



Review

Performance and toxicity assessment of nanoscale zero valent iron particles in the remediation of contaminated soil: A review

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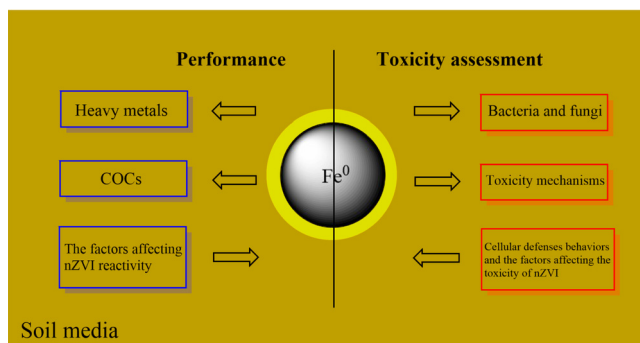
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HIGHLIGHTS

- The immobilization mechanisms of heavy metals by nZVI were summarized.
- Reaction behaviors of chlorinated organic compounds associated nZVI were summarized.
- The biological toxicity of nZVI in soil was reviewed with critical analysis.
- Future challenges in application of materials in soil remediation are discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanoscale zero valent iron (nZVI) particles have been studied in recent years as a promising technology for the remediation of contaminated soil. Although the potential benefits of nZVI are considerable, there is a distinct need to identify possible risks after environmental exposure to nZVI. This work firstly introduced the remediation of nZVI for heavy metals and chlorinated organic compounds in contaminated soil. And the corresponding stabilization mechanisms were discussed. We also highlighted the factors affecting nZVI reactivity, including nZVI surface area, nZVI stabilizers, soil pH, soil organic matter and soil types. In addition, this review shows a critical overview of the current understanding of toxicity of nZVI particles to soil bacteria and fungi. The toxicity mechanisms, cellular defenses behaviors and the factors affecting the toxicity of nZVI were summarized. Finally, the remaining barriers to be overcome in materials development for environment application are also discussed.

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1. Introduction

As a result of improper industrial discharge, waste disposal, mining tailings and stockpiles, there is a wide range of soil contaminants and it has become a critical issue worldwide, considered as a major barrier to sustainable human development (Wall et al., 2015; Vilardi et al., 2018a). Heavy metals and toxic organic compounds constitute the majority of the prevailing soil contaminants (Cheng et al., 2016; Ding et al., 2017; Huang et al., 2017a), which hardly degrade and may accumulate in soils, and will pose risks and hazards to ecosystem and humans (Huang et al., 2008; Suominen et al., 2014). Consequently, there is an imperative to restore contaminated soils.

Extensive work has been performed for the development of soil remediation techniques (Kumpiene et al., 2017; Sivaram et al., 2017; Vilardi et al., 2017a; Zulfiqar et al., 2017). Among these treatment techniques, nanoremediation strategies have been studied in recent years as a promising technology for the remediation of contaminated soil (Gil-Díaz et al., 2016). Recently, nano-scale zero-valent iron (nZVI) particles have attracted much concerns. Due to the high reactivity and large specific surface area, nZVI can transform various contaminants such as toxic heavy metals (Gil-Díaz et al., 2017), chlorinated organic compounds (COCs) (El-Temsah and Joner, 2013), and inorganic compounds (Vilardi et al., 2017b; Vilardi and Palma, 2017) to low toxicity or inert compounds. In the soil system, the toxicity of metals mainly depends upon their speciations, including acid soluble fraction, reducible fraction, oxidizable fraction and residual fraction (Fajardo et al., 2015). It is more easy for heavy metals in non-residual fractions to be mobilized by biotic and abiotic changes (e.g. the changes of soil organic matter and pH), which has higher bioavailability with potential pernicious influences on ecosystem health (Fonti et al., 2015). Therefore, an effective remediation strategy should be provided by transforming the unstable fractions of heavy metals to stable form. At present, few review was paid on the variation of heavy metal speciation in soil under nZVI application. In addition, although nZVI particles for the degradation of several organic contaminants have been studied and are typically promising for chlorinated organic compounds (COCs) (Gomes et al., 2015;

Varanasi et al., 2007), limited work has been done to study the reaction behavior of COCs contacted with nZVI in contaminated soil. Moreover, in many fundamental ecosystem processes, as key players, microorganisms are firstly exposed to nZVI particles (Kocur et al., 2016). Some studies have confirmed the negative impacts of nZVI on many microorganisms, particularly for the gram-negative bacteria (Fajardo et al., 2013; Saccà et al., 2014b). Despite the environmental concerns, the potential negative effects of nZVI on organisms are still poorly known (Fajardo et al., 2015; Saccà et al., 2014a), and most studies have only investigated the short term toxic effects of nZVI on bacteria. Consequently, it is necessary to understand the toxicity effect of nZVI on soil microorganism.

The aims of the present study are to: (i) summarize and discuss the heavy metals immobilization mechanisms by means of nZVI in soil matrix; (ii) summarize and discuss about the chlorinated organic compounds/nZVI reaction mechanisms; (iii) discuss the factors affecting nZVI reactivity in soil; (iv) summarize and discuss the possible nZVI toxicity effects on soil bacteria and fungi; (v) discuss the toxicity mechanisms, cellular defenses behaviours and the features influencing the nZVI toxicity; (vi) propose the future challenges regarding the material development for environmental restoration.

