent (Sigma Aldrich) and put on a shaker for 30 seconds. Samples were left to incubate for 30 minutes before 100 µl was transferred to a 96 well plate (COSTAR) and absorbance was measured at 595 nm (BMG POLARstarOmega). Protein concentration was plotted against absorbance (OD) to create a linear plot for the standard curve and was expressed as mg BSA (eq)/ mL *H. annuus* husk extract.

2.2.4. 3,5-Dinitrosalicylic Acid (DNS) Assay

Total reducing sugar content of plant extracts was determined using the 3,5-dinitrosalicylic acid (DNS) method as outlined by King, et al. 39. Briefly, DNS working solution was prepared by combining 10 g DNS with 403 g KNaC₄H₄O₆•4H₂O made up to 1 L in 0.4 M NaOH (heated at 50°C till dissolved).

Sixty microliters of glucose standard (0.0-3.0 mg/mL) prepared in 0.1 M sodium acetate buffer pH 5.0 or *H. annuus* husk extract was added to 120 µl DNS working solution in PCR tubes. PCR tubes were then placed into a PCR thermocycler (Bio-Rad, T-100) and an incubation cycle was set to at 95°C for 5 min, cooling to 48°C for 1 min, and holding at 20°C.

Fifty microliters of the completed DNS reaction was then added to 150 µL of ultrapure water (18 MΩ) in flat-bottom microplates (COSTAR) and absorbance was measured at 540 nm. Findings are expressed as glucose equivalents/ ml *H. annuus* husk extract (mg glucose (eq)/ ml)

2.2.5. Total Lipid Extraction and Purification

*Helianthus annuus* seed husk (100g) was blended to a fine powder and made up to 700 mL in ultrapure water (18 MΩ). This solution was heated at 90°C for 2 hours, filtered through a 0.45µm filter and freeze dried to a powder. Fat was extracted using chloroform/methanol or petroleum ether/iso-propyl alcohol via Mojonnier Extraction. The extract was evaporated under nitrogen gas and fat was esterfied using a methanolic sodium methoxide solution and treatment with sulphuric acid in methanol. The solution was neutralised and re-extracted using n-hexane. The hexane layer is removed, dried using anhydrous sodium sulphate and made to volume, with hexane. The relative proportion of each fatty acid methyl ester in the prepared sample is determined using gas chromatography with flame ionisation detection.

2.3. Synthesis of Silanol Wafers

Silanol wafers were prepared via hydrothermal method using APTES. Briefly, 1 mL of APTES was added to 14 mL of ultrapure water (18 MΩ) or *H. annuus* husk extract prior to being subjected to hydrothermal treatment at 200°C for 2 h using a ramp up speed of 6.6°C per minute. The resulting water soluble silanol wafers were filtered through a 0.2 µm syringe filter and subjected to dialysis for 48 h (MWCO 2000 Da). The dialysed solution was then snap frozen using liquid nitrogen and lyophilised until desiccated. Un-functionalised silicon dioxide nanopowder (10-20 nm particle size) was purchased Sigma Aldrich.
2.4. Characterisation

2.4.1. High Resolution-Transmission Electron Microscopy (HR-TEM)

Silanol wafers and SiO₂ nanoparticles were prepared by drop coating the samples on a carbon grid. The grid was allowed to dry, and the morphological characteristics of the particles were visualised using a JEOL2010 TEM operated at an accelerating voltage of 200 kV.

2.4.2. Scanning Electron Microscope Energy Dispersive X-ray Spectroscopy (SEM-EDS)

Elemental surface analysis of silanol wafers and SiO₂ nanoparticles was determined by subjecting them to EDS analysis using Oxford X-MaxN 20 EDXS Detector (2014) and AztecEnergy analysis software in conjunction with a Phillips XL30 SEM. Nanoparticles were freeze dried prior to use and were imaged as prepared.

2.4.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of dried dye, silanol wafers and SiO₂ nanoparticles before and after remediation were determined by FTIR (Perkin Elmer Frontier). An average of 32 scans was collected for each measurement with a resolution of 4 cm⁻¹ in the range of 4000–400 cm⁻¹ using a percentage transmission spectrum.

2.4.4. X-ray Diffraction (XRD)

The crystallinity of silanol wafers and SiO₂ nanoparticles was determined by XRD using a Bruker AXS D8 Discover diffraction instrument equipped with a Cu-Kalpha radiation source (wavelength 0.1542 nm) operating at 40 kV and 35 mA. All X-ray data was obtained in the θ–2θ locked-couple mode over a 2θ interval of 10–60.

2.4.5. Zeta Potential

Particles charge dynamics were measured using a Malvern 2000 Zetasizer following appropriate dilution and sonication within a DTS 1060C, Malvern cuvette.

2.4.6. Brunauer–Emmett–Teller (BET) surface area measurements

Nitrogen adsorption-desorption isotherms were performed on a Micromeritics (ASAP 2400) analyser at -196 °C using liquid nitrogen (N₂). BET analysis was used to determine the surface area of the samples by N₂ multilayer adsorption measured as a function of relative pressure using a fully automated analyser. The pore characteristics of the synthesized samples were measured using the Barrett-Joyner-Halenda (BJH) formulae from N₂ isotherms. Prior to BET analysis, samples were degassed at 160 °C in a vacuum overnight.

2.5. Dye removal

Methyl Blue (certified), acid red 88, remazol brilliant blue R and acid green 25 dyes were mixed together in an equal concentration and a 1:1:1:1 (v/v) ratio (adjusted as per the manufacturer’s percentage dye concentration) to make a concentrated dye mixture of 0.51 mM.
Silanol wafer and SiO$_2$ nanoparticles were resuspended in phosphate buffered saline (PBS) (pH 7) and added to the dye at 5, 2.5, 1.25 and 0.6325 g/L. After 5 min and 30 min, $\lambda_{\text{max}}$ (OD$_{610}$) measurements were taken using a BMG CLARIOStar® spectrophotometer using Costar flat bottom 96 well plates.

Mixed dye removal efficiency (%) was calculated as follows:

$$\text{Mixed dye removal efficiency (\%)} = 100 - \left( \frac{C_t}{C_0} \right) \times 100$$

Where $C_0$ is the OD$_{610}$ of the mixed dye solution at time 0 and $C_t$ is the OD$_{610}$ of mixed dye solution at time $t$.

The amount of dye adsorbed at equilibrium $q_e$ (mg/g) was determined from the following equation.

$$q_e = \frac{(C_0 - C_e)V}{W}$$

Where $q_e$ is the adsorbents adsorption capacity (mg/g) at equilibrium, $C_0$ and $C_e$ are the initial and equilibrium dye concentrations (mg/g), $V$ is the volume of dye solution (L) and $W$ is mass of adsorbent at time (g).

2.6. Relationship of Carbon Concentration and Dye Removal

Relative surface carbon concentrations over all samples (obtained from SEM-EDS analysis) were plotted against dye removal capacity in an un-categorised manner. Linear line of best fit was applied along with corresponding $R^2$ value.

2.7. Bacterial Toxicity

Marine broth (concentrated to 6 X the manufacturer’s suggested concentration) was added to the supernatant from remediation treatments (1:6 v/v ratio respectively) and was sterilized via autoclaving for 20 min at 121°C. The resulting solution was inoculated with *Vibrio fisherii* (Modern Water Microtox Acute Reagent) resuspended in ultrapure water (18 MΩ) (150 µL; OD$_{600}$ of 0.15) and incubated at 25°C for 48 h. Periodical OD$_{600}$ measurements were measured using a BMG CLARIOStar® spectrophotometer with Costar flat bottom 96 well plates.

3. Results:

3.1. HR-TEM

APTES was utilised as a silicon source for the generation of silanol nanoparticulate wafers (Figure 1 and Supplementary Figure 3) and subsequent functionalization of these wafers via hydrothermal treatment. APTES was added to either water or aqueous *H. annuus* husk plant extract and subjected to hydrothermal treatment for 2 h at 200°C. Synthesis of silanol nanoparticles within a reaction medium of *H. annuus* husk phytochemicals induced an increased porosity to the nanoparticles when compared to the treatment without *H. annuus* extract. This can be seen by the < 0.5 nm sized speckles identifiable on the HR-TEM image (Figure 1a & Supplementary Figure 4).

The presence of these speckles on silicon materials instils a higher surface area and increased pore volume (Supplementary Table 2) to the particles when compared to non-porous materials. Silanol wafers generated in water alone showed negligible porosity (Figure 1b) indicating that the plant extract was responsible for
increasing porosity (Figure 4). APTES has been shown to be thermostable and functionalised onto TiO₂ nanoparticles over 200°C, with loss of integrity only between 230-400°C due to combustion of the organic part of the silane 49. Therefore, carbon structures from APTES or its hydrolysed form are unlikely to be altered during the heat ranges experienced within this study and functionality remains intact 49-51.

3.2. XRD

P-Silanol-NP wafers and SiO₂-NPs exhibited an amorphous nature with the Silanol-NP sample showing initial stages of crystalline lattice formation; however, the bulk sample remained mostly amorphous in nature (Figure 1b & supplementary Figure 5). A comparison of the XRD fingerprint between samples shows only a slight increase in crystallinity with Silanol-NP wafers over the P-Silanol-NP treatment (Figure 1d & Supplementary Figure 5). Conversion of amorphous silicon to crystalline silicon at 200°C is dependent on exposure time, with exposures less than 3 h producing minimal crystallinity 52,53. The lack of crystalline phases in the P-Silanol-NP sample is likely due to the complex multiple morphologies of C-C, C-O, Si-O and Si-Si bonds derived from the plant material. In addition, the carbon capping of silicon can reduce the degree of silicon crystallisation, especially at lower temperatures (< 230°C) 49,54,55.

