Chapter 10

Environmental effects of nZVI for land and groundwater remediation

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1. Main characteristics and applications of nZVI

Nanotechnology is one of the fastest growing sectors of the high-tech economy, with more than 800 consumer products using nanomaterials (NMs) with personal, commercial, medical, and military uses (http://www.nanotechproject.org/cpi/). The term nanoparticle (NP) is generally used to refer to a small particle with at least two dimensions < 100 nm. The small size and large surface area per unit mass reveal characteristics that can be useful in hazardous waste site remediation and contaminant removal such as in the case of nano-zerovalent iron (nZVI). This type of material has a wide use in land and groundwater applications. The power of iron-based technologies in remediation activities is widely known due to the ability of this metal to be a great reductant (Elliott and Zhang, 2001) or sorbent (Liu et al., 2014) for soil contaminants.

Over the past decade the development of nanostructured materials has led to the upgrade of traditional treatment with macro- and micro-sized iron (Kim et al., 2010). The introduction of nano-scale is explained from physical characteristics: nano-metals have high surface-area-to-volume ratio, high levels of stepped surface, and high surface energies. Particularly, nZVI can remove contaminants through reduction, oxidation, adsorption, precipitation or co-precipitation (Yang et al., 2013). Since the conventional micro-sized ZVI (mZVI) has low reactivity towards lightly chlorinated hydrocarbons, Wang and Zhang (1997) pioneered the application of nZVI for environmental remediation. Due to its unique properties, nZVI is able to effectively eliminate or neutralize certain recalcitrant pollutants that can be found in contaminated soils and groundwater. nZVI particles are typically 5-40 nm sized Fe^0/Fe-oxide particles and can rapidly transform many environmental contaminants into less harmful products being promising as an *in situ* remediation agent. Due to its small size and increased reactivity, nZVI has the potential to be more effective than mZVI that is already in use for contaminant remediation in soil and groundwater aquifers. However, little information is known about the environmental fate of nZVI once it has undergone biological and non-biological processes within a contaminated aquifer. For this reason, it is important to find out what are the possible impacts of these NMs once they enter the environment and how they could potentially affect human health or the ecosystem. Despite these concerns, nZVI technology and its application seem a very promising, efficient and cost-effective method for remediating contaminated soil and groundwater aquifer sites (Lee et al., 2014).

The scale of soil and groundwater contamination is enormous and its complexity seemingly intractable. In the US, more than 1500 sites have been put on the Superfund list (Zhang,

2003), but less than one third of them have been cleaned up in the past decade. In Italy, 39 sites of national interest (in 2013) and other 12943 local sites affected from low to extremely high levels of contamination are waiting for some kind of remediation (MinAMb, 2015). Agricultural soil is decreasing both in terms of quantity and quality. The problem of water scarcity, which from a strictly economical point of view translates into higher costs for clean water and wastewater treatment (WWT) due to the higher technological effort necessary, is not solely restricted to developing countries in dry areas, but it is also of high concern for developed countries worldwide (e.g., in the USA like California, Arizona, New Mexico, or Australia, India and Europe). In the EU, 44% of freshwater abstraction is used for cooling in energy production, 24% for agriculture, 21% for public water supply, and 11% for industry, even though geographical differences exist in the designated use (Hussain et al., 2002).

The average cost for cleaning up a Superfund site has been reported to be more than 25 million dollars (US). According to the United States Environmental Protection Agency (USEPA), over 418,000 underground storage tank (UST) leakages had been confirmed as of September 30, 2001. While much work has been and continues to be done, there are still about 150,000 UST sites remaining to be cleaned up (Zhang, 2003). The numbers of other contaminated sites such as abandoned mining sites, landfills, and industrial sites are just as staggering. If \$100/ton or \$100/m^3 (a commonly used yardstick by consultants) is needed to clean up the contaminated soil and/or water, the aggregated financial burden for site cleanup is truly colossal. Thus the problem of land as well as water contamination is extremely challenging.

ZVI iron has been used successfully in the past to remediate groundwater by construction of a permeable reactive barrier (PRB) intercepting and dechlorinating chlorinated hydrocarbons such as trichloroethylene (TCE) in groundwater plumes (Lee et al., 2014). Currently, ZVI (micro and macro-scale) is used in PRBs for contaminated sites' remediation. Commonly, a PRB contains granular iron as the reactive medium degrading chlorinated organics into potentially non-toxic dehalogenated organic compounds and inorganic chloride. In its simplest form, a PRB is a trench built across the flow path of a groundwater plume. The trench is filled with a suitable reactive or adsorptive medium capable of removing contamination from the groundwater and protecting down gradient water resources or receptors. Despite their minuscule status, nanoscale particles may hold the potential to cost-effectively address some of the challenges of site remediation (Masciangioli and Zhang, 2003;

Zhang, 2003; Libralato et al., 2016). Two factors contribute to NPs' capabilities as an extremely versatile remediation tool. Microscale ZVI (granular ZVI) has been used as a treatment reagent for *in situ* groundwater remediation for many years, in particular within permeable reactive barriers (NATO, 1998). nZVI is a type of iron NP that has been already injected *in situ* as a groundwater and aquifer treatment. Whilst the possibility of unique characteristics gives nZVI promise for beneficial applications, it is simultaneously a cause of concern, as there is a degree of uncertainty with regards to particle behavior, fate and toxicity. As produced, most nZVI tested falls into the 10−100 nm size range (Nurmi et al. 2005), although it tends to agglomerate to form larger particles.