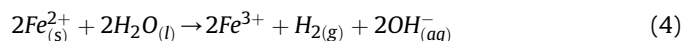
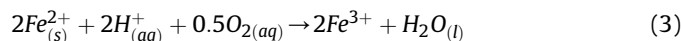
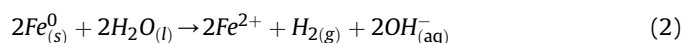
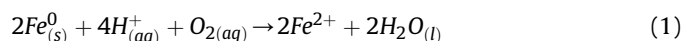
2. Performance of nZVI in the remediation of contaminated soil

As described above, the speciations of heavy metals were divided into four forms (Wei et al., 2016). The acid soluble fraction of heavy metals is composed by the most mobile metal species, thus characterized by the highest toxicity and bioavailability. The reducible and oxidizable fraction could be transformed to more readily available form when the redox potential changes implying a potential toxicity of these forms. The residual fraction (i.e. the metals in primary and secondary minerals) of metals is quite stable (Fonti et al., 2015; Huang et al., 2016). Based on this theory, the remediation strategies designed to promote the transformation of heavy metals from unstable fraction to stable fraction become a promising technique. To make a better understanding of heavy metals stabilization by nZVI in soils, the possible immobilization mechanisms of heavy metals are discussed in the following section.

Moreover, the soil contamination with COCs is gradually serious in the last years. nZVI has been shown to successfully degrade a large number of COCs. However, most of the researches are focused on the degradation of COCs by nZVI in groundwater or wastewater. More research is needed to study the reaction behavior or reaction mechanisms of COCs/nZVI in contaminated soil.

2.1. Immobilization mechanism of heavy metals by nZVI

The dimensions of the nZVI particles are less than 100 nm, the form of nZVI is mostly composed of a Fe^0 core and an iron oxide shell (Vilardi et al., 2017c). Iron oxides were formed *via* corrosion of the iron core by water and oxygen during the nZVI synthesis process with a thickness of 2–4 nm (Mu et al., 2016), which include goethite ($FeOOH$), wüstite (FeO), magnetite (Fe_3O_4), maghemite (γ - Fe_2O_3) and hematite (α - Fe_2O_3). The pathway to form iron oxides can be explained by the following equations (Wang et al., 2005):

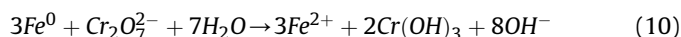


The iron oxide shell enables sorption, surface complexation metal ions and plays an important role in contaminants removal and immobilization with nZVI (Mu et al., 2016). However, nZVI tends to agglomerate to micro-sized particles because of its magnetic attraction, causing the rapid loss of soil mobility and reactivity (He and Zhao, 2005; He et al., 2007; Xue et al., 2018). So, various stabilization techniques have been studied. The most used methods of nZVI modification consist of admixtures of other metals with nZVI, surface modification of nZVI, and immobilization of nZVI onto supports, Fig. 1 presents an illustration about the methods of

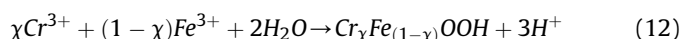
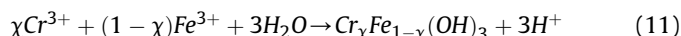
modification of nZVI.

Chromium commonly enters the soil environment through anthropogenic activities, including wood processing, metal corrosion inhibition, leather tanning, and pigment production (Xu and Zhao, 2007). Chromium has two different states in soil: the immobile form, Cr(III), and the more mobile Cr(VI) forms, as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$). While Cr(VI) form is a dangerous species and human carcinogen (Megharaj et al., 2003). Some studies have demonstrated that Cr(VI) could be decreased “in situ” and precipitates as an insoluble hydroxide compound by directly adding a suspension containing stabilized nZVI particles in soil (Di Palma et al., 2015; Singh et al., 2012; Wang et al., 2014). Wang et al. (2014) investigated the mobility of Cr(VI) in soil with the treatment of carboxymethyl cellulose (CMC) stabilized nZVI and the results showed that acid soluble Cr(VI) was converted to relatively stable speciations, thus enhancing the immobilization of Cr in soils. Xu and Zhao (2007) confirmed that stabilized nZVI could convert all leachable Cr(VI) to its less toxic form Cr(III) in Cr-contaminated sandy soil and the highly effective reduction of chromate was found following pH range of 5.0–9.0. In addition, Singh et al. (2012) observed that the remediation rate of Cr(VI) in soil reached 99% with the reduction and immobilization by nZVI (5 g/L) after 40 days of treatment. nZVI can reduce Cr^{6+} to Cr^{3+} and Cr^{3+} can be immobilized into $Cr(OH)_3$ by precipitation or form into alloy-like $Cr^{3+}-Fe^{3+}$ hydroxides by incorporation existing in the iron (hydr)oxide shell. Related reactions occurred between Cr and nZVI are presented as follow:

(1) Reduction of Cr^{6+} to Cr^{3+} :



(2) Formation of mixed $Cr^{3+}-Fe^{3+}$ hydroxides:



At later reaction times, further electron may not be transferred from the Fe^0 core to Cr^{6+} considering $Cr^{3+}-Fe^{3+}$ hydroxides form on the surface layer of oxidized nZVI. Consequently, the reduced chromium does not undergo re-oxidation.