Figure 1: HR-TEM of (a) P-Silanol-NP (b) Silanol-NP (c) SiO₂-NP and (d) XRD of P-Silanol-NP
3.3. EDS

SEM-EDS spectra of Silanol-NP wafers revealed a composition of 27.6% carbon, 38.0% oxygen, 24% silicon and 9.5% nitrogen (Table 2). The relatively high concentration of carbon and nitrogen confirms the functionalization of APTES derived ligands onto the nanoparticles surface; in contrast SiO$_2$-NP's possessed a high percentage of silicon and oxygen (44.1 and 51.2% respectively) and a low carbon content (< 4.5%). Energy-dispersive X-ray spectroscopy is often used as a surface analysis tool as its sample penetration depth is only 1-2 µm. However, as the nanoparticles within this study are smaller than this size range, an accurate measure of sample composition can be determined with this technique. It is predicted that the carbon derived from phytochemicals is adsorbed to the surface of the particles and burrow into them. Removal of these compounds following hydrothermal treatment may be partly responsible for generating pores. In addition, hydrothermal treatment of the sugar rich *H. annuus* husk extract is likely to form quantum carbon dots (QCD) which, if imbedded into the silanol wafer, would increase their surface area $^{56-58}$. Evidence for this can be seen in Supplementary Figure 8 where the P-Silanol-NP show a higher fluorescence intensity than Silanol-NPs when excited at 360nm. The increased carbon concentration of the P-Silanol-NP (57.8%) is likely derived from nanoparticle-capping phytochemicals from the *H. annuus* husk extract and the formation of QCD's; The presence of nitrogen for P-Silanol-NP is confirmed with EDS showing a more prominent peak than that of Silanol-NP (Figure 2a) however, its representation in percentage composition (Table 2) is not present due to the high percentage of carbon in the sample overshadowing its representation.

Table 2: SEM-EDS surface elemental composition (%) between silanol wafer nanoparticles and SiO$_2$ nanoparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbon</th>
<th>Oxygen</th>
<th>Silicon</th>
<th>Nitrogen</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Silanol-NP</td>
<td>57.8</td>
<td>24.7</td>
<td>15.2</td>
<td>ND</td>
<td>2.3</td>
</tr>
<tr>
<td>Silanol-NP</td>
<td>27.6</td>
<td>38.0</td>
<td>24.1</td>
<td>9.5</td>
<td>0.8</td>
</tr>
<tr>
<td>SiO$_2$-NP</td>
<td>4.2</td>
<td>51.2</td>
<td>44.1</td>
<td>ND</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* ND (Non detectable)

Figure 2: SEM-EDS surface elemental composition (%) spectra between 0 - 2.4 KeV; (a) P-Silanol-NP (b) Silanol-NP (c) SiO$_2$-NP

3.4. FTIR

Silicon dioxide has been shown to possess three distinct FTIR frequency regions, 1000-1100 cm$^{-1}$ corresponding to Si-O-Si vibrations, 700-800 cm$^{-1}$ corresponding to O-Si-O vibrations and 440-460 cm$^{-1}$ corresponding to Si-O entities $^{22,59,60}$. Taking into account the sample composition, the greater number of oxygen atoms bound to silicon
Atoms results in a shift in the peaks to a higher wavenumber. In this study, P-Silanol-NP showed peaks within this region at 1033, 748 and 442 cm\(^{-1}\) whilst Silanol-NP had peaks located at 1043, 776 and 442 cm\(^{-1}\). SiO\(_2\)-NP without functionalization possessed peaks at 1076, 796 and 455 cm\(^{-1}\) (Figure 3b). This indicates that all samples contained silicon dioxide moieties and the lower surface functionalization of Silanol-NP wafers with carbon entities results in a greater degree of silicon-oxygen bonds within the sample. This assumption is supported by the SEM-EDS data (Table 2 & Figure 2).

Although the source of silicon and nitrogen was derived from APTES, this precursor rapid hydrolyses in water. The absence of bands near 2975, 1104 and 1089 cm\(^{-1}\) indicate that the Si-OC\(_2\)H\(_5\) group from APTES is absent and that it has been hydrolysed into silanetriols ((OH)\(_3\)Si(CH\(_2\))\(_3\)NH\(_2\)) \(^{61,62}\).

Nitrogen groups responsible for providing a positive charge within the silicon-oxygen rich matrices of both silanol treatments are located at 1558 and 1634 cm\(^{-1}\) confirming the presence of asymmetric -NH\(^{3+}\) deformations and -NH\(_2\) bending groups respectively \(^{10,22}\). These groups are responsible for adding a positive charge to the particles. This is supported by differences in zeta potentials between SiO\(_2\) and silanol particles (Table 5).

Bonds showing the attachment of carbon to silicon were visualised at 691 cm\(^{-1}\) confirming the presence of Si-CH\(_2\) stretch/ Si-O-C moieties. Additional carbon bonds were visualised within both silanol samples. P-Silanol-NP sample –CH\(_2\) stretching modes were identified at 3046, 2933 and 2880 cm\(^{-1}\) frequencies (Figure 3a). The presence of –CH stretching modes were also identified on Silanol-NP wafers at 2973 2989, and 2903 cm\(^{-1}\) representing -CH\(_3\) and –CH attachments respectively \(^{63-67}\). The different types of silanol and SiO\(_2\) bonds found within the nanoparticles are presented in Supplementary Figure 6. The lack of ordered structure of the bonds is representative of amorphous matrices.

Specific carbonaceous bonds derived from the *H. annuus* husk extract was not easily deduced from FTIR peaks; however, the sample was light brown in colour compared to the bright white powder of the silanol sample. This indicates that capping of the particles with plant extract phytochemicals has occurred. Several very broad peaks between 3600-2500 cm\(^{-1}\), 1250-875 cm\(^{-1}\) and between 500-400 cm\(^{-1}\) are likely to be masking any organic FTIR signature peaks. An increase in the carbon concentration found in the P-Silanol-NP though SEM-EDS analysis supports this finding.

Following dye adsorption studies, the precipitated adsorbent with bound dye was subjected to FTIR analysis (Figure 3c). P-Silanol-NP and Silanol-NP show similar FTIR profiles to the dye itself indicating that it had adsorbed onto the surface without modification. SiO\(_2\)-NP had a dissimilar profile compared to the other treatments, possessing dominant peaks at 1076, 796 and 455 cm\(^{-1}\) indicating that the adsorbed dye was not in high enough concentrations to dwarf the SiO\(_2\) signature peaks (Figure 3c).
Figure 3: FTIR profiles for P-Silanol-NP (green solid), Silanol-NP (blue dash) SiO₂-NPs (red dotted) and Dye (black solid) between wavenumbers (a) 4000 - 2000 cm⁻¹ (b) 2000- 400 cm⁻¹ (c) comparison of the surface bonding of samples prior to and following dye adsorption.

3.5. BET Surface Area Analysis

BET analysis was carried out in order to determine the influence of the H. annuus extract on silanol porosity (Figure 4). The nitrogen adsorption capacity of P-Silanol-NP show a typical type IV isotherm according to the IUPAC classification indicating a mesoporous nature. A reduced adsorption profile with differing desorption characteristics (as seen by the absence of the hysteresis loop) was observed within the Silanol-NP. This is representative of a type II isotherm indicating a non-porous or macroporous nature.

A significant increase in surface area, indicated by an increased nitrogen adsorption capacity was observed with the P-Silanol-NP (15.26 m² g⁻¹) compared to the nitrogen adsorption capacity of the Silanol-NP (4.88 m² g⁻¹) (Supplementary Table 2). This is due to an increase in the number of pores and subsequent pore volume (0.0753 cm³ g⁻¹ and 0.0062 cm³ g⁻¹ respectively) of the bulk P-Silanol-NP compared to the Silanol-NP. The measurement of pore diameters below 2 nm is inaccurate using nitrogen adsorption BET analysis; this may be why pore sizes of below 0.3 nm (as seen in the HR-TEM images – Figure 1a & Supplementary Figure 4) may not have been identified in the Barrett–Joyner–Halenda (BJH) pore-size distribution curve (Supplementary Figure 7).
3.6. Mechanism of Increased Porosity

Table 3: Phytochemical analysis of *H. annuus* husk extract

<table>
<thead>
<tr>
<th>Phytochemical Test</th>
<th>Plant Extract</th>
<th>Antioxidants (mM Trolox® (eq)/ml)</th>
<th>Phenolics (mg GA (eq)/ml)</th>
<th>Reducing Sugars (mg Glucose (eq)/ml)</th>
<th>Proteins mg (BSA (eq)/ml)</th>
<th>Fat Content (g/100ml extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Helianthus annuus</em></td>
<td>0.75 (+/-)</td>
<td>0.11 (+/-)</td>
<td>1.49 (+/-)</td>
<td>0.23 (+/-)</td>
<td>N.D</td>
<td>&lt;0.200</td>
</tr>
<tr>
<td><strong>Helianthus annuus</strong></td>
<td>0.044</td>
<td>0.008</td>
<td>0.008</td>
<td>0.010</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Templating of silicon nanoparticles with surfactants, fats and oils is a well-established approach for inducing mesoporosity within silicon nanoparticles. Ravber, et al. 74 have shown that at elevated temperatures, fats and oils can be extracted from *H. annuus* seeds in an aqueous medium. This was not shown during our aqueous extraction of *H. annuus* seed husk at 90°C for 2 hours and was confirmed by our freeze-dried extract possessing <0.2g/100 mL of fat content by means of solvent extraction (Table 3 & Supplementary Table 3). Considering this, a surfactant/fat templating mechanism for inducing porosity of particles was not possible.

Antioxidants and phenolic compounds have been used to cap silica nanoparticles but have not been implicated with increasing their porosity. Proteins however, have been utilised for increasing porosity in silica nanoparticles via a templating approach. Both Antioxidant and protein concentrations were low (Table 3) within the *H. annuus* husk extract (0.75 mM Trolox® (eq)/ml and 0.23 mg (BSA (eq)/ml respectively) and are therefore unlikely to have significant interactions with the silanols.

*Helianthus annuus* husk extract contains approximately 4% protein, 5% lipid, 85% carbohydrate and 6% water. Reducing sugars are a major component of *H. annuus* husk with an approximate total biomass concentration of 26%. Reducing sugar concentrations of our extract were determined via the DNS assay and found to be at 1.49 mg/ml glucose equivalents (Table 3). When considering the total volume of *H. annuus* husk extract and silicon
precursor (APTES) in the reaction vessel, the concentration of reducing sugars was 2% (mg/L) of the amino-silanol concentration present. This is supported by FTIR analysis of the freeze dried H. annuus extract which shows the presence of bonds at 1143 and 970 cm\(^{-1}\) which are representative of stretching vibrations of the C-O-C within the glycosidic bridge (β-glycosidic bonds) and an α-glycosidic bond at 837 cm\(^{-1}\) which are all characteristic of sugars\(^{80,81}\) (supplementary Figure 1).