In 2000, the first documented field trial of nZVI involved treatment of TCE in groundwater at a manufacturing site in Trenton (New Jersey, USA) (Elliott and Zhang, 2001). Several commentators anticipated that nZVI technology would take off rapidly because of its perceived benefits such as rapid and complete contaminant degradation. In 2007, a European report forecast that the 2010 world market for environmental nanotechnologies would be around $6 billion (Rickerby & Morrison 2007), but in practice, at present, this market has not been achieved. To date, the use of nZVI in remediation is largely a niche application for chlorinated solvents in aquifers, competing with more established techniques such as *in situ* bioremediation, chemical reduction and granular ZVI. Lee et al. (2014) reviewed 60 field applications worldwide. Bardos et al. (2014) identified about 70 projects around the world at pilot or full scale. Within the identified case studies, only 17 were in Europe (Czech Republic, Germany and Italy) focusing mainly on the degradation of chlorinated solvents for plume (i.e. pathway interruption) management although pilot studies have also demonstrated successful treatment of benzene, toluene, ethylbenzene and xylene (BTEX), perchlorates, hexavalent chromium, diesel fuel, polychlorinated byphenyls (PCBs) and pesticides. O'Carroll et al. (2013) detailed the chemical processes involved in the treatment of chlorinated solvents and various metals by nZVI.

Several approaches can be taken to NP deployment for contaminant remediation, including direct injection. The limited adoption of nZVI is linked to uncertainty over the balance of benefits versus risks from NP use in remediation. Additional factors that are likely to have hindered the development of nanoremediation market are its cost compared to other technologies and its social acceptance. The latter has been a particular impediment to the adoption of nZVI because of a heightened perception of risk of nanotechnology's use

amongst the public and other stakeholders including landowners (Bardos et al., 2014). Process based remediation techniques seen as "new" within a particular jurisdiction have historically encountered significant market barriers and required verified field based performance data to gain widespread regulatory and market acceptance. It is not unusual for such evidence to be demanded by regulators and landowners for specific conditions encountered or perceived in their country.

Given the heightened perception of potential risks from NPs in the environment, as well as the limited evidence base related to nZVI use in the field, particularly for its modified forms, it is likely that a higher burden of proof will be required by regulators prior to permitting of nZVI based *in situ* remediation techniques, compared with other *in situ* remediation techniques.

Although the establishment of nanoremediation market has been slow, there are a number of initiatives and development opportunities to expand its uptake. The use of nZVI instead of using micro/macro-scale Fe^0 materials could potentially eliminate the need for using PRBs and be more effective in both cost feasibility and contaminant remediation. Laboratory studies indicated that a wider range of chlorinated hydrocarbons may be dechlorinated using various nanoscale iron particles, including chlorinated methanes, ethanes, benzenes, and potentially, PCBs, some pesticides, heavy metals, and dyes (EPA, 2007).

Several factors play a role in determining a nanoscale iron product's reactivity, including particle size, reactive surface area, the presence or absence of hydrogenation catalysts (e.g., palladium), the method of manufacture, the morphology of the particle (porosity), its crystalline structure, impurities and coating, and whether or not particles have been exposed to acid washing (Dupont Chemicals, 2007).

nZVI can be distributed into the subsurface using a variety of carrying fluids; water, nitrogen gas, and vegetable oil being the most commonly used. Slurries of water and nZVI powder can be injected into the contaminated zone using nitrogen gas as a carrier. This helps the iron powder dispersal in the subsurface and creates contact between contaminants and iron. Alternatively, nZVI can be mixed with vegetable oil and water to create an emulsion, which is then injected into the contaminant zone (ESTCP, 2006).

Bare nZVI is composed of a core, which consists primarily of zerovalent or metallic iron while the mixed valence (i.e., Fe (II) and Fe (III)) oxide shell is formed as a result of oxidation of the metallic iron. Iron typically exists in the environment as iron (II)-and iron (III)-oxides, and as such, ZVI is a manufactured material. Thus far, applications of ZVI have focused primarily on the electron-donating properties of nZVI. Under ambient conditions, nZVI is fairly reactive in water and can serve as an excellent electron donor, which makes it a versatile remediation material (Stumm and Morgan, 1996). According to the core-shell model, the mixed valence iron oxide shell is largely insoluble under neutral pH conditions and may protect the ZVI core from rapid oxidation. A report by Tratnyek and Johnson (2006) stated that the greater reactivity that is often ascribed to NPs can be the result of larger overall surface area, greater density of reactive sites on the particle surfaces, and/or higher intrinsic reactivity of the reactive surface sites. Together, these factors have produced three operationally distinct results for nZVI: i) degradation of contaminants that do not react detectably with larger particles of similar material (e.g., PCBs); ii) more rapid degradation of contaminants that already react at useful rates with larger particles (e.g., chlorinated ethylene); or iii) more favorable products from contaminants that are rapidly degraded by larger materials, but that yield undesirable byproducts (e.g., carbon tetrachloride) (Tratnyek and Johnson, 2006).