Regarding arsenic (As), current studies have confirmed that the employment of iron oxide nanoparticles or zero valent iron (ZVI) is effective in soil samples (Gil-Díaz et al., 2017; Shipley et al., 2010). The addition of nZVI significantly decreased the As concentration in more available fractions and increased As in the residual fraction in brownfield soil (Gil-Díaz et al., 2017). Gil-Díaz et al. (2014) demonstrated the effectiveness of nZVI for reducing the availability of As in highly As-contaminated soil. Results from the sequential extraction procedure indicated a significant reduction of As in more available fractions after treatment by stabilized nZVI. Waychunas et al. (2005) investigated the adsorption mechanisms of As by nano iron oxides in the soil, and demonstrated that surface complexation (co-precipitation) was the main mechanism for immobilization of As by nano iron oxides. Similar mechanisms have been confirmed by other studies (Vítková et al., 2017; Zhang et al., 2010). Scorodite ($FeAsO_4 \cdot 2H_2O$) was predicted as a potential mineral phase for As in soils amended with nZVI (Vítková et al., 2017).

Cadmium (Cd)-contaminated soil has obtained great concern owing to its potential to cause various disorders such as liver, kidney damage and cancer even in low concentration (Huang et al., 2018; Khan et al., 2017; Li et al., 2016). In our previous studies, we demonstrated that most acid soluble fraction and oxidizable

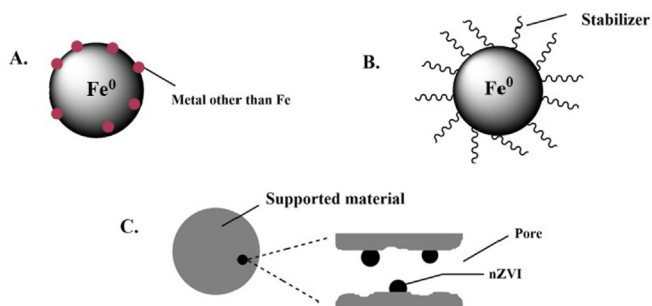
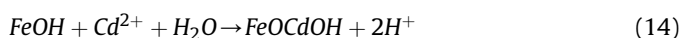
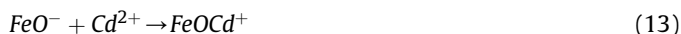


Fig. 1. The schematic presentation of nZVI modification methods. (A) Other metal doping. (B) Surface modification of nZVI. (C) nZVI deposition on the support.

fraction of Cd were converted to residual fraction (the maximum residual percentage of Cd increased from 15.49% to 57.28% after 30 days of incubation at 0.1 wt% sodium alginate (SA)-modified nZVI) (Huang et al., 2016). Similar to the metal ion adsorption on Fe(III)(oxyhydro) oxide, this surface oxide layer provides surface sites for coordination with Cd. Li et al. (2017) suggested the use of zeolite supported nZVI (Z-nZVI) for Cd immobilization from soil and found that the acid soluble Cd in soil was significantly reduced by 30% with the treatment of 30 g/kg Z-nZVI. Muehe et al. (2013) demonstrated that Cd was mainly associated with iron, showing that Cd was complexed or sorbed with Fe(III) minerals. The surface reactions of Cd immobilization by nZVI can be explained according to the following equations:



As for various metals, the metal-nZVI interactions can be summarized as:

1. Reduction-Cr, As, Cu, U, Pb, Ni, Se, Co, Pd, Pt, Hg, Ag.
2. Adsorption-Cr, As, U, Pb, Ni, Se, Co, Cd, Zn, Ba.
3. Oxidation/reoxidation-As, U, Se, Pb.
4. Co-precipitation-Cr, As, Ni, Se.
5. Precipitation-Cu, Pb, Cd, Co, Zn.

Generally, nZVI-heavy metals removal mechanism can be described through one or a combination of the processes reported above. For example, Vilardi et al. (2018b) reported that adsorption and reduction were the removal mechanism of Cr(VI) by nZVI in polluted water. However, soil is a very complex system, because composed of solid, liquid and gas phases. The complex nature of soil systems means that the reduction and oxidation/reoxidation between metal and nZVI are hard to occur. The possible mechanisms involved in nZVI-induced heavy metals immobilization are summarized in Fig. 2. Few studies have investigated kinetics of metal removal by nZVI from soils. Cao et al. (2018) demonstrated that pseudo-first-order model was better for describing the kinetic processes of metal washing from soils by most washing agents, but a small part of agents were still fit the pseudo-second-order kinetic model. Generally, the pseudo-first-order was used to describe the initial kinetics process (Meng et al., 2017), while the pseudo-second-order suggested the whole process of chemical adsorption/desorption and complexation reactions on soil surface active sites. More research is needed to investigate the kinetics aspects of

nZVI application to contaminated soil.