Wei et al, 1998 showed that the use of non-surfactant organic molecules composed of the reducing sugars D-glucose and D-maltose were able to act as templates for generating mesoporous silica with large surface areas and pore volumes as well as narrow pore size distributions\(^31\). The morphology of these silica particles and their disordered mesopores were comparable to findings from this study (Figure 1 and supplementary Figure 4).

The mechanism underlying the increased silanol porosity can be explained by the interaction and binding of the carbohydrate derived phytochemicals from the H. annuus husk extract (i.e. reducing sugars) which create cavities in their surface. Following hydrothermal treatment, interactions between these compounds and the silanol nanoparticles become weak and dislodge from the silanol. However, the cavities they have created in the surface remain, increasing porosity\(^{40,70-73}\). This phenomenon has been demonstrated with surfactants i.e. (polystyrene-b-poly(acrylic acid) (PS-b-PAA) and CTAB as co-templates\(^30\) and organic non-fatty compounds i.e. reducing sugars\(^{31,32}\). The formation of QCD’s is likely to further increase the surface area of these silanol nanoparticles.

### 3.7. Concentrated Dye Removal

Adsorption is currently the most utilised dye remediation strategy as it is a rapid and environmentally friendly approach which does not degrade the parent molecule which could result in the release of toxic intermediates\(^9-11\). Activated carbon is a commercialised dye adsorbent with removal rates of between 14.7 - 603.3 mg/ g, dependant on the biological source of the carbon and the dye type removed\(^82-84\). As un-functionalised silicon nanoparticles possess a negative charge, functionalization is required for the binding of anionic dyes. It is not only the electrostatic nature of nanoparticles which determines its ability to serve as functional adsorbents; if ligands are weakly bound to the nanoparticles surface they may dislodge and if the functional groups present are not capable of strong binding to organic molecules, significant adsorption will not occur.

To determine if the functionalised nanoparticles were able to adsorb dye via a monolayer or stacking phenomenon, Langmuir and Freundlich isotherms were applied to our data.

Langmuir isotherms were generated by plotting \(C_e \div q_e \text{ vs } C_e\) where \(q_{\text{max}} = 1 \div \text{slope}\) and \(b = \text{slope} \div \text{intercept}\). The Langmuir equation can be expressed linearly as:

\[
\frac{C_e}{q_e} = \frac{1}{b \cdot q_{\text{max}}} + \frac{C_e}{q_{\text{max}}}
\]

Where \(C_e\) is the equilibrium dye concentration, \(q_e\) is the amount of dye adsorbed at equilibrium and \(q_{\text{max}}\) and \(b\) are the Langmuir constants.

The feasibility of absorption was determined by the separation factor (\(R_L\)) which is dimensionless and can be expressed as:

...
Where $R_L$ is dimensionless, $C_0$ is the initial dye concentration and $b$ is one of the Langmuir constants.

Freundlich adsorption capacities were determined by plotting $\log q_e$ vs $\log C_e$ and applying this data to the following equation:

$$q_e = K_F \cdot C_e^{1/n}$$

Where $q_e$ is the amount of dye adsorbed at equilibrium, $C_e$ is the equilibrium dye concentration, $1/n$ is the slope of the line and $K_F$ is the Freundlich constant.

The results from this study have shown a calculated dye removal capacity of 731.04 and 454.96 mg/g for P-Silanol-NP and Silanol-NP respectively, based on the average dye removal capacity of the unsaturated treatments (area of the graph which had not plateaued) at 1.25 g/L dosage (Table 4 & Figure 5). This is in good agreement with their corresponding Langmuir isotherm adsorption capacity extrapolations of 714.29 and 416.67 mg/g respectively. The $R^2$ values for the Langmuir isotherms for both treatments were between 0.96-0.99 respectively which supersede that of the Freundlich model with $R^2$ values between 0.73-0.77 respectively (Table 4). It is therefore anticipated that the adsorption process proceeds via a monolayer adsorption route where dye molecules bind to free binding sites on the adsorbent and do not stack on top of each other in multiple layers 85-87.

The feasibility of adsorption was determined by the separation factor ($R_L$) where $R_L > 1$ indicates desorption where $R_L = 0$ shows a very strong binding affinity, $R_L > 0$ but $< 1$ indicates a favourable adsorption and $R_L = 1$ relates to a linear adsorption 10,88. P-Silanol-NP and Silanol-NP possessed $R_L$ parameters of $1.4 \times 10^{-6}$ and $2.4 \times 10^{-6}$ representing a very strong binding affinity.

To date, in vitro studies of dye removal with functionalised silica nanoparticles have focused on single dye types and if mixtures have been used, their concentrations are low and only 2 dyes were blended 11,89-97. This is not representative of real-world textile waste contamination events where concentrated dye mixtures i.e. blends of azo, anthraquinone, acid, basic and reactive dyes, are blended together and released into the environment 98-100. In fact, to the best of our knowledge, our P-Silanol-NP treatment shows the highest reported concentrated mixed dye removal efficiency when compared to other silicon derived treatment approaches.

Dye removal curves for P-Silanol-NP (Figure 5) show saturated removal efficiency at 2.5 and 5 g/L with no statistical difference (one-way ANOVA coupled with Tukey and Duncan post-hoc tests). It was therefore important to extrapolate the dye removal efficiency at the lower concentration of 1.25 g/L. After 30 min 91.37% of the 0.51 mM dye solution had been removed from solution. The value for the Silanol-NP and SiO$_2$-NP treatments was 56.87% and 15.06% removal respectively for the same adsorbent dosage. Non-functionalised SiO$_2$ nanoparticles
showed a maximum dye adsorption capacity of 41.25% with 5 g/L adsorbent. Silicon dioxide functionalization is therefore required for elevated dye removal efficiencies.

Figure 5: Dye removal capacities with different adsorbent dosages following 5 min and 30 min of exposure to (a) P-Silanol-NP (b) Silanol-NP (c) SiO$_2$-NP (d) relationship between carbon concentration and dye adsorption capacity.

3.8. Langmuir and Freundlich isotherms

Table 4: Langmuir and Freundlich Parameters for P-Silanol-NP and Silanol-NP

<table>
<thead>
<tr>
<th></th>
<th>P-Silanol-NP</th>
<th>Concentrated Dye (0.51 mM)</th>
<th>Silanol-NP</th>
<th>Concentrated Dye (0.51 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Langmuir</td>
<td>$q_{max}$ (mg g$^{-1}$)</td>
<td>714.29</td>
<td>$q_{max}$ (mg g$^{-1}$)</td>
<td>416.67</td>
</tr>
<tr>
<td>$R_s$</td>
<td>(L mg$^{-1}$)</td>
<td>11.31</td>
<td>$R_s$</td>
<td>15.31</td>
</tr>
<tr>
<td>$R^2$</td>
<td></td>
<td>0.96</td>
<td>$R^2$</td>
<td>0.99</td>
</tr>
<tr>
<td>Calculated $q_{max}$ (mg g$^{-1}$)</td>
<td>731.04</td>
<td>Calculated $q_{max}$ (mg g$^{-1}$)</td>
<td>454.96</td>
<td></td>
</tr>
<tr>
<td>Freundlich</td>
<td>$K_f$ (mg g$^{-1}$)</td>
<td>60.46</td>
<td>$K_f$ (mg g$^{-1}$)</td>
<td>137.45</td>
</tr>
<tr>
<td>$1/n$</td>
<td></td>
<td>0.41</td>
<td>1/n</td>
<td>0.25</td>
</tr>
<tr>
<td>$R^2$</td>
<td></td>
<td>0.73</td>
<td>$R^2$</td>
<td>0.77</td>
</tr>
</tbody>
</table>
3.9. Relationship between Surface Carbon Content and Dye Removal

A scatter plot of surface carbon concentrations against dye removal efficiency was created from data over all treatments (Figure 5d). The plot shows a strong linear relationship ($R^2 = 0.96$) between surface carbon concentration and dye removal efficiency. Raw plant extract and hydrothermally treated raw plant extract exhibited no dye removal activity. Increased surface functionalization of nanoparticle wafers by APTES derived tri-silanols may be represented by an increase in carbon concentration. Carbonaceous phytochemical capping may increase the binding efficiency of such silanols by the interaction of the nitrogen containing groups with carbon from the silanol chain upon oxygenation within the hydrothermal vessel.

3.10. Zeta Potential

The zeta potential of un-functionalised silicon dioxide nanoparticles has been shown to be highly negative which is consistent with our findings (-18.33 mV). Both P-Silanol-NP and Silanol-NP contain SiO$_2$ bonds as can be seen in the FTIR spectra; however, they possess a strong positive charge with zeta potentials between 8.77-9.20 mV (Table 5). The reverse in charged state can be explained by the presence of amino groups within the silica matrix. Kim et al. (2011) reported that in PBS aqueous solutions, the amino group of APTES has an affinity for the negatively charged silicon surface whilst negatively charged and neutral silanols point away from the silicon’s surface. As the silanol matrices are made up of branching functional groups (Supplementary Figure 6) there are surplus amino groups that are not bound to the silanol surface. The strong binding affinity between silanols and the dye can be explained by electrostatic attraction between the negatively charged dye molecules and positively charged amino groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Silanol-NP</td>
<td>8.77</td>
</tr>
<tr>
<td>Silanol-NP</td>
<td>9.20</td>
</tr>
<tr>
<td>SiO$_2$-NP</td>
<td>-18.33</td>
</tr>
<tr>
<td>Dye</td>
<td>-48.73</td>
</tr>
</tbody>
</table>

3.11. Bacterial viability

A key factor for any environmental remediation is whether the treatment itself poses any environmental harm. A number of metal nanoparticles and carbon nanotubes have been shown to induce microbial and mammalian toxicity. Any chemical treatment may be hazardous and pose an environmental threat themselves.