Elemental iron slowly oxidizes to ferrous iron and releases two electrons. These electrons begin to function in a variety of reactions that lead to the transformation of target contaminants as shown in Figure 1. It is intended that toxic contaminants (e.g., TCE, trichloroethylene) would be reductively dechlorinated to an essentially non-toxic mixture of ethane, ethene, and acetylene. nZVI works best under anaerobic conditions, especially in the case of beta-elimination reactions. Decreased dissolved oxygen (DO) and oxidation-reduction potential levels (ORP) ($\leq$ -400mV) are ideal conditions to facilitate target contaminant degradation (USEPA, 2005b). ORP depends on the transfer of electrons between chemical species and determines the reduction potential of an aqueous solution. Like pH, the reduction potential represents an intensity factor. Reduction potential measurement is useful as an analytical tool in monitoring changes in an aqueous system rather than determining their absolute value.
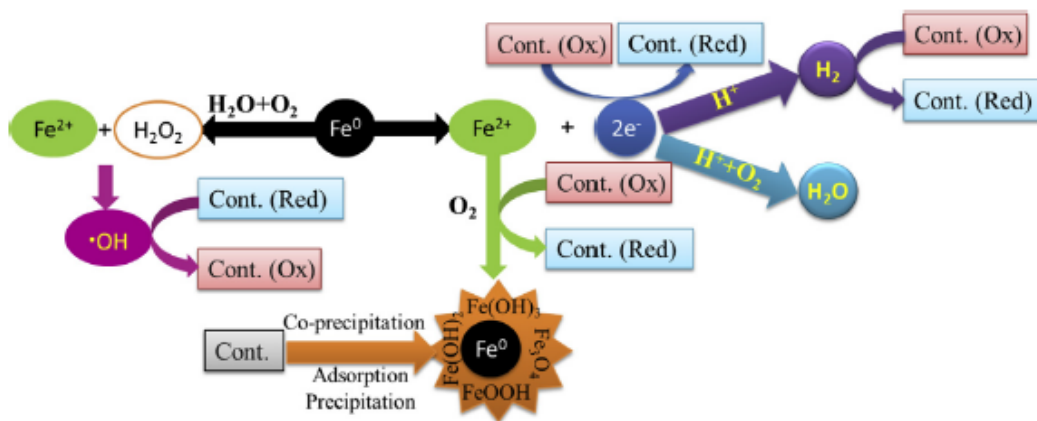
Figure 1 Illustration of the major reactions occurrring in the Fe^0/H$_2$O system and the mechanisms of contaminants removal (Reproduced from Guan et al., 2015)

With regard to the interaction between NPs and biota, it was observed that some NMs that enter animal tissues may be able to pass through cell membranes or cross the blood-brain barrier. This may be a beneficial characteristic for such uses as targeted drug delivery and other disease treatments, but could result in unintended impacts in other uses or applications. Inhaled NPs may become lodged in the lung or be translocated, and the high durability and reactivity of some NMs raise issues about their fate in the environment (Lockman et al., 2008). At present, not enough information exists to assess environmental exposure for most engineered NMs. This information is important because governments will need a sound scientific basis for reducing/limiting/avoiding unforeseen future impacts resulting from the introduction of NPs and NMs into the environment. A NMs risk assessment work sheet by the DuPont Chemical Company in 2007 stated that toxicity, human hazard, and environmental fate and effects data available from potential suppliers of nZVI are highly variable in both quality and completeness. It also stated that, in many cases, it is not clear from the information provided whether the Environmental Health and Safety (EHS) data are based on nZVI or on larger sized iron powder. Typical warnings include: i) Skin irritation; ii) Eye irritation; iii) Harmful if inhaled - irritating to mucous membranes and upper respiratory tract; iv) may be harmful if swallowed. Large doses will produce a laxative effect; v) Toxicological properties have not been thoroughly investigated; vi) Dissolved iron and manganese concentrations in groundwater may increase. nZVI that is released into the air in the form of particulate matter could cause the first three aforementioned effects (i-iii). Particulate matter triggers an acute inflammatory response in lungs, thus stimulating the secretion of cytokines, chemokines, reactive oxidation species (ROS), and transcription factors (Gwinn and Vallyathan, 2006). Naturally occurring and engineered NPs can remain airborne over a long

period because of the small size and light weight (Biswas and Wu, 2005). Therefore, an increased likelihood that they will travel long distances, and interact with gases and other airborne particles is possible. When considering the fact that most NPs are suspended in some type of liquid, surfactant medium, or pumped directly into the soil and water for contaminated groundwater remediation purposes, this may not be an important factor. Several studies are currently available on nZVI (eco) toxicological effects (Bardos et al., 2014; Libralato et al., 2016), but just few studies took into consideration field concentrations and the effects on environmental matrices post-treatment.

Currently, the advantages of nZVI over mZVI for aqueous contaminants removal are as follows (Comba et al., 2011): (1) some aqueous contaminant species that have been proven unsuccessful for remediation using mZVI can be effectively removed using nZVI because of its large reactive surface area, (2) nZVI can be used for more rapid degradation of contaminants, (3) the formation of some undesirable by-products during remediation using mZVI can be avoided by using the more reactive nZVI, resulting from a more complete degradation of pollutants, and (4) injecting nZVI to the source of contamination is believed to be faster and more effective for groundwater treatment than either the pump-and-treat or PRB methods (EPA, 2008).

Despite the potential for the use of manufactured NPs, there are still particular concerns that must be addressed in regard to the effectiveness, application and safety of this new technology. These issues include i) the mobility of NPs under subsurface conditions; ii) the kinetics and products of contaminant degradation by nZVI; iii) whether the nZVI maintains its reactivity during the time period of treatment; iv) human (occupational exposure) and environmental health (fate and effects of nZVI after the treatment).