2.2. Degradation of organic contaminants by nZVI

COCs, such as, trichloroethylene (TCE), tetrachloroethylene (PCE), polychlorinated biphenyls (PCBs) and many organochlorine pesticides are widely known chemicals that are highly toxic to human health and the environment (El-Temsah and Joner, 2013; He et al., 2010). nZVI has been shown to successfully degrade COCs and gained very promising results aiming at the development of remediation technologies to treat COCs-contaminated soils (El-Temsah and Joner, 2013). For example, Wise et al. (2009) studied the remediation of TCE contaminated soil using emulsified zero-valent iron. The results indicated that the TCE concentrations were below USEPA industrial soil standards. El-Temsah and Joner (2013) investigated the effect of nZVI on organochlorine insecticide DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane] degradation in soil. The results showed that 1 g nZVI/kg soil caused in 56% degradation of DDT after 7 d treatment. However, the degradation rate of DDT in water was higher than that in soil (El-Temsah et al., 2016). A similar phenomenon has also been confirmed by Wang and Zhang (1997) and Varanasi et al. (2007). Wang and Zhang (1997) found that the highest degrading rate of PCB reached 90% in aqueous solution, while Varanasi et al. (2007) showed that the degradation rate of PCB was low by 38% in soils. They concluded that the difference of reaction rate was the result of lower diffusion of PCB from soil particles to nZVI particles surface. The fact that PCBs are strongly bound to soil particles (sorbed on soil mineral surface or partitioned into organic matter) makes major challenge for applying nZVI for remediation of PCB-contaminated soils. Some studies have focused on employing nZVI with organic cosolvents simultaneously for remediation of contaminated soil (Chen et al., 2014; Gomes et al., 2015; Yu et al., 2016). Organic cosolvent could increase PCB desorption into the liquid phase, thus, enhancing the PCB dechlorination with nZVI. Yu et al. (2016) assessed the treatment efficiency of PCB in contaminated soil using bentonite clay modified nZVI (CZVI) and ethanol. Environmental data revealed that the dichlorination of PCB became more rapid as the ethanol fraction increased from 10% to 50%, due to the increasingly greater PCB desorption and thus facilitating the contact between PCB and CZVI. In addition, nZVI has been used to restore PCB-contaminated soil under high temperature conditions. Varanasi et al. (2007) investigated the dechlorination of PCB-contaminated soil over nZVI under a nitrogen atmosphere at 473–673 K with magnetic stirring and results showed that the increase of the temperature results in fast desorption of PCBs from soil contributing to a higher destruction rate. Because nZVI is ferromagnetic and can produce heat under electric field stimulation. Phenrat et al. (2015) reported that nZVI together with low frequency (150 kHz) AC electromagnetic field (AC EMF) thermally increased the desorption of TCE from soil media and enhanced the degradation rate of TCE up to 5.36 times compared with the absence of AC EMF.

Gomes et al. (2015) also found that lower nZVI consumption and the use of half of the voltage gradient could cause a higher dechlorination percentage of PCB in contaminated soil compared with the traditional electrokinetic setup. The remediation methods were employed to increase the contaminants desorption from soils and increased the contacts between the nZVI and chlorinated organic contaminants, thus, enhancing the dechlorination of COCs by nZVI (Fig. 3). The mechanism put forward to interpret the observed dechlorination process is as followed (Gomes et al., 2015; Wu et al., 2012):

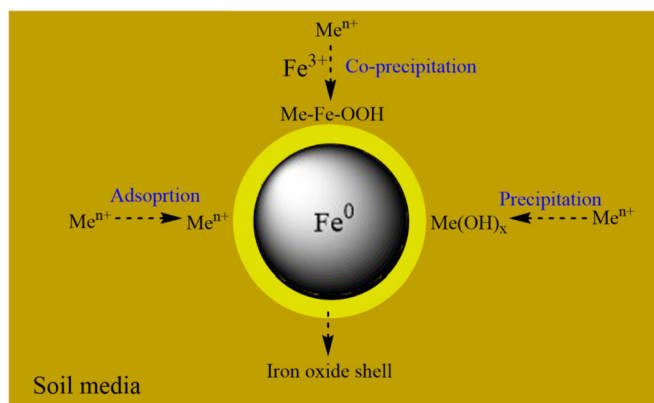
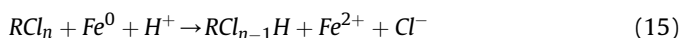


Fig. 2. The possible mechanisms involved in nZVI-induced heavy metals immobilization.