Within this study treated concentrated dye solutions were assessed for their ecotoxicity via a $V. fischeri$ growth capacity assay. $Vibrio fischeri$ is a model microorganism routinely used to study the toxicity of anthropogenic pollutants and infer ecosystem health.

Titanium dioxide nanoparticles functionalised with APTES have been implicated with microbial toxicity as they bind to the bacterial cell wall and allow Ti$^{2+}$ ions to interact with genomic DNA and other biological moieties. Silicon dioxide nanoparticles however, have been shown to be generally non-toxic to bacteria and therefore offer a suitable template for environmental applications. Within this study the silanol wafers showed only minor effects on bacterial growth, reducing the growth by around 20% when compared with the positive control (Table 6). Removal of un-bound silanols via filtration and dialysis is likely to decrease the particles toxicity as microbial cultures subjected to APTES alone, were shown to be significantly inhibited, with a 95.5% reduction in growth observed.
Both SiO₂-NP and the concentrated dye mixture both inhibited bacterial growth by around 70% compared with the positive control. SiO₂ nanoparticles have previously been reported to be ecologically benign; the reduction in bacterial growth in this study may therefore be attributed to the presence of un-degraded dye (around 625 mg/L).

**Table 6: Relative growth of *V. fisherii* in the presence of remediated dye supernatants compared to growth in the positive control.**

<table>
<thead>
<tr>
<th></th>
<th>P-Silanol-NP (5.0 g/L)</th>
<th>Silanol-NP (5.0 g/L)</th>
<th>SiO2-NP (5.0 g/L)</th>
<th>Dye (0.51 mM)</th>
<th>APTES (5.0 g/L)</th>
<th>Bacterial control (+)</th>
<th>Bacterial control (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. fisherii</em> growth following 48 hour exposure period</td>
<td>++++ (83.97% ±19.3)</td>
<td>+++ (78.54% ± 7.9)</td>
<td>++ (30.46% ±9.1)</td>
<td>++ (32.90% ± 9.2)</td>
<td>+ (4.50% ±3.0)</td>
<td>++++ (100% ± 8.0)</td>
<td>- (-0.66% ± 1.5)</td>
</tr>
</tbody>
</table>

100-75% = ++++, 74-50% = ++++, 49-25% = ++, 24-1% = +, < 1% = -

**4.0. Conclusions**

Functionalization of SiO₂ nanoparticles with alkylsilanes has been suggested as the basis for a number of technologies due to their inherent affinity for binding biomolecules and heavy metal ions. Here we reason that the use of a template to attach our functional ligands (i.e. TiO₂, SiO₂, zeolites etc.) is unnecessary as nanoparticulate powders composed of a disordered functional silanol ligand matrix serve the same purpose and do not contain void space below the functionalised layer. Therefore, if environmental conditions change, the stability of the complex and resulting affinity of the ligand for the template is inconsequential. This study utilised APTES as a silicon and amine precursor for the generation of amino silanol nanoparticles for the purpose of concentrated dye removal from aqueous solutions.

The addition of *H. annuus* husk extract to the synthesis solution resulted in a porous silanol nanoparticle assembly which led to increased dye removal capacity when compared to silanol nanoparticles alone. The mechanism of increased porosity is likely due to a reducing sugars templating phenomenon along with the formation and immobilization of QCD’s. Both bare silanol nanoparticles and *H. annuus* husk infused silanol nanoparticles showed a 4-6-fold increase in dye removal capacity when compared to that of SiO₂ nanoparticles at a concentration of 1.25 g/ L and in the presence of 0.51 mM mixed dye solution. The findings were substantiated by HR-TEM, FTIR, SEM-EDS and zeta potential analyses. The silanol dye removal strategy is one of monolayer adsorption affinity with Langmuir isotherms closely resembling the calculated adsorption capacity for 1.25 g/ L adsorbent. The strong binding affinity was supported by *Rₛ* values of between 1.40 x 10⁻⁶ - 2.40 x 10⁻⁶.

The high binding affinity of the dye to adsorbent can be explained by the electrostatic attraction between positive amine groups from the silanol nanoparticles and the negatively charged concentrated dye mixture. Enhanced dye removal from P-Silanol-NP’s has been attributed to increase dye-particle interactions due to increased particle porosity resulting from phytochemicals (i.e. reducing sugars). *Helianthus annuus* husk phytochemicals bind to the silanol nanoparticles and create nano-craters in the surface. Upon hydrothermal treatment, the phytochemicals are dislodged, increasing the porosity of silanols and subsequent surface area (from 4.88 to 15.26 m² g⁻¹) and pore volume (from 0.0062 to 0.0753 cm³ g⁻¹). The environmental suitability of these particles is supported as the bacterial toxicity of remediated dye solutions using these particles was shown to be minor. The results from this study show a promising textile dye treatment technology with significant dye adsorption capacity and minimal potential for environmental harm.
Funding

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References


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**Supplementary Information**

Supplementary Figure 1: FTIR spectra of freeze dried sunflower husk extract
Supplementary Figure 2: UV-vis Absorbance of sunflower husk extract

Supplementary Figure 3: Representative nanoparticle morphology of silanol nanoparticles under HR-TEM
Supplementary Figure 4: HR-TEM of P-Silanol-NP

Supplementary Figure 5: X-ray diffraction spectra for Silanol-NP & SiO2-NP treatments (left to right respectively).

Supplementary Table 1: FTIR peaks of P-Silanol-NP, Silanol-NP and SiO2-NP treatments

<table>
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<th>Sample</th>
<th>Peak</th>
<th>Assignment</th>
<th>Reference</th>
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<tr>
<td>MP-Silanol-NP</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Wavenumber</td>
<td>Description</td>
<td>References</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------------------</td>
<td>-------------</td>
<td></td>
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<tr>
<td>3046</td>
<td>CH2 stretch and/or Si-OH</td>
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<td>2933</td>
<td>CH2 stretch (from methylene)</td>
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<tr>
<td>2880</td>
<td>CH2 stretch</td>
<td>[122-124]</td>
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<tr>
<td>1634</td>
<td>-NH₂ bending groups</td>
<td></td>
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<tr>
<td>1558</td>
<td>asymmetric -NH³+ deformations</td>
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<tr>
<td>1033</td>
<td>Si-O-Si</td>
<td>[121, 125, 126]</td>
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<tr>
<td>982</td>
<td>Si-O-Si (framework defects i.e. Silanol groups)</td>
<td>[127]</td>
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<tr>
<td>860</td>
<td>Si-O-Si stretch</td>
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<td>748</td>
<td>O-Si-O bending</td>
<td>[128-130]</td>
<td></td>
</tr>
<tr>
<td>691</td>
<td>Si-CH2 stretch/ Si-O-C</td>
<td>[121, 131-134]</td>
<td></td>
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<tr>
<td>542</td>
<td>Si-O-Si bending/ Si-OH</td>
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<td></td>
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<tr>
<td>527</td>
<td>Al-O-Si/ Si-O</td>
<td>[138-140]</td>
<td></td>
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<tr>
<td>442</td>
<td>Si-O bending</td>
<td>[121, 130, 141]</td>
<td></td>
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<td>3666</td>
<td>HO-Si-OH stretch (free OH groups)</td>
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<tr>
<td>2989</td>
<td>CH stretch</td>
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<tr>
<td>Wavenumber</td>
<td>Functional Group</td>
<td>References</td>
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<tr>
<td>------------</td>
<td>------------------</td>
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<tr>
<td>2973</td>
<td>CH3 stretch</td>
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<td>Si-O-Si stretch</td>
<td>[124]</td>
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<td>776</td>
<td>O-Si-O bending</td>
<td>[130]</td>
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<tr>
<td>691</td>
<td>Si-CH2 stretch/ Si-O-C</td>
<td>[121, 131-134]</td>
<td></td>
</tr>
<tr>
<td>542</td>
<td>Si-O-Si/ Si-OH (vibrations of the bridging oxygen in the Si-O-Si linkage)</td>
<td>[135-137]</td>
<td></td>
</tr>
<tr>
<td>442</td>
<td>Si-O bending</td>
<td>[121, 130, 141]</td>
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<th>Functional Group</th>
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<td>1076</td>
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<td>[121, 154]</td>
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<td>961</td>
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<td>796</td>
<td>O-Si-O bending</td>
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<tr>
<td>455</td>
<td>O-Si-O bending</td>
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Supplementary Figure 6: Diagrammatic representation of bonds on (a) P-Silanol-NP (b) Silanol-NP (c) SiO$_2$-NP’s.
Supplementary Figure 7: Pore size distribution and pore volume of P-Silanol-NP and Silanol-NP treatments respectively.

Supplementary Figure 8: Fluorescence intensity of P-Silanol-NP and Silanol-NP treatments excited at 360nm.
Supplementary Table 2: Textural and structural properties of treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surface area (m² g⁻¹)</th>
<th>Pore Volume (cm³ g⁻¹)</th>
<th>Pore diameter (nm)</th>
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</thead>
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<tr>
<td>P-Silanol-NP</td>
<td>15.26</td>
<td>0.0753</td>
<td>15.10</td>
</tr>
<tr>
<td>Silanol-NP</td>
<td>4.88</td>
<td>0.0062</td>
<td>5.14</td>
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<tr>
<td>SiO₂-NP</td>
<td>163.49</td>
<td>0.9944</td>
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### Supplementary Table 3: Total fat and fatty acid analysis of sunflower husk extract