1.1 Tools in nanoremediation

Nanoremediation is supported by a growing number of nFe^0 based engineered NPs including bare nZVI, bimetallic NPs associated nFe with other elements and Fe NPs coated with various biopolimers. The first work about nZVI was published by Wang and Zhang (1997) reporting an efficient method of synthesizing nanoscale iron and palladized iron particles and evidences about their use for the elimination of TCE as shown in Figure 2. The first pilot tests about the use of nZVI were conducted in the summer of 2000 in Trenton (USA) for the purification of groundwaters contaminated with TCE, tetrachloroethylene (PCE), vinyl

chloride (VC), and chloroform. Approximately, 1.7 kg of modified Pd/nZVI was applied at an average concentration of 1.125 g/L. After four weeks of remediation a 96.5% reduction of chloroorganic contaminants was obtained (Elliott and Zhang, 2001).
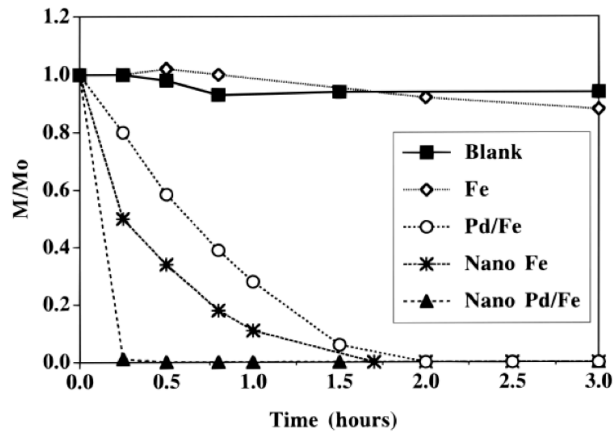

Figure 2 Degradation kinetics of TCE using commercial Fe powders (Fe), Pd-modified commercial Fe powders (Pd/Fe), nanoscale Fe particles (nFe), and nanoscale Pd/Fe particles (nano Pd/Fe). Initial TCE concentration was 20 mg/L; metal to solution ratio was 2 g/100 mL (Reproduced from Whang and Zang, 1997).

1.2 Environmental behavior

The environmental behavior of NPs and NMs is controlled by their intrinsic physico-chemical properties, the specific modifications of native NPs to allow for their desired uses and/or limit unwanted environmental impacts and their overall interactions with master environmental variables such as pH, ionic strength, redox conditions and composition of relevant environmental matrices (Park et al., 2016; Stefaniuk et al., 2016). The main use of nZVI being for the remediation of contaminated soils and groundwater (Lefevre et al., 2016), most research is currently devoted to understand their behavior in these two matrices. Indeed, contrary to the unwanted release of other NMs, nZVI is purposely introduced into the environment to react with contaminants. Understanding the reactions that nZVI will undergo under variable (realistic) scenarios is therefore necessary to maximize the efficiency of the intended remediation process while limiting uncontrolled long-distance transport and adverse effects to biota.

The complexity of real environmental settings and the specificity of contamination at various sites still prevent a unifying model of nZVI fate in the environment. However, available knowledge highlights some points of general validity that will serve to foster further

responsible use of nZVI. NMs in the environment can undergo four main types of transformation (Lowry et al., 2012): chemical, physical, biological and transformations following interactions with macromolecules. These reactions control persistence, reactivity and bioavailability of NMs in the environments. It is noteworthy that some transformations (e.g., dissolution and release of toxic cations) will increase the environmental mobility and toxicity of metallic NMs, while other processes (e.g., adsorption onto natural organic matter) will have opposite effects. ZVI NPs are often specifically modified to increase their dispersion in the environment, limit their aggregation and slow down passivation in order to guarantee maximum efficiency for the intended remediation purposes at a specific site (Stefaniuk et al., 2016). However, the mechanism of contaminant removal by nZVI involves in itself the loss of the nano-character of nZVI (Peeters et al., 2016, Noubactep, 2010). The commonly accepted mechanisms by which nZVI remove contaminants is via the oxidation of Fe(0) to Fe(II) with the concomitant reduction of the contaminant. Noubactep (2010) also points out the importance of adsorption processes following these initial steps by highlighting that Fe(II) is unstable under typical environmental conditions and tends to further oxidize to Fe(III) which then precipitates in the form of oxides or oxyhydroxides. As a consequence, the natural tendency of Fe(0) to oxidize to Fe(II), which is part of the cleaning action of nZVI, leads to the progressive loss of its nano-character. Apart from this behavior which is essentially linked to the intrinsic properties of nZVI (even in the presence of modifications), nZVI mobility can be further modified by specific environmental factors.