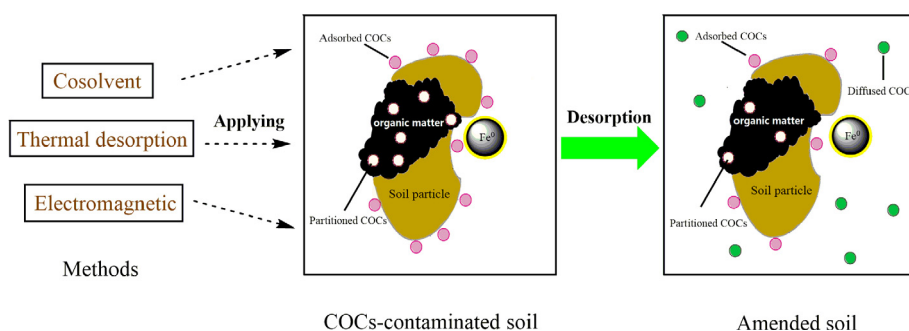


Fig. 3. A schematic illustrating the sorption/partition behavior of COCs and adhering characteristics of nZVI in soil.

The hydrogen atom involved in the reaction may be derived from organic impurities in the soil. This adventitious water can also be used as proton donor (Chuang et al., 1995). Aristov and Habekost (2010) studied on the dechlorination of PCBs in a flow system containing commercial iron powder with toluene and $\text{Ca}(\text{OH})_2$ as proton source. Dehalogenation was not observed without the addition of such a proton source. In addition, when adding a small fraction of a transition metal (e.g., Pd, Ni or Cu) into nZVI, the degradation rate of COCs can be greatly enhanced. For example, Chen et al. (2014) reported that the removal efficiency of PCBs in the soil with the treatment of Pd/Fe bimetallic nanoparticles was higher than using nZVI. He et al. (2009) studied the dechlorination of 2,2',4,5,5'-pentachlorobiphenyl contaminated soil using synthesized Pd/Fe bimetallic particles suspensions. There are two reasons for the positive effect of Pd on the dechlorination of pollutants: Pd can promote dechlorination by forming a strong Pd–Cl bonds, thus accelerating the dissociation of chlorinated hydrocarbons; The hydrogen gas generated during reaction process is sorbed on Pd and dissociated into atomic hydrogen, which the strongest reductants for the dechlorination reactions. Therefore, it is presumed that the dechlorination of 2,2',4,5,5'-pentachlorobiphenyl by Pd/Fe bimetallic probably happens on the surface of Pd. 2,2',4,5,5'-pentachlorobiphenyl molecules are adsorbed on the surface of Pd by forming a strong Pd–Cl bonds. The H atoms, formed on the surface of Pd, and the electrons released from iron move to the Cl atoms.

2.3. Factors affecting reactivity of nZVI with heavy metals and organic contaminants

Previous studies have shown that many factors affect nZVI reactivity with heavy metals and organic contaminants. Factors that may affect nZVI reactivity include surface area (Phenrat et al., 2015; Zhang et al., 2017), nZVI stabilizers (Wang et al., 2014; Zhang et al., 2011), soil pH (He et al., 2009; Vítková et al., 2017; Wang et al., 2012), soil organic matter (Vítková et al., 2017; Zhang et al., 2011), and soil types (El-Temsah and Joner, 2013; Zhang et al., 2011). These factors will be elaborated more specifically in the following sections.

2.3.1. Surface area

Zhang et al. (2017) investigated the effects of ordinary zero-valent iron (ZVI) and nZVI on the immobilization of Cr(VI) in spiked soil. Sequential extraction procedure showed that nZVI could significantly increase the proportion of residual Cr compared with ZVI. This phenomenon was attributed to the higher specific surface area of nZVI. Research shows that the dechlorination reactivity of nano-sized Fe/Pd is over 600 times better than commercial bulk Fe deposited with Pd (Fe metal size = 120 μm) (Wu

et al., 2012). Generally, the surface area of the nZVI was 15–34 m^2/g , at least 15 times larger than that of the ZVI (<1 m^2/g) (Wang et al., 2010; Zhang et al., 2017). Compared to ZVI particles, the larger surface area of nZVI exists generous of reactive sites for adsorption and reduction or complexation of contaminants thus increasing the removal rate (Gil-Díaz et al., 2017). However, due to the high surface area, nZVI particles tend to aggregate, leading to the decrease of reaction activity and mobility in soil and how to improve its stability is the key to field application.

2.3.2. nZVI stabilizers

Because agglomerated nZVI particles are usually at the micron scale level, which are essentially not deliverable or transportable in soils, thus, cannot be used effectively for in situ applications. To improve delivery and mobility of nZVI in soil, various particle stabilization methods have been developed (He and Zhao, 2005; He et al., 2007). He and Zhao (2005) and He et al. (2007) synthesized stabilized Fe/Pd nanoparticles modified by low concentrations of a starch or CMC. Results showed that the stabilized nanoparticles had remarkable soil mobility. Reddy et al. (2014) showed that the stabilizer lactate significantly increases the mobility of nZVI suspension (1 g/L and 4 g/L) in a sandy soil. Schrick et al. (2004) also studied that anionic, hydrophilic carbon and poly (acrylic acid)-supported nZVI particles could enhance the transport of nZVI in soils. This phenomenon was mainly due to the stabilizers improved the colloidal stability of nZVI suspensions and reduced the adhesion to the soil surface. And similar results were observed by Sirk et al. (2009). In addition, the reactivity of stabilized nZVI was higher than that of bare nZVI. Quinn et al. (2005) also showed that significant reductions in TCE soil concentrations (>80%) by a non-ionic surfactant sorbitan trioleate emulsified nZVI injection at NASA locations in 90 days. However, Wang et al. (2010) demonstrated that there exists competition for contaminant between the reactive sites of nZVI and the adsorption sites of stabilizers. But the loss of reactivity was not significant, and it must be weighed against the benefits supported by the stabilizers. These studies show that the reactivity of nanoparticles is dependent on particle characteristics and is an important consideration for remediation activities.