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<th>Lab Reg No.</th>
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<th>Sample Description</th>
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<tr>
<td>V18/015564</td>
<td>SF-Husk Extract</td>
<td>Freeze dried water extract of sunflower husk</td>
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<table>
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<tr>
<th>Sample Reference</th>
<th>Units</th>
<th>SF-Husk Extract</th>
<th>Method</th>
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<tr>
<td><strong>Proximates</strong></td>
<td></td>
<td></td>
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<tr>
<td>Fat (Mojonnier extraction)</td>
<td>g/100g</td>
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<td>VL302</td>
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<td><strong>Saturated Fatty Acids</strong></td>
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<td>C4:0 Butyric</td>
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<td>C14:0 Myristic</td>
<td>%</td>
<td>&lt;0.1</td>
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<tr>
<td>C15:0 Pentadecanoic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C16:0 Palmitic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C17:0 Margaric</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C18:0 Stearic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C20:0 Arachidic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C22:0 Behenic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C24:0 Lignoceric</td>
<td>%</td>
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<td>VL289</td>
</tr>
<tr>
<td>Total Saturated</td>
<td>%</td>
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</tr>
<tr>
<td><strong>Mono-unsaturated Fatty Acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:1 Myristoleic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
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<tr>
<td>C16:1 Palmitoleic</td>
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<td>VL289</td>
</tr>
<tr>
<td>C17:1 Heptadecanoic</td>
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<tr>
<td>C18:1 Oleic</td>
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<td>&lt;0.1</td>
<td>VL289</td>
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<tr>
<td>C18:1 Vaccenic</td>
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<td>&lt;0.1</td>
<td>VL289</td>
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<tr>
<td>C20:1 Eicosenic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C22:1 Cetoleic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C22:1 Docosenoic (Erucic)</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C24:1 Nervonic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>Total Mono-unsaturated</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
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<tr>
<td><strong>Poly-unsaturated Fatty Acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:4 Hexadecatetraenoic</td>
<td>%</td>
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Poly-unsaturated Fatty Acids
<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>%</th>
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<th>VL289</th>
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</thead>
<tbody>
<tr>
<td>C18:4 Moroctic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C18:2w6 Linoleic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C18:3w6 gamma-Linolenic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C18:3w3 alpha-Linolenic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C20:2w6 Eicosadienoic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C20:3w6 Eicosatrienoic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C20:3w3 Eicosatrienoic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C20:4w6 Arachidonic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C20:5w3 Eicosapentaenoic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C22:2w6 Docosadienoic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
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<tr>
<td>Omega 3 Fatty Acids</td>
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<tr>
<td>Omega 6 Fatty Acids</td>
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<td>VL289</td>
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<tr>
<td>C22:4w6 Docosatetraenoic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C22:5w3 Docosapentaenoic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>C22:6w3 Docosahexaenoic</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>Total Poly-unsaturated</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
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<tr>
<td>Total Mono Trans Fatty Acids</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
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<tr>
<td>Total Poly Trans Fatty Acids</td>
<td>%</td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
<tr>
<td>P:M:S Ratio</td>
<td></td>
<td>&lt;0.1</td>
<td>VL289</td>
</tr>
</tbody>
</table>

References


33 Kaur, A., Chattopadhyay, S., Jain, S., Tyagi, A. & Singh, H. Preparation of hydrogel impregnated antimicrobial polyurethane foam for absorption of radionuclide contaminated


Chapter 5

General Discussion

Nanoparticles for contaminant remediation have received extensive focus in recent years with many different template and functionalization strategies employed. Researchers have focused their attention on carbon nanotubes (single-walled, SWCNTs or multi-walled, MWCNTs), fullerenes, graphene, zero-valent iron (nZVI), TiO$_2$, Ag, ZnO, iron/oxide, nickel/oxide, cobalt/oxide, clay, SiO$_2$, alginate and chitosan, to name a few of many. Combinations of the aforementioned nanoparticles along with different surface functionalisation strategies and catalyst additions have been shown to aid in remediative enhancement.

Zero valent iron (Fe$^0$) and iron oxides are currently used as a remediation approach for both reactive degradation and adsorption of PAHs (including textile dyes) and heavy metals for wastewater treatment. Advances in nanoparticle technology over the last decade have shown that specific nanoparticle attributes have a considerable impact on the rate of reaction, subsequent pollution remediation capacity and treatment times. Fine tuning of nanoparticle traits is being researched to infer optimal properties.

Table 1: Iron nanoparticle properties, their significance and optimal parameters

<table>
<thead>
<tr>
<th>Nanoparticle attribute</th>
<th>What this provides for the nanoparticle</th>
<th>Optimal traits</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>A decrease in nanoparticle size leads to an increase in surface area</td>
<td>Smaller nanoparticles will have increased surface area and as a result, faster remediation rates.</td>
<td>124</td>
</tr>
<tr>
<td>Shape</td>
<td>Rods, rosettes, wires, spheres, squares, triangles etc...</td>
<td>Each application may prefer a specific nanoparticle shape i.e. groundwater remediation prefers circular nanoparticles as they are more likely to percolate through the underground matrix to seek out pollutants.</td>
<td>125,126</td>
</tr>
<tr>
<td>Monodispersity</td>
<td>Stable nanoparticle suspensions and increased overall surface area.</td>
<td>Even size distribution and un-agglomerated particles.</td>
<td>127</td>
</tr>
<tr>
<td>Species of iron/oxide</td>
<td>Reactive or adsorbent properties depending on species of iron present.</td>
<td>For reactive organic pollution degradation nZVI is more efficient however, for adsorption pollution remediation routes, iron oxides are beneficial.</td>
<td>128</td>
</tr>
<tr>
<td>Surface area</td>
<td>Increases in surface area leads to faster reaction rates.</td>
<td>Maximum surface area possible which will support adequate shelf lives and stability (i.e. if nZVI particle is too small the particle may rust and disintegrate before it reaches the pollutant).</td>
<td>126</td>
</tr>
<tr>
<td>Functionalised surface</td>
<td>Additional capacity for nanoparticles to bind</td>
<td>Functionalization which provides the nanoparticle with stability and has a strong affinity for the pollutant of</td>
<td>127</td>
</tr>
</tbody>
</table>
and interact with pollutant. Interest. Optimal traits are dependant on the pollutant of interest.

<table>
<thead>
<tr>
<th>magnetism</th>
<th>Capacity for nanoparticles to be removed from a system via a magnet. Magnetism is beneficial if nanoparticles require removing from a system (if nanoparticles are not immobilised or removable in other ways).</th>
</tr>
</thead>
<tbody>
<tr>
<td>reactivity</td>
<td>Capacity to catabolise pollutant chemical structures. Fast degradation rates with the capacity to completely catabolize organic pollutants.</td>
</tr>
<tr>
<td>Adsorbent capacity</td>
<td>Capacity to bind pollutant and remove from solution without degradation. Strongly bind to pollutant of interest without desorption in environmental settings. Adsorbent ideally shows specificity for the pollutant of interest.</td>
</tr>
<tr>
<td>Crystallinity</td>
<td>Ordered atomic crystal lattice structure of nanoparticles. Crystallinity is linked with superior decontamination capacity.</td>
</tr>
<tr>
<td>Shelf life/stability</td>
<td>Determines the storage conditions and practicability of nanoparticles for use in environmental settings. Long shelf life with particles that do not passivate at ordinary storage conditions or do not passivate quickly upon environmental exposure.</td>
</tr>
</tbody>
</table>

With this in mind, an approach to generate nano-sized reactive zero valent iron nanoparticles was proposed to elevate dye degradation reaction rates when compared to conventional millimetric sized particles. Current nZVI synthesis approaches largely rely on the toxic iron reducing agent, sodium borohydride. Synthesis of nZVI by this method results in toxic borohydride waste that needs specific and costly disposal. Further, particle rinsing needs to be conducted to remove all traces of this precursor and the particles generated agglomerate reducing surface area and nanoparticulate nature, size, shape, monodispersity and morphology are unable to be controlled. In addition, nZVI particles rapidly oxidise in air and water reducing their reactivity. Segura, et al. showed that after 5 minutes of environmental exposure nZVI reactivity slowed due to passivation of its surface forming iron/hydrous oxides.

To generate nZVI nanoparticles without the use of sodium borohydride as an iron reducing agent the literature was consulted to find viable and environmentally considerate alternatives. Research showed that many studies had generated ‘nZVI’ with plant extracts. These plant extract-generated nanoparticles were reported to have phytochemical particle capping preventing passivation and TEM images tended to show some tuneable control of particle size, shape and monodispersity parameters (Table 1). This, information led to a direction of research which proved troublesome due to a commonly unfounded assumption that the reduction of yellow iron salts to black nanoparticles is representative of nZVI (Appendix Table 1). In fact, only limited studies have confirmed the presence of nZVI from reaction with iron salts and plant extracts and most of these show low proportions of nZVI compared to iron oxides within the final product.

In order to confirm the presence of nZVI one or more of the following should be measurable; magnetism with non-zero magnetic susceptibility above 585 °C corresponding to Fe0 (Curie temperature 770 °C), X-ray absorption near edge structure (XANES) to rule out the dominance of iron oxide Fe3+ and Fe2+ related ions, XRD with Fe0 representative peak at 45° (for crystalline nZVI), an XPS peak found at approximately...
706.5 eV and Raman spectroscopy to identify different species of iron present against a Fe⁰ standard.

The methodology for Chapter 2 entitled ‘Iron nanoparticles synthesized using green tea extracts for the Fenton-like degradation of concentrated dye mixtures at elevated temperatures’ originated with the assumption that reactive and magnetic nZVI nanoparticles would be formed. Initial dye degradation microcosms showed no intrinsic reductive capability of particles (data not shown). X-ray diffraction analysis showed no crystallinity relating to nZVI and there was no apparent magnetism of particles against a high-powered magnet (compared to the magnetic attraction witnessed with nZVI particles generated with 0.1M FeCl₃ solution reacted with 0.05M NaBH₄ solution in a 1:1 ratio). It was therefore realised that particles were in fact iron oxides and a Fenton-like degradation approach for dye removal was implemented.

The Fenton reaction involves the reaction between Fe⁴⁺ and H₂O₂ in acidic conditions to produce hydroxyl radicals (• OH) and proceeds via one or more of the following cascades of reactions:

\[
\begin{align*}
\text{Fe}^{3+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{2+} + \text{H}_2\text{O} + \text{H}^+ \quad (1) \\
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \text{• OH} + \text{OH}^- \quad (2) \\
\text{Fe}^{2+} + \text{• OH} & \rightarrow \text{Fe}^{3+} + \text{OH}^- \quad (3) \\
\text{• OH} + \text{H}_2\text{O}_2 & \rightarrow \text{OOH} + \text{H}_2\text{O} \quad (4) \\
\text{• OH} + \text{Dye} & \rightarrow \text{Intermediate degradation products} + \text{H}_2\text{O} + \text{CO}_2 \quad (5)
\end{align*}
\]

This process has been widely used to treat numerous complex organic pollutants, however, reagents are costly and iron sludge is a waste product of the process. Iron nanoparticles, including iron oxide and nZVI species have been applied to form a Fenton-like remediation approaches with enhanced efficiency, increased controllability and reduced cost when compared to conventional Fenton methods.