Dong et al. (2016) examined the effects of pH and fulvic acid concentration on the stability and reactivity of nZVI (synthesized using borohydride reduction) in a background electrolyte solution of 1 mM NaCl, which was considered as representative of groundwater conditions. An increase in pH from 5 to 7 units resulted in a faster sedimentation of nZVI, indicating enhanced aggregation. On the other hand, the sedimentation at pH 9 was similar to what was observed at pH 5. The effect of pH follows from the related changes in particle surface charge which varied from positive (pH = 5) to neutral (pH 7) and, eventually, negative (pH 9). The presence of particle charges augments the inter-particle repulsive forces, thus increasing the stability of the nZVI suspension. However, aggregation was observed at all the examined pH values with about 80% of the nZVI particles having aggregated at pH 5 and 9 (almost 100% at pH 7) after 60 min from the preparation of the suspensions. Addition of increasing concentrations of fulvic acids slowed down the aggregation kinetics at pH 5 and 7, but not at pH 9. In any case, even in the presence of 10 mg/L of fulvic acids, about 70% of the particles

had aggregated after 60 minutes at pH 7. An increase in ionic strength also seems to favor nZVI aggregation as documented by studies showing an increased retention of nZVI in laboratory experiments with packed sand columns (Raychoudhury et al. 2012; Lin et al., 2010). Liu et al (2016) also examined the behavior of freshly prepared nZVI under anoxic and oxic conditions over 72 h. Most importantly, nZVI remained stable over 72 hours indicating that, in field situations, transport into anoxic zones may enhance the environmental persistence of nZVI. Considering that nZVI is most effective under anoxic conditions, joint studies on process efficiency, environmental behavior and interactions with biota in such conditions are granted.

The effect of the presence of humic acids (HA) in groundwater can vary depending on the type of surface modification of nZVI. Adsorption of HA to nZVI was shown to enhance the stability of nZVI coated with polycarylic acid (PAA), but to favor aggregation of nZVI modified with Tween-20 or starch (Dong and Lo, 2013). However, as already pointed out for pH, the eventual trend of nZVI to aggregate remained visible in both presence and absence of HA. Thus, after an incubation time of 60 minutes, about 80% of PAA coated nZVI had aggregated in the absence of HA and about 60% in the presence of 20 mg/L HA. On the other hand, in similar experimental conditions, only 20 % of the starch-coated nZVI aggregated in the presence of HA compared with the lack of significant aggregation in the absence of HA (Dong and Lo, 2013). In the case of nZVI modified with carboxylmethyl cellulose (CMC), a HA concentration of 1 mg/L reduced aggregation by about 40%, but HA concentrations of 5 and 10 mg/L did not have any major effect on the NPs' stability (Jung et al., 2014). The interactions between HA and CMC-modified ZVI can be further modified by the presence of electrolytes with the actual effects depending on the specific combinations of HA and electrolyte concentrations (Jung et al., 2016).

Comparison of nZVI behavior in artificial versus real-world matrices suggests that nZVI has a strong tendency to aggregate (i.e., to become less mobile) in ultrapure water, forest spring water and landfill leachate, although the distribution of the aggregates and the kinetics of their formation can be somewhat variable (Peeters et al., 2016). The aggregation behavior of nZVI was not markedly changed by the addition of a dispersant (tetramethylammonium hydroxide – TMAH) although TMAH increased the stability of FeO NPs to a variable extent depending on the tested matrix (Peeters et al., 2016). The relative constancy of nZVI behavior is noteworthy considering the ranges of pH (5.5–8.0 units), conductivity (0.055–6800 μS cm^{-1})

and total organic content (<0.005–760 mg L^{-1}) covered by the three matrices. These observations are a promising for the definition of a general behavioral pattern of nZVI under realistic operating conditions. Park et al (2016) suggest two general patterns for the environmental mobility of engineered NMs in porous media: a favorable pattern leading to NP immobilization and an unfavorable pattern conductive to increased mobility. Besides the master parameters examined above, Park et al. (2016) highlight that the final environmental behavior of nZVI will also be linked to the soil hydraulic properties and specific site characteristics. Considering that the most typical application of nZVI is injection into soils and/or groundwater, it is also important to understand how far the injected nZVI can travel after its reaction with the soil matrix under variable *in situ* conditions (Lefevre et al, 2016). The actual injection technology will determine the mobility of nZVI during the operational phase of the remediation, but it is desirable that the injected nZVI will remain immobile once delivered to the intended place of action. In the absence of unwanted environmental migration (which may occur under anoxic conditions enhancing the stability of nZVI (Liu et al., 2016), nZVI will gradually transform into iron minerals that should remain immobile in the soil matrix (Zhao et al., 2016).

2. (Eco)toxicology

2.1 Bacteria

Ecotoxicity can be caused by direct nZVI association with biological components, oxidative stress compounds generated by nZVI, and membrane permeability. Kim et al. (2010) studied the mechanism of *Escherichia coli* (Figure 3) inactivation by nZVI by monitoring ROS (Figure 3). Using fluorescence assay [3' -(*p*-aminophenyl) fluorescein (APF) and 3'-(*p*-hydroxyphenyl) fluorescein (HPF) (Invitrogen)], they concluded that the bactericidal activities of nZVI under de-aerated conditions involved the generation of intracellular oxidants, such as $^-$OH or Fe(IV) produced by the reaction with H_2O_2 or other reactive species, resulting in serious damage to cell membrane integrity and respiratory activity.
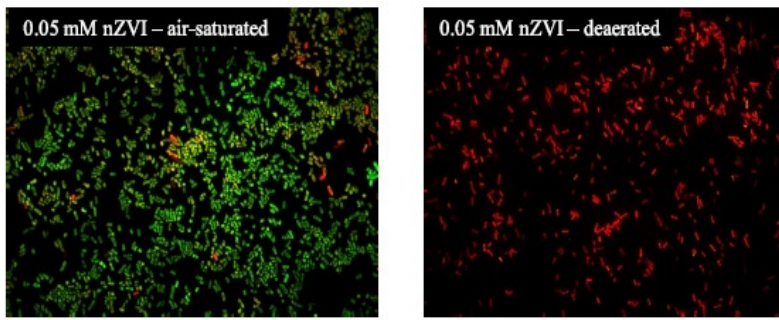
Figure 3 Microscopic images of BacLight Live/Dead staining for *E. coli* inactivated by nZVI (Reproduced from Kim et al., 2010).