2.3.3. Soil pH

In terms of dominating metals transfer behavior in soil, the pH was a key parameter. Gil-Díaz et al. (2016) applied nZVI to remediate As contaminated soil (acidic and calcareous), and results showed that the As availability was lower in acidic soils than in calcareous ones. The pH affecting As immobilization can be explained by the reason: at pH below point-zero-charge, adsorption of H^+ is in excess of that of OH^- , the surface of becomes positively charged, the adsorption of arsenate anions is favored (Wang and Mulligan, 2006). Similar to As, Cr(VI) in soil is found as oxyanion and the presence of contaminants of an opposite charge

can increase its immobilization due to the increase of positive charge on the soil particles (Gil-Díaz et al., 2016). Zhu et al. (2017) demonstrated that the reduction rate of Cr(VI) could reach 99.84% with the treatment of nZVI/Ni bimetallic in the soil with a pH value of 5. In addition, previous studies have indicated that weak acidic condition was beneficial to the removal of PCBs from soil (He et al., 2009; Wang et al., 2012). He et al. (2009) studied on 2,2',4,5,5'-pentachlorobiphenyl transformation with the treatment of Pd/Fe in soils. Pd/Fe particles were added to the spiked soil samples with 2 mL deionized water and the removal efficiency of 2,2',4,5,5'-pentachlorobiphenyl increased more rapidly at pH value 5.5 than other pH conditions. This is mainly because the amount of H⁺ is enough to produce H₂ through the corrosion of iron, thus the reduction of 2,2',4,5,5'-pentachlorobiphenyl progresses more readily (see Section 2.2).

2.3.4. Soil organic matter

Adsorption of organic matter on nZVI surface is known to reduce its reactivity because of accumulation of humic acid or fulvic acid on the surface active sites of the nZVI interface with soil (Giasuddin et al., 2007). Zhu et al. (2017) demonstrated that humic acid had an inhibitory effect on the Cr(VI) reduction by nZVI/Ni bimetallic. Gueye et al. (2016) also found that an inhibitory effect on the Cr(VI) reduction by nZVI was generally shown in the presence of high level of organic matter in soil. A decrease of Cr(VI) reduction yield by nZVI from 91% to 12% was observed after 2 h of treatment in a soil containing 35.71 g/kg of organic matter. Zhang et al. (2011) studied the dechlorination of TCE in two different types of soil (a potting soil and Smith Farm soil) by CMC coated Fe/Pd nanoparticles. Under the same conditions, about 44% of TCE existed in the potting soil (organic matter content = 8.2%) was degraded within 30 h, however, about 82% of TCE was degraded in Smith Farm soil (organic matter content = 0.7%). In general, organic matter can inhibit the reactivity of CMC-coated nZVI from these two aspects. On the one hand, the CMC macromolecules sorbed on nZVI are loosely structured due to the large CMC molecular structure (molecular weight = 90,000). The attachment of the smaller OM molecules on nZVI particles may form a denser OM film, resulting in elevated mass and electron transfer resistance. Second, some OM molecules may compete with contaminants and/or its intermediates for the electrons on nZVI particles.

2.3.5. Soil types

Previous studies have shown that the degradation rate of chlorinated compounds in an aged soil is slower than the spiked soil because of its lower bioavailability. El-Temsah and Joner (2013) investigated the degradation rate of DDT in the spiked sandy loam soil and the historically contaminated soil with the treatment of nZVI, respectively. The degradation rate of DDT in the spiked sandy loam soil was higher than that in historically contaminated soil. Zhang et al. (2010) used nZVI to stabilize As in two representative As-contaminated soils (a sandy soil and a clay soil). The stability of As was higher on the sandy soil that has a lower iron content and higher initial leachability than on the clay soil that already has a high iron content. Because the higher iron content in soil could reduce the leachability and bioavailability of As (Yang et al., 2002). Chen et al. (2014) also reported that the lower the content of clay and the higher the content of sand, the higher the removal rate would be, because of less facile desorption of PCBs from clay particles. It can be found that the removal efficiency of contaminants was related to the leachability and bioavailability in different types of soils.

3. Toxicity assessment of nZVI

Nanoparticles surreptitiously enter the environment through water, soil, and air during various human activities. However, the application of nanoparticles for environmental treatment deliberately injects or dumps engineered nanoparticles into the soil or aquatic systems. This has resultantly attracted increasing concern from all stakeholders (Van et al., 2018; Yopasá Arenas et al., 2018). Studies also illustrated that nanoparticles have the ability to enter organisms during ingestion or inhalation (Abudayyak et al., 2017). Although some studies have also addressed the toxicological effects of nZVI on animal cells (Liang et al., 2018), plant cells (Gong et al., 2017), microorganism cells (Chaithawiwat et al., 2016), the toxicological studies with nZVI nanoparticles on soil bacterial and fungal species to date is still limited.