Iron oxide nanoparticles generated within this study were capped with green tea polyphenol compounds i.e. ECGC, and this was supported by representative FTIR peaks and increased carbon content of samples as confirmed by SEM-EDS. Dye degradation microcosms using these particles showed that within an hour, over 90% of concentrated dye mixture was degraded at pH 3, and increased temperature further elevated degradation rates to over 90% degradation within 20 minutes at 70°C.

These findings showed that iron oxide nanoparticles produced via green methods were able to participate in a Fenton-like reaction and rapidly degrade concentrated dye mixtures. Traditional approaches of Fenton reactions use iron salt solutions which do not provide a controlled reaction rate. The use of nanoparticles allows surface interactions between iron species and H₂O₂ resulting in controlled nanoparticle surface disintegration and the subsequent generation of reactive hydroxyl radicals. In addition, the surface capping of nanoparticles from green tea may prevent the exposure of iron to moisture and oxygen, extending their shelf life over uncoated nZVI nanoparticles which rapidly oxidize and lose reactive functionality. Following treatment of pollutants, green tea capped iron...
nanoparticles which have not taken part in the Fenton-like reaction will break down over time in environmental settings and convert to rust which is ubiquitous in the environment.

Most researchers only assess the capacity of iron nanoparticles to degrade individual dye types and not complex mixtures. This is not indicative of real world textile effluent waste. Shahwan, et al. 151 showed dye removal kinetics of green tea synthesized nanoparticles with an initial Methyl Blue (MB) and Methyl Orange (MO) dye concentration of 50 mg/L. Complete dye degradation was achieved after 200 mins for MB and 350 for MO. In the current study faster degradation rates for RBB-R (150 mg/L) but slower degradation rates for Direct red 80 (25 mg/L) was observed. The structure of the dyes differ along with their ease of destruction with hydroxyl radicals; consequently they cannot be compared against one another. This study, and other studies do not assess the effect of elevated temperatures. This sets the study apart from others with findings showing that faster and more concentrated dye removal (500 mg/L) is achieved at elevated temperatures.

The successful synthesis and capping of iron nanoparticles using green tea extract confirmed that synthesis of nanoparticles was possible with water soluble plant extracts and capping with green tea derived phytochemicals was present. It also, showed that reactive nZVI nanoparticles were not formed with green tea but rather, iron oxides were present. Although green tea was not capable of producing nZVI under the conditions tested, other plants with different phytochemical make-up may reduce iron salts in a differing manner. In addition, these varying phytochemicals may provide different surface functionalities and optimal nanoparticle morphologies 152. This led to the rationale for the formation of the second research chapter (Chapter 3) entitled ‘Phytofabrication of Iron Nanoparticles for Hexavalent Chromium Remediation’.

Within this study a number of different plant species, namely *P. undulatum*, *S. molle*, *M. azedarach* and *S. paniculatum* were used to generate iron nanoparticles. All plants had differing phytochemical profiles linked to either medical or antimicrobial applications. The rationale was to assess how the plants reducing power, total antioxidant concentration, phenolic concentration, reducing sugars concentration, protein concentration and iron chelation capacity impact nanoparticle formation and morphology. We wanted to determine if differing plant species possess phytochemicals which may instil beneficial nanoparticle morphologies and increase pollution removal capacity.

The iron oxide nanoparticles generated with green tea extract in Chapter 2 showed considerable variation in particle size and showed a tendency to agglomerate. This was consistent with other research conducted using green tea as an iron salt reducing agent 153-155. Although rapid and high degrees of dye degradation were present with the green tea synthesized nanoparticles, the process required highly acidic conditions and therefore post treatment of remediated textile effluent would need costly pH balancing during large scale application. In addition, the requirement for H₂O₂ significantly increases the cost of this technology. For these reasons, instead of coupling green generated nanoparticles with H₂O₂ for a Fenton-like remediation approach, a decision was made to determine their adsorption capacity for hexavalent chromium instead.

The findings from Chapter 3 confirmed the assumption that differing plant phytochemicals result in the formation of iron nanoparticles with differing size, shape, monodispersity and capping properties. Many researchers have selected plant species for nanoparticle generation based on their high antioxidant concentrations which are known to rapidly reduce iron salts into nanoparticles 156-159. The results from Chapter 3 confirm that plant extracts which possess high antioxidant concentrations also show high
Reducing power capacity. However, antioxidant concentration was not linked to optimum nanoparticle morphology. A high yield of nanoparticles through elevated reduction of iron salts is desirable but if nanoparticle morphologies are not favourable, alternative, cheap and mass-produced reductants will likely be employed over the green alternatives to save cost. However, if beneficial nanoparticle morphologies cannot be obtained through these reductants, phytochemicals which instil beneficial traits can be used as a viable alternative.

Within this study all plant species were able to reduce iron salts into iron oxide nanoparticles with no nZVI signatures as determined by XPS, XRD and magnetism measurements. This same outcome was also found with green tea generated nanoparticles synthesized in Chapter 2. Linking plants reducing power, total antioxidant concentration, phenolic concentration, reducing sugars concentration, protein concentration and iron chelation capacity to nanoparticle morphology was not possible as no specific property definitively resulted in a specific morphology. However, different plant extracts did result in the generation of particles with varying traits. It is suggested that phytochemicals from each plant extract need to be separated and purified to test their influence on nanoparticle morphology.

Of the four-plant species, *P. undulatum* and *S. molle* were able to generate monodispersed nanoparticles with spherical shape and sizes between 5-15 nm. Plant extracts with the highest and lowest antioxidant concentrations were *S. paniculatum* and *M. azedarach* respectively. These two species both showed agglomerated chains of spherical nanoparticles with *S. paniculatum* possessing sizes between 30-60 nm whilst that of *M. azedarach* exceeded 100 nm. This shows that nanoparticle size, shape and monodispersity are independent of antioxidant concentration.

These iron nanoparticles were used as adsorbents for hexavalent chromium and their capacity was compared using a diphenylcarbazide assay. Certain researchers choose to assess plant derived nanoparticles efficiency for pollution removal by comparing volumes of the crude plant extract reduced nanoparticle solution as opposed to nanoparticle concentration 160-162. In this study, nanoparticles were separated from their reaction solution so that the effect of nanoparticles (without competing species from the iron salts or plant extract) were the only drivers of chromium removal.

The potential hexavalent chromium adsorption of nanoparticles was linked with nanoparticle size and monodispersity. Plant extracts of *P. undulatum* and *S. molle* which showed the smallest nanoparticle size (5-15 nm) and were monodispersed. Their chromium adsorption efficiency was 96.2 and 84.1% respectively following 9 hours of exposure compared to larger, agglomerated particles from *S. paniculatum* and *M. azedarach* with 58.7 and 43.7% removal respectively. A higher hexavalent chromium removal efficiency can be attributed to the increase in surface area from smaller, mono-dispersed particles. This is consistent with the research from Huo, et al. 163 who showed that elevated rates of arsenic(V) adsorption with iron nanoparticles is attributed to increases in surface area as a result of smaller particle size.

Generating small, monodisperse nanoparticles is a focus for many researchers as with even size distributions, more accurate predictions of nanoparticle shelf-life, reaction rates, interactions with biological/inorganic entities, environmental dispersion estimates and delivery to site of application can be ascertained. This is not only applicable for environmental remediation applications but for many other fields of research e.g. attaching medication to nanoparticles for targeted drug delivery 164 and increased hydrophobic surfaces for water repellent surfaces in materials chemistry 165. Plant extracts of *P. undulatum* and *S. molle* may be of considerable interest for researchers from many fields for providing
favourable nanoparticle dimensions and dispersity. In addition, these plant extracts may also transfer similar traits to metallic nanoparticles other than iron i.e. green generated silver nanoparticles for antimicrobial applications.

Plant phytochemical composition may differ, and concentrations may fluctuate depending on environmental conditions, seasonal variation and geographical location. This makes it difficult to accurately replicate exact test conditions for freshly picked and dried specimens from non-commercial plantations. Plant material sourced from mass produced crop species may therefore provide more consistent phytochemical makeup and reproducible results.

Although results from Chapters 2 and 3 both showed appreciable degradation and adsorption rates of concentrated dye and hexavalent chromium respectively, there were a number of downfalls with the use of iron which may be prevented by the use of silicon derived nanoparticulate templates. These include;

• Iron nanoparticles need to be removed from the system to provide environmentally benign treatment options. The Fenton process results in the build-up of sludge which needs to be removed and disposed of in landfill and requires costly H\textsubscript{2}O\textsubscript{2} and pH adjustment.
• Nanoparticles used for adsorption need to be recoverable and as they are not magnetic, immobilised or collectable via sedimentation, and therefore remain in the environment along with the pollutant.
• Surface functionalization of nanoparticles is likely to provide optimal pollution remediation for adsorption approaches. However, functionalization of iron nanoparticles may be problematic due to passivation and rusting of iron below the modified surface leading to flaking off of surface coatings and reduced stability.
• Iron nanoparticles have been praised for their environmental compatibility; however, direct application into waterways will prevent sunlight from penetrating surface waters resulting in aquatic plant death. In addition, iron nanoparticles have been shown to be toxic to fish and their embryos.
• Iron is heavy, and the shelf life of commercial products may be short due to rusting and passivation of the surface layers. This will result in additional costs for transport and appropriate storage.

Silicon dioxide and other porous silicon nanoparticles have been shown to be efficient adsorbents for differing pollutants i.e. heavy metals \textsuperscript{166}, dyes \textsuperscript{167}, volatile organic contaminants (VOC's) \textsuperscript{168} and pesticides \textsuperscript{169}. Silicon dioxide is unreactive, cheap to produce on mass scale, light (in comparison to iron nanoparticles), possesses good environmental compatibility and low microbial toxicity \textsuperscript{170,171}. Their adsorbent capacity for cationic pollutants requires surface modification to change their inherent negative charge into positive charged particles for electrostatic attractive purposes \textsuperscript{172}.