Liu et al. (2014) studied the influence of nZVI on nitrate removal by the coccoid *Paracoccus sp*. (Figure 4) known for its NO_3^- reducing ability. They compared the nitrate removals using nZVI-free cells and nZVI-amended cells under both aerobic and anaerobic conditions. Under aerobic conditions, 66.92% of nitrate was removed by free cells while addition of nZVI (50 mg/L) into cells promoted nitrate removals with an efficiency of 85.2%. They associated this increase with the formation of H_2 produced by iron corrosion, which could be used as an electron source for free cells. However, as nZVI concentration increased, the removal efficiency decreased. This was due to the fact that the Fe^{2+} concentration increased with increased nZVI concentration, leading to increased Fe^{2+} toxicity to cells. Similar results were obtained under anaerobic conditions (Liu et al., 2014).
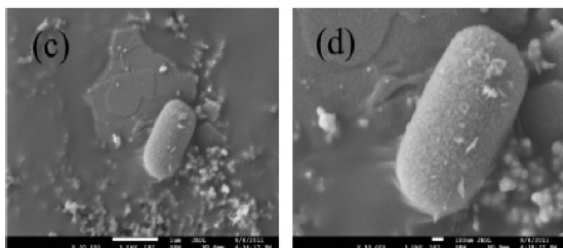

Figure 4 (c) and (d) Scanning electron microscopic images of *Paracoccus* sp. strain YF1 treated with 50 mg/L of Fe NPs suspension, (c) 20000x and (d) 50000x (Reproduced from Liu et al., 2014).

Yang et al. (2013) studied the potential negative impact of nZVI on anaerobic digestion methanogenesis resulting in its partial inhibition due to cell integrity disruption. The inhibition was coincident with the fast hydrogen production and accumulation due to nZVI dissolution under anaerobic conditions with a methane reduction of > 20%. Conversely, other authors (Kirschling et al., 2010; Xiu et al., 2010) found a stimulation of sulfate reducing bacteria (SRB) and methanogen growth in TCE contaminated aquifer materials. Marsalek et

al. (2012) highlighted the presence of inhibitory effects of nZVI on the photosynthetic activity of cyanobacteria because of rapid coating of Fe and Fe(III) hydroxide onto their cells. As shown in Figure 5, a green-colored dispersion of untreated cyanobacteria at an ecologically relevant concentration of 250 μg/L of cyanobacteria was stable for 24 h, while an identical dispersion treated with nZVI (50 mg/L) after 24 h reaction appeared clear and free of live cyanobacteria (Marsalek et al., 2012). The decomposed biomass containing heavy Fe(III) hydroxide settled down and scanning electron microscopy (SEM) analysis of filtered and air-dried sediment indicated that the reaction product was not a nano-sized material (Marsalek et al., 2012).

Figure 5 Picture obtained immediately after the dispersion of nZVI (50 mg/L) in cyanobacteria cells suspension (left) and after 24 h treatment (right), demonstrating the decomposition and gradual sedimentation of the cyanobacteria biomass (Reproduced from Marsalek et al., 2012).

2.2 Macrophytes and soil organisms

Macrophytes are essential components of our ecosystem, regulating the carbon and nitrogen cycles and constituting an important food source for humans and wildlife. Impact assessment of nZVI used for land remediation must then consider the potential effects on plants. Libralato et al. (2016) investigated the effects of nZVI on *Lepidium sativum, Sinapis alba* and *Sorghum saccharatum* up to concentrations used in field activities (50 g/L) and also compared the effects of nZVI with those of ionic- and micro-sized iron. Results reported no significant adverse effects even at 50 g/L of mZVI and nZVI, while some effects were evidenced for the ionic form. Biostimulation effects occurred at higher levels of nZVI. Microscopic analysis of

S. saccharatum roots (Figure 6) exposed to nZVI revealed the presence of black spots and coatings, but, apparently, no uptake by tissues (Libralato et al., 2016).
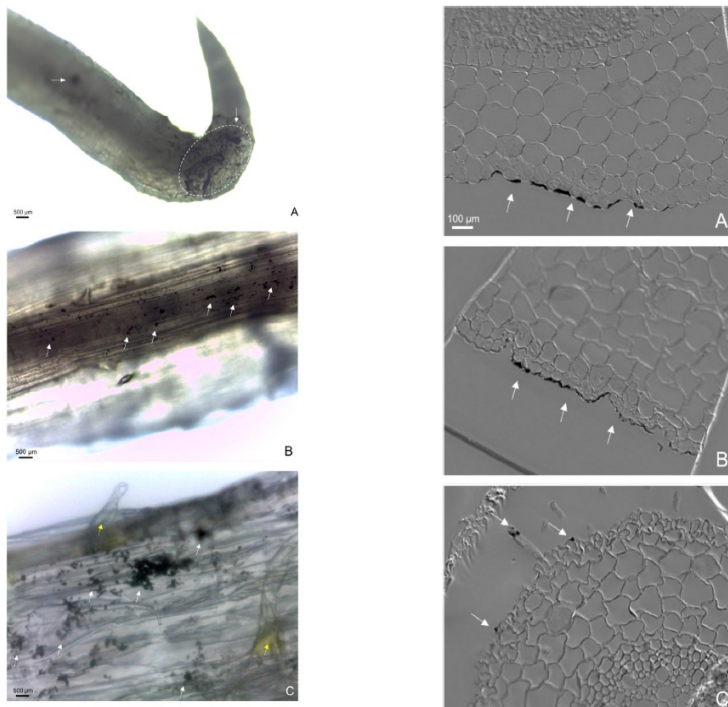