3.1. Toxicity studies on bacterial and fungal species

The advantages of nZVI like high reactivity, small size, may has potential fatal factors through causing adverse cellular toxic and detrimental effects to soil microorganism when it was used to study on the remediation of contaminated soil. The cytotoxicity of nZVI mainly includes damage to the cell membrane integrity, interference with respiration, and oxidative damage of DNA or enzymatic proteins by the generation of reactive oxygen species (ROS) (Xie et al., 2016). For the gram-positive/-negative bacteria and the fungus, they have different cellular structures and characteristics that their sensitivities to nZVI are also obviously distinct (Fajardo et al., 2012, 2013; Pawlett et al., 2013). At present, toxicity studies primarily based on *in vitro* assays (Huang et al., 2017b). Generally, *in vitro* toxicity assays were primarily used to detect and measure cell biological activity of various microbial species under nZVI application. Most researches have shown that nZVI has an adverse impact on viability, activity and cell integrity. nZVI with high concentrations has no any toxic effects on *Klebsiella planticola* (Gram⁻) or *Klebsiella oxytoca* (Gram⁻) (Fajardo et al., 2012; Saccà et al., 2013), but may produce toxic effect on *Bacillus nealsonii* (Gram⁺) (Fajardo et al., 2012). Fajardo et al. (2012) tested the cytotoxicity of the nZVI toward bacteria and results indicated that under the same experimental conditions, Gram-positive bacteria *Bacillus nealsonii* has a lower resistance to nZVI particles compared with Gram-negative bacteria *Klebsiella planticola* (Fig. 4 a and b). Fig. 4c shows the biological activity of the strains treated with nZVI. It was found that a slight decrease in the biological activity (O₂ mg/L) was observed only for the Gram-positive strain after 24 h of exposure to 10 mg/mL of nanoparticles. Fungal species appears to have higher tolerance to nZVI toxicity in comparison with bacterial species (Diao and Yao, 2009; Shah et al., 2010). When nZVI concentrations came up to 10,000 mg/L, there was no influence in the growth of *Aspergillus versicolor* (Diao and Yao, 2009), and although *Trametes versicolor* cellulolytic enzyme produced, their integrity was not affected deeply (Shah et al., 2010). The reason of the phenomenon was mainly attributed to the different cell wall constituents. The major constituent of the cell wall of Gram-positive bacteria is lipoteichoic acids, but the content of lipoteichoic acids is less in Gram-negative bacteria. And the discrepancy of bacteria cell wall structure may lead to their different absorption ability on the nZVI particles. Moreover, fungi have an extremely rigid chitinous cell wall as a barrier to decrease nZVI toxicity (Diao and Yao, 2009). It can be explained this phenomenon that fungal cells are more resistant to nZVI particles than that of bacteria. Representative studies about the effects of nZVI particles (either positive or negative effects) on soil bacterial and fungal species are summarized in Table 1.