Silicon dioxide nanoparticles have been functionalised with amines and folate for targeted drug delivery applications \textsuperscript{173}, n-octyltriethoxysilane (OTES) for increased hydrophobicity \textsuperscript{174}, 3-aminopropyl triethoxysilane (APTES) coupled with 2-bromoisobutyryl bromide (BIBB) for gene transfection \textsuperscript{175}, iron-silicon hybrid nanoparticles functionalised with tetraethyl orthosilicate (TEOS) and 3-aminopropyl trimethoxysilane (APTMS) for acrylamide adsorption \textsuperscript{176} amongst many functionalization studies.
Due to the environmentally friendly nature of silicon oxides, the successful functionalization of these particles with various ligands for environmental pollutant removal applications and the scarce literature utilising green technologies for their synthesis and functionalization, a project was designed to combine all the aforementioned benefits and unknowns.

Chapter 4 entitled ‘Amino-Silanol Nanoparticles Modified with Waste Aqueous Helianthus annuus Husk Extract for Increased Porosity and Rapid Adsorption of Concentrated Dye Mixtures’ focused on the use of waste Helianthus annuus husk extract along with amine containing APTES to generate porous amino-silanol nanoparticles with beneficial properties for concentrated dye removal.

Researchers functionalising silicon nanoparticles routinely use a silicon template followed by subsequent surface modification steps (Table 2). Within this chapter we utilised the silicon directly from APTES’s chemical structure for the generation of silanol nanoparticulate wafers. APTES rapidly hydrolyses in water to produce silanols. These silanols contain a complex network of silicon, oxygen, carbon and amine constituents. With this approach, an unutilised SiO$_2$ core is not required and functionalization is not required as the entire nanoparticulate mass is composed of the same functional matrix. This removes the void space present below the functionalised surface of other silicon dioxide nanomaterials.

Within this study, APTES was subjected to hydrothermal treatment in water or H. annuus extract and then purified with dialysis. The resulting silanol nanoparticles were assessed for surface charge, size, shape, morphology, crystallinity, bonds present, concentrated dye removal efficiency and bacterial toxicity.

Silanol nanoparticulate wafers without H. annuus extract showed rapid mixed dye removal rates with dye having a very high initial concentration of 1000 mg/L. These nanoparticles had a very high dye adsorption efficiency and removed up to 455 mg of dye removal per gram of adsorbent. Other researchers have been using functionalised silicon nanoparticles for dye removal. Some of the highest reported removal capacities are presented in Table 2. Upon the addition of H. annuus extract to the reaction solution prior to hydrothermal treatment, the concentrated dye removal capacity increased from 455 mg/g adsorbent to 714.29 mg/g adsorbent. Little statistical deviation between 5 and 30-minute treatment exposures were shown, making this treatment both rapid and effective. To the best of our knowledge, this is the highest concentrated mixed dye adsorption capacity for silicon-based materials.
<table>
<thead>
<tr>
<th>Dye</th>
<th>Adsorbent</th>
<th>$Q_{\text{max}}$ (mg/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congo red</td>
<td>Guar gum modified SiO$_2$ nanoparticles</td>
<td>233.24</td>
<td>21</td>
</tr>
<tr>
<td>Reactive blue 4</td>
<td>Guar gum modified SiO$_2$ nanoparticles</td>
<td>579.01</td>
<td>21</td>
</tr>
<tr>
<td>methylene blue</td>
<td>mesoporous silicon doped with phosphomolybdic acid (HPMo-SiO$_2$)</td>
<td>87.80</td>
<td>377</td>
</tr>
<tr>
<td>Rhodamine B (RhB)</td>
<td>Mg$_3$Si$_2$O$_5$(OH)$_4$ microspheres</td>
<td>300.08</td>
<td>278</td>
</tr>
<tr>
<td>phenol red</td>
<td>bis(triethoxysilyl) ethane &amp; bis(3-trimethoxysilyl-propyl)amine nanoparticles further functionalised with Hexadecyltrimethylammonium bromide (CTAB)</td>
<td>201.00</td>
<td>9</td>
</tr>
<tr>
<td>bromophenol blue</td>
<td>nanosilica-supported poly β-cyclodextrin</td>
<td>41.2</td>
<td>379</td>
</tr>
<tr>
<td>Methylene Blue</td>
<td>Polyacrylamide Grafted Xanthan Gum and Incorporated Nanosilica</td>
<td>497.5</td>
<td>380</td>
</tr>
<tr>
<td>methyl orange</td>
<td>polyhedral oligomeric silsesquioxane nanohybrid</td>
<td>237.5</td>
<td>381</td>
</tr>
<tr>
<td>Concentrated mixed dyes - Methyl Blue (certified), acid red 88, remazol brilliant blue R and acid green 25 (1:1:1:1 rato)</td>
<td>AminoSilane nanoparticulate wafers</td>
<td>455</td>
<td>Present Study</td>
</tr>
<tr>
<td>Concentrated mixed dyes - Methyl Blue (certified), acid red 88, remazol brilliant blue R and acid green 25 (1:1:1:1 rato)</td>
<td>AminoSilane nanoparticulate wafers with Sunflower husk extract</td>
<td>714.29</td>
<td>Present Study</td>
</tr>
</tbody>
</table>
The elevated dye adsorption capacity for *H. annuus* husk generated silanol nanoparticles can be attributed to (1) an increase in porosity leading to an elevated surface area (shown by TEM and BET analysis) and (2) the electrostatic attraction between negatively charged dye molecules and positively charged silanol nanoparticulate wafers (confirmed by zeta potential measurements).

Phytochemicals from the *H. annuus* husk extract interact/bind with APTES derived silanols. The increase in the porosity of silicon nanoparticles from hydrothermal treatment has been shown in the presence of oleic acid, symmetric triblock copolymer (Pluronic P123) and various surfactants 73,182-184. *Helianthus annuus* husks contain the surfactant oleic acid along with waxes and oils which are slightly soluble in water. However, fatty acid analysis of the aqueous extract showed negligible fat and fatty acid content suggesting a different mechanism in this study.

During the phytochemical extraction procedure at 80°C, various non-fatty acid compounds leach out from the *H. annuus* husk into the aqueous media 74. Anionic water soluble/semi-water soluble *H. annuus* husk constituents i.e. reducing sugars, galacturonic acid, cinnamic acid, eriodictyol, luteolin, salicylic acid, trans-m-hydroxycinnamic acid, ferulic acid, rutin, p-coumaric acid, syringic acid, vanillic acid, caffeic acid amongst other compounds may interact with the silanol nanoparticles under hydrothermal conditions 185,186. Interactions between these compounds are dependent on their affinity for the silanols functional groups.

The mechanism for increased silanol porosity is shown in Figure 1 and can be explained by the phytochemicals from the *H. annuus* husk extract interacting and or binding to them and creating cavities in their surface. Following hydrothermal treatment, interactions between phytochemical constituents (mainly reducing sugars) from the plant extract become weak and they dislodge from the silanol if they are not converted to quantum dots as a result of the high temperatures and pressures experienced. However, the cavities they have created in the surface remain, increasing porosity 40,70-73.

Wei et al. 1998 showed that the use of non-surfactant organic molecules composed of the reducing sugars D-glucose and D-maltose were able to act as templates for generating mesoporous silica with large surface areas and pore volumes as well as narrow pore size distributions. The morphology of these silica particles and their disordered mesopores were comparable to findings from this study 187.

Chapter 4 revealed that dye removal efficiency exhibited a linear trend with surface carbon concentration (0.96 $R^2$ value). Although carbon is unlikely to be responsible for significant dye binding, it highlights that nanoparticles synthesized with plant extract possessed superior dye removal capacity above naked silanol nanoparticles (which still contain hydrocarbon chemical bonds from the APTES precursor) and well above commercially purchased SiO$_2$ nanoparticles which possessed negligible carbon concentrations as shown by SEM-EDS analysis. FTIR investigation of nanoparticle dye binding showed that representative peaks of the dye by itself were present on both silanol nanoparticle treatments, confirming strong adsorption of un-modified dye molecules. Silicon dioxide nanoparticle FTIR peaks showed little similarity with that of the dye, indicating that strong adsorption was not present. Again, this is due to the electrostatic repulsion between negatively charged dye molecules and negatively charged SiO$_2$ nanoparticles.

Utilisation of plant waste products for instilling beneficial properties for materials offers an economically favourable approach (the market value of *H. annuus* husk is 0.03 $/kg) and utilises a resource which was
previously regarded to be a negative value waste residue. Their use reduces the strain on natural resources and provides beneficial traits with compounds more likely to be biocompatible due to their natural origin.

Figure 1: Mechanism of action for generating porous silanol nanoparticles with *H. annuus* husk extract; (1) APTES and plant extract are mixed (2) Rapid hydrolysis of APTES occurs and phytochemicals (i.e. reducing sugars and other anionic phytochemicals) interact with silanol surface as it condenses (3) Phytochemicals embed themselves into the silanol matrix (4) Hydrothermal treatment destroys bonds between phytochemical and silanol but leaves craters from their attachment on their structure. Purification removes dislodged traces of phytochemicals which cannot cope with temperatures from hydrothermal treatment. As a result, porous silanol nanoparticles are formed.

Current research for silicon-based dye removal applications show proof of concept for pollution control but do not incorporate microbial toxicity experiments to ensure that the treatment itself does not pose an environmental threat. Many studies showing the biocompatibility of silicon nanoparticles for medical applications have been conducted; however, environmental focused technologies rarely include this confirmation step. However, Gopalu, et al. showed that SiO$_2$ nanoparticles were nontoxic to the green algae (*Porphyridium aerugineum Geitler*) and Chia and Leong showed that the bacterial toxicity of ZnO nanoparticles was significantly reduced with a silica coating. Amine functionalization on the surface of SiO$_2$ nanoparticles has been shown to increase their biocompatibility in mammalian cells but this may also cross over to microbial cells.
Chapter 4 assessed the bacterial toxicity of remediated dye solutions with a *Vibrio fischeri* proliferation assay. *Vibrio fischeri* is a model indicator for environmental health and its use for ecotoxicity is commonplace. Remediated dye solutions using silanols and sunflower husk synthesized silanols showed little bacterial toxicity with growth between 78.5-84.0% respectively when compared to the positive control. Conversely, SiO$_2$ nanoparticles and the crude dye solution showed approximately 30% proliferation exhibiting obvious growth stunting. APTES alone was shown to be highly toxic with negligible growth observed.