Figure 6 On the left, bright-field micrographs illustrating root apex of *S. saccharatum* treated with 992 mg/L of nZVI for 72 h at 25 °C. White arrows indicate nZVI aggregates, while yellow ones root hairs. On the right, differential interference contrast images of unstained, transverse sections of leaf (A), shoot (B) and root (C) of *S. saccharatum* treated with the same concentration at the same condition. White arrows indicate the presence of nZVI deposits/agglomerates/precipitates (Reproduced from Libralato et al., 2016).

Similar results were highlighted by Ma et al. (2013) considering *Typha latifolia* and hybrid poplars. However, in contrast with Libralato et al. (2016), they reported a drastic reduction in *T. latifolia* weight and shoot height after four weeks of exposure to 1 g/L of nZVI (nominal concentration). El-Temsah et al. (2016) showed the effect of two differently synthesized types of nZVI on barley and flax. Adverse effects were observed on nZVI-B (produced by precipitation with borohydride) while stimulatory or no effects were detected on nZVI-T treated organisms (produced by gas phase reduction of iron oxides under H_2 - NANOFER 25S, NANOIRON, Czech Republic), stating that the way a NM is synthetized can greatly influence its toxicity. Wang et al. (2016) assessed the effects of nZVI on rice up to 1 g/L. nZVI induced visible iron deficiency chlorosis and significant inhibition of seedlings growth.

nZVI transferred to the rice seedlings caused the cortex tissue of root to be seriously damaged, inducing the inhibition of active Fe transported from the root to shoot.

Since nZVI is intended for use in soil, there is an evident need to evaluate the potential toxicity of Fe NPs on soil biota both to preserve soil ecosystem and prevent the elimination of organisms useful in contaminants' degradation. Among soil organisms, earthworms play a key role in terrestrial ecosystems by recycling organic matter and mineral nutrients and maintaining soil structure (Blouin et al., 2013). El-Temsah et al. (2016) verified an increase of earthworm mortality (*Eisenia fetida*) after 14 d exposure to the solid fraction of soil slurry from 15% of negative controls up to 33% and 22% in soil treated with nZVI-T and nZVI-B, respectively. After 28 d, corresponding values were 46, 33 and 67% mortality, respectively. For soils treated in columns, no significant increase in mortality was observed for treatments with nZVI compared to controls. Significant reduction in earthworm body weight was observed only for nZVI-B. Other treatments showed higher body weights compared to controls. Reproduction was completely inhibited in both column soil and slurry soil after treatment with nZVI-B. No reduction in production of juveniles or cocoons in slurry soil was observed with nZVI-T, except for a 50% reduction of cocoons in column soil.

2.3 Human cells

The behavior of NPs in complex systems like the human body is quite unknown and a case-by-case basis survey is required (Gornati et al., 2015) considering also the results from Čábalová et al. (2015). They estimated the presence and quantity of micro- and nanosized particles and inter-individual differences in their distribution and composition in nasal mucosa obtained by mucotomy from patients with chronic hypertrophic rhinosinusitis (age 19 - 74, n = 6 including student and industrial worker). In all samples, NPs of different elemental composition were found with Fe being the most abundant element. Several studies indicated that ingested or inhaled NPs can cross biological barriers and migrate in small numbers to various organs and tissues (Lockman et al., 2004; Gornati et al., 2015) where they can potentially damage organ systems particularly sensitive to oxidative stress (OS). Gornati et al. (2015) carried out nZVI cytotoxicity and gene expression experiments on SKOV-3 (human ovarian carcinoma) and U87 (human glioblastoma) cells line investigating cell viability, cellular localization and uptake quantification by internalized metals. Results showed that nZVI are readily internalized by the cells (< 5%) (Figure 7). On gene expression, Fe NPs caused effects similar to their corresponding ions.

The small size and high-surface area of NPs, combined with their ability to generate reactive oxygen species (ROS) or release toxic metals at cell surfaces, may contribute to their final toxicity. To identify the mechanism through which nZVI can damage cells, Keenan et al. (2009) carried out a series of experiments considering nZVI in phosphate-buffered saline (PBS) solution exposed to oxygen in the presence and absence of human bronchial epithelial cells. Results evidenced a production of oxidants causing lung irritation. Ferrous iron (Fe^{2+}) is converted to ferric iron (Fe^{3+}) leading to oxidative damage over longer time periods. Further studies were carried out by Phenrat et al. (2009) about the effects of four ZVIs on rodent cells like cultured microglia (BV2) and neurons (N27) (Figure 8) including fresh (bare) nZVI, oxidized nZVI ("aged") and two poly-aspartate surface-modified (SM) nZVI (SML, laboratory generated; MRNIP, commercially available product). They conducted this experiment to examine the possibility that nZVI or its by-products could produce OS-mediated neurotoxicity. Intracellular H_2O_2 generated from the oxidative burst was produced in response to both fresh nZVI and "aged" nZVI. Depolarization of the mitochondrial membrane, an index of apoptosis, occurred only in response to fresh nZVI. Ultrastructurally, nZVI produced a perinuclear floccular material and cytoplasmic granularity. Reductions of intracellular ATP affected the microglia in the following rank order: fresh nZVI > "aged" ZVI > SM ZVI NPs.
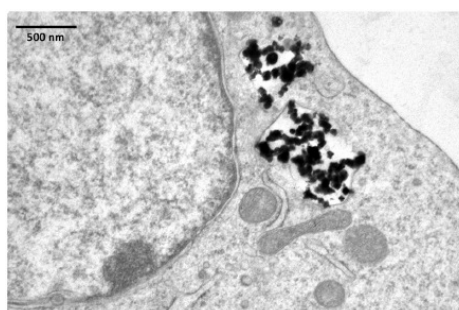

Figure 7 Transmission electron microscopy picture of SKOV-3 cells exposed to nZVI NPs. Inside the cell, nZVI is localized inside vesicles but not in the cytoplasm or in nucleus and mitochondria (Reproduced from Gornati et al., 2015)
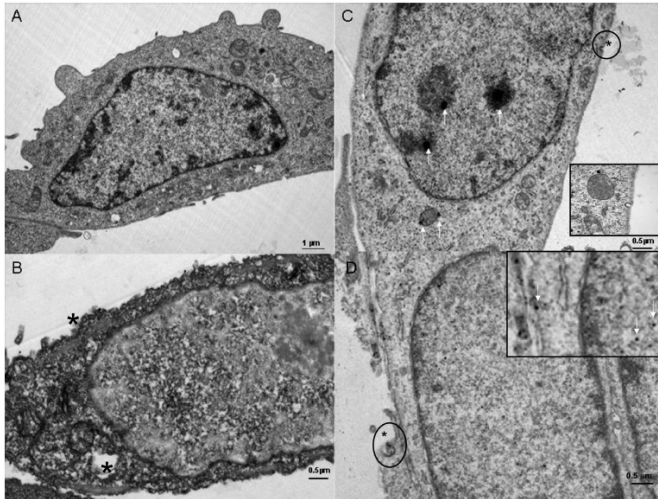
Figure 8 Nuclear chromatin of control N27 neurons displayed a high contrast appearance and ultrastructurally normal cytoplasm (A). In contrast, the cytoplasm of N27 neurons exposed to fresh nZVI (1 ppm, 3 h) appeared darker and more condensed, and displayed a fibrillar material around the cell's internal membrane (asterisks) and a perinuclear distribution of floccular material (B). The cytoplasm of N27 neurons, treated with either laboratory-generated SML-nZVI (C) or MRNIP (D) (1ppm, 3 h) appeared relatively normal ultrastructurally, although ZVI agglomerates (~200-500 nm) and single NPs were noted in the nuclei and cytoplasm in response to both treatments (C and D, insert). Invaginations of the neuron's cell membrane, suggestive of clathrin-lined endocytotic vesicles, were also noted in both SM-nZVI treated samples (C and D, circles, asterisks) (Reproduced from Phenrat et al., 2009).

3. Outlook

This overview provided a basic knowledge about the discussion on hurdles for the commercialisation of nZVI, case study applicability, potential authority acceptance considering that the environmental marketplace is driven and constrained by regulations and laws, supporting funding for controlled pilot and field projects, clarifying the potentiality of using these new technologies because of their inherent risk and largely unknown effects. Only a few studies to date have investigated the potential for nZVI to pose an environmental risk, including a very small number of toxicity and ecotoxicity researches of nZVI's potential to cause adverse effects in laboratory studies. This is to substitute one set of risks (*i.e.* site contamination) by another set of risks (potential risk from nZVI application) in terms of "risk–risk trade-off" illustrating the need to thoroughly analyze technology options before

their full-scale introduction and commercialisation. In particular, such analyses should be performed at the early stages of development on the basis of the precautionary principle. In the perspective of life cycle impact analysis, factors such as persistence, bioaccumulation and toxicity, the Best Available Technology, and the use of zero-emission and zero-discharge approaches should be considered.

Given the expected benefits of using nZVI as an environmental remediation option and the largely unknown risks of its use, it is imperative that environmental engineers, scientists and decision makers are made fully aware of these benefits, risks, and uncertainties. Research needs also concern some of the challenges that traditional quantitative risk assessments face as a decision support tool under extreme conditions of uncertainty for ENMs and the need to better anticipate potential environmental risks of nZVI. These aspects are important given the early stage of use and development of nZVI, in which identified potential risks may still have the possibility to be mitigated or managed during the technology's maturing and development process while also aiming to reap the benefits of its use as an environmental remediation option.

Further assessment is surely required to fully understand nZVI environmental risk considering the fact that most studies are currently only laboratory based. Although some common patterns on nZVI environmental behaviour and interactions mechanisms with biota are emerging, site-specific conditions and organism-specific characteristics still remain important variables in real-world applications. Further guidance on how to comprehensively and transparently make decisions regarding nZVI use in relation to the potential risks for human and environmental health therefore remains a priority.

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