The outcome of this research chapter was the development of a highly rapid and effective treatment approach for concentrated dye removal which is environmentally compatible and utilises an otherwise discarded resource (*H. annuus* husk) for increased efficiency.

**Future perspectives**

Due to the numerous differing types of phytochemicals present within plant extracts, it is unlikely that reproducible synergistic interactions will occur between plant extract and other nanoparticle precursors to generate nanoparticles with optimal properties. It is therefore suggested that phytochemical fractionation take place to determine if similar compounds possess beneficial nanoparticle synthesis and morphological interactive qualities. From crude fractions which show promise, further refinement can be attempted to isolate individual chemicals responsible for inferring specific qualities.

Due to the deterioration of iron nanoparticles with the Fenton-like process and subsequent sludge formation, it is not suggested that this treatment approach be implemented in environmentally sensitive areas. Adsorption of pollutants onto iron oxide nanoparticles showed promising results. However, the nanoparticles remain in the environment and may pose a concern to aquatic species.$^{192,193}$ It is therefore suggested that these particles be immobilized onto solid supports so that following contaminant adsorption, the nanoparticles, along with their supports can be removed and replaced.

Further research into incorporating catalysts, reactive nanoparticles or slow release oxidant/reductants onto porous amino-silanol particles for degradation of dye molecules is likely beneficial for preventing the treatment recovery step associated with sedimenting adsorbents. If removal of the adsorbent is necessary, the post treatment of dye loaded amino-silanol particles with microbial bioremediation (bioaugmentation and biostimulation) approaches may destroy the dye structure in an environmentally friendly manner.

If economically feasible, the recycling and reuse of desorbed dye along with re-use of the amino-silanol adsorbent may be of interest. Several studies show the re-use of adsorbents following desorption of the dye.$^9,194-196$

More detailed ecotoxicity analysis of amino-silanol nanoparticles is required to determine its influence on different microorganisms, algae, invertebrates, plants and mammals. If biocompatible for the aforementioned organisms, upscaling the technology for site evaluation of pollutant removal may be investigated.
Conclusions

Nanoparticles for site remediation have received extensive focus in recent years with many different template and functionalization strategies employed. Fenton like processes for dye degradation using green synthesized iron oxide nanoparticles have benefits over the traditional Fenton process i.e. sustained and more controlled reaction rates and degradation over a wide pH range. It is a low-cost technology with the capacity to destroy numerous organic contaminants however, as green tea synthesized nanoparticles are not magnetic or immobilised, their recovery following treatment is impossible and nanoparticulate matter remains in the environment. This can lead to the build-up of iron oxide sludge and nanoparticles may be harmful to aquatic species.

Control of nanoparticle morphology and composition is of importance to all nanoparticle research as it allows for predictive modelling of reaction rates, site transport efficiency and particle behaviours. The use of different plant extracts to instil specific qualities for nanoparticles has been shown in Chapter 4 however, linking the phytochemical driving forces for these specific morphologies is complicated and cannot be linked with iron reducing capacity. Nanoparticles generated with plant extracts possessing particle sizes below 15nm and showing monodispersity had elevated heavy metal (Cr6+) removal rates when compared to larger nanoparticles possessing agglomerated chain forming tendencies. This is consistent with the literature, outlining that smaller nanoparticles possess larger surface areas and pollutant removal capacities.

Surface functionalization is key to efficient adsorption capacity of various pollutants. The mechanism of binding is strongly linked to opposing surface charges between functionalised ligands on nanoparticles and chemical groups on dye molecules. However, chemical ligands often possess their own inherent toxicity and therefore natural alternatives may offer suitable alternatives unless exotoxicity studies can confirm their environmental suitability. Hydrolysed APTES in the presence of *H. annuus* husk extract showed low bacterial toxicity towards *V. fischeri* and plant extract enhanced porosity and surface area through deposition and removal of phytochemicals on the silanol nanoparticle following hydrothermal treatment. This presents an alternative for the use of chemical surfactants by utilising cheap organic waste materials for inducing mesoporosity on silicon nanoparticles. Further refinement can always be made with novel technologies however, primary results from Chapter 5 show rapid and high adsorbent capacities for concentrated dye mixtures with subsequent remediated treatments showing low bacterial toxicity.

References


Das, S., Berke-Schlessel, D., Ji, H.-F., McDonough, J. & Wei, Y. Enzymatic hydrolysis of biomass with recyclable use of cellobiase enzyme immobilized in sol–gel routed


Freundlich, H. & Hatfield, H. *Colloid [and] capillary chemistry*. (Methuen, 1926).


Kim, J. in *Interfaces and Interphases in Analytical Chemistry* Vol. 1062 ACS Symposium Series Ch. 6, 141-165 (American Chemical Society, 2011).


128 Di Palma, L., Gueye, M. T. & Petrucci, E. Hexavalent chromium reduction in contaminated soil: A comparison between ferrous sulphate and nanoscale zero-valent


https://www.nature.com/articles/srep24358#supplementary-information (2016).


https://www.nature.com/articles/srep40765#supplementary-information (2017).


Appendices:

Supplementary Table 1: Literature review of green synthesis approaches for generating nZVI nanoparticles and confirmation whether nZVI is present.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Plant extract used</th>
<th>Claiming formation?</th>
<th>nZVI</th>
<th>XRD</th>
<th>XANES</th>
<th>Magnetism</th>
<th>XPS</th>
<th>RAMAN</th>
<th>nZVI confirmed?</th>
<th>Comments</th>
</tr>
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<tr>
<td>[240]</td>
<td>Green tea</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>[241]</td>
<td>Green tea</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>[192]</td>
<td>Green tea, pomegranate, black tea, oak, strawberry, mulberry, eucalyptus, mandarin, plum, apricot, medlar, lemon, raspberry, apple, peach, quince, passion fruit, orange, cherry, olive, pine, walnut, pear, vine, kiwi, avocado</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Claims that the reaction with Fe$^{2+}$ salts and plant extract result in the formation of nZVI and has not conducted any characterization to confirm this.</td>
<td></td>
</tr>
<tr>
<td>[177]</td>
<td>Rosa damascene, Thymus vulgaris, and Urtica dioica</td>
<td>Yes</td>
<td>yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>XRD peak present at 45° however there are many peaks relating to iron oxides also and XRD spectra is noisy with low peak definition. Concentration and contribution of nZVI for degradation is unknown.</td>
<td></td>
</tr>
<tr>
<td>[242]</td>
<td>Mangifera indica</td>
<td>Yes</td>
<td>No (report claims XRD peaks which are not prominent)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>[195]</td>
<td>tea powder (Red label from Tata, India Ltd. 99%)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[243]</td>
<td>Citrus limettiioides Tan</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Extracts Used</td>
<td>nZVI Production</td>
<td>Presence of nZVI</td>
<td>Claim to Have Generated nZVI</td>
<td>All References</td>
<td>Review Notes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>-----------</td>
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<td>----------------</td>
<td>----------------</td>
<td></td>
<td></td>
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<tr>
<td>[244]</td>
<td>black tea, grape marc, and vine leaves. Camellia sinensis (green tea, GT), Syzygium aromaticum (clove, CL), Mentha spicata (spearmint, SM) and Punica granatum (pomegranate, PG). lemons, mandarins, limes, oranges or vine pomace, oolong tea Eucalyptus leaves</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Review article claiming that nZVI has been produced by a number of researchers, however, all researchers do not show evidence of nZVI and many do not claim to have generated nZVI.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[245]</td>
<td>grape marc, black tea and vine leaves</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>[246]</td>
<td>Camellia sinensis (green tea, GT), Syzygium aromaticum (clove, CL)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[247]</td>
<td>Mentha spicata (spearmint, SM) and Punica granatum (pomegranate, PG) Numerous already listed plant extracts</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>All references relating to nZVI being created from plant extracts are in reference to Machado (with one exception that does not confirm the presence of nZVI). In addition, the second author of this review is Machado.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[248]</td>
<td>grape seed extract</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Only paper I can find with convincing XRD signature of nZVI from interaction with plant extracts. The nanoparticles generated were synthesized with NsB4, and capped by plant extract and therefore the plant extract was not involved in the synthesis of nZVI.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[178]</td>
<td>Nettle and Thyme</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>XRD peak present at 45° however there are many peaks relating to iron oxides also and XRD spectra is noisy with low peak definition. Concentration and contribution of nZVI for degradation is unknown.</td>
<td></td>
</tr>
<tr>
<td>[249]</td>
<td>Mango peel</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>XPS analysis shows a potential reflectance of Fe⁰ however this may require further supporting characterization. In addition, the total predicted percentage of nZVI in the iron oxide mixture was approximately 15%</td>
<td></td>
</tr>
<tr>
<td>[250]</td>
<td>oak, mulberry and cherry leaf</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[251]</td>
<td>Roselle flower</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[252]</td>
<td>coffee, green tea, and Virginia creeper (Parthenocissus tricuspidata)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Only reaction mediums which contained NaBH₄ or Na₂S₂O₄ generated nZVI</td>
<td></td>
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<tr>
<td>[253]</td>
<td>Banana peel</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<td>No</td>
<td>No</td>
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<tr>
<td>[254]</td>
<td>Mediterranean cypress (Cupressus sempervirens)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>XRD peak present at 45° however there are many peaks relating other entities including iron oxides. XRD spectra is noisy with low peak definition. Concentration and contribution of nZVI for degradation is unknown.</td>
<td></td>
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<tr>
<td>[180]</td>
<td>oak leaves</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Machado is still claiming nZVI without characterization in 2017 (and getting published)…</td>
<td></td>
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<tr>
<td>[180]</td>
<td>Pure tea polyphenol</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Questionable XRD peak at 45°</td>
<td></td>
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<tr>
<td>[255]</td>
<td>Neem leaves</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
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