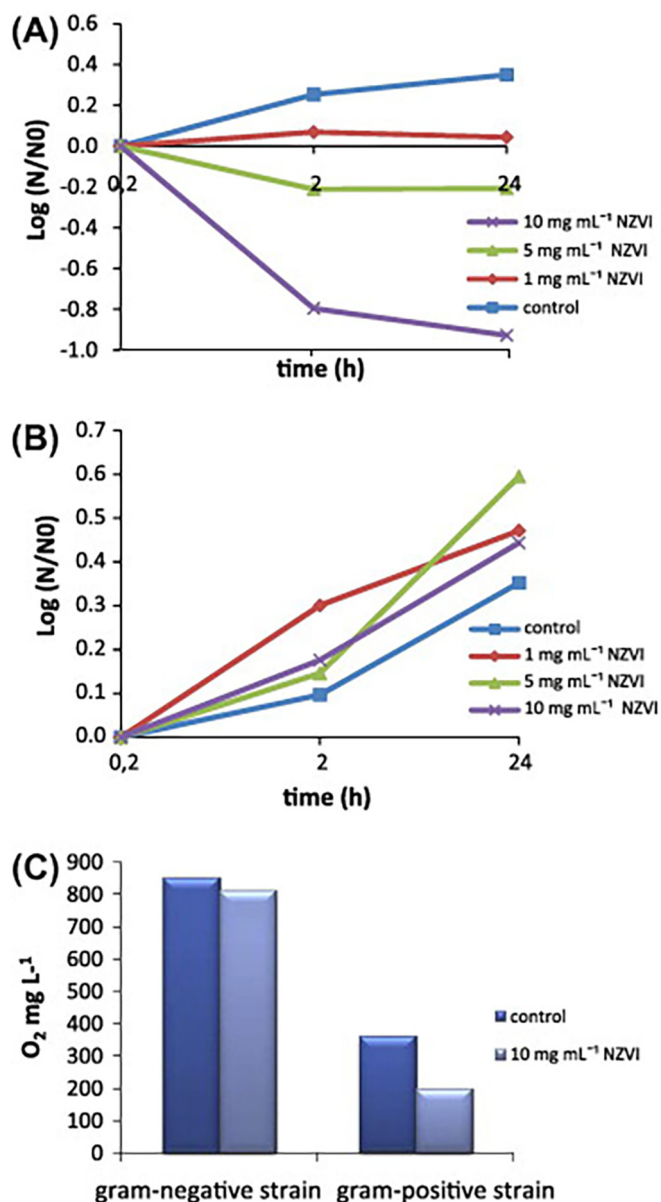


Fig. 4. Cell viability of the Gram positive strain *B. nealonii* (A) and Gram negative strain *K. planticola* (B) after exposure to nZVI. Biological activity (O₂ mg/L) of the strains (C) after 24 h of exposure to 10 mg/mL nZVI (Adopted from (Fajardo et al., 2012)).

3.2. Toxicity mechanisms

Many studies have shown that reactive oxygen species (ROS) and Fe²⁺ generated by nZVI, are probably the main mechanisms, damaging the cell intracellular structures and cell membrane, eventually causing the death of cells. The generation of ROS may be caused by nZVI particles directly or indirectly when they enter the cell. The nZVI particles were adsorbed to the surface of cells, and then started releasing Fe²⁺, increasing the concentration of Fe²⁺ around the cell and promoting the migration of ions into the membrane. Once internalized, Fe²⁺ can react with the hydrogen peroxide produced by mitochondria and form reactive oxygen species (such as OH•, O₂⁻, or FeO²⁺) and then further damage macromolecules including proteins, lipids, and nucleic acids, which ultimately lead to cell death. Saccà et al. (2013) used electron microscopy to investigate the surface morphology and the molecular

and cellular interaction between nZVI and *Klebsiella oxytoca* strain, isolated from the nZVI-treated soil. Results indicated that some bacteria are around nZVI particles, but the morphological of the cells shows no obvious change. The transmission electron microscopy (TEM) confirmed that nZVI was attached to the surface of bacteria and also showed nZVI penetrated some of the cells, damaging the bacterial membrane (Fig. 5d). Another possible toxicity mechanism is that nZVI can complex with lipoteichoic acids, the main components of the cell wall of Gram⁺ bacteria or anionic structures of bacterial cell walls, which can increase the precipitation of iron oxides in the cells. The processes can block the pores of the outer membrane of the cell and stop taking up nutrient from the outside environment (Diao and Yao, 2009). Diao and Yao (2009) found that the cell wall of the fungi *Aspergillus Versicolor* existed with iron oxide, but its growth was not affected. It may be due to the presence of chitin in the cell wall of fungi, acting as a shield to resist nZVI toxic effects (Diao and Yao, 2009; Pawlett et al., 2013; Shah et al., 2010).

3.3. Cellular defenses behaviors

Resistance mechanisms responses caused by bacterial or fungal cells to against nZVI toxicity are being unraveled. According to previous studies, the molecular approach is employed to provide a new insight to improve our understanding about the cellular homeostasis against nZVI. Changes in protein expression signals a molecular response set off by cells to against the nZVI effects, which was mainly by two main mechanisms: the restraint of membrane proteins to oppose iron uptake; the overproduction of proteins to scavenge ROS, thus decreasing intracellular oxidative stress (Saccà et al., 2014b). Saccà et al. (2014b) demonstrated that some membrane proteins and transport proteins were downregulated after *Pseudomonas stutzeri* was exposed to nZVI, which played an important role in nutrient and iron uptake, and the decrease in proteins abundance could reduce the availability of iron in intracellular and cause intracellular oxidative damage. A unique defense measure has been found in *Bacillus cereus*, one common gram-positive bacterium in the soil. When *Bacillus cereus* exposed to nZVI particles, an asymmetric septum was formed in cells, which was typical of an early forespore structure. Then, the cell rapidly entered the early spore formation stage, therefore, it could survive under the stressful circumstances and regulate the expression rate of specific proteins (Fajardo et al., 2013). According to Saccà et al. (2013), *Klebsiella oxytoca* may build up an adaptive stress response including indole and tryptophanase, which could be considered as a signal molecule. The overexpression of tryptophanase may show that the protein is participated in the *Klebsiella oxytoca* cellular adaptive process, capable of reducing and even offset the negative effects of nZVI. In addition, the activity of some common intracellular antioxidants, for example glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT), will also change in different levels when the intracellular ROS levels increase. For instance, catalase, an enzyme encoding by the *katB* gene, is involved in cellular oxidative stress detoxification. The *katB* occurred upregulation after *Pseudomonas stutzeri* was exposed to nZVI. The phenomenon indicated that an oxidative stress induced by H₂O₂ was participated (Saccà et al., 2014b). Fig. 6 summarizes the potential toxicity mechanisms of nZVI particles and the cellular defenses behaviors.

3.4. Factors affecting toxicity of nZVI

3.4.1. nZVI concentration and exposure time

Generally, the toxicity of nZVI to bacterial species increases with

Table 1
Review of the representative studies investigating the impact of nZVI particles on soil bacterial and fungal species.

Species	nZVI particles	NZVI concentrations	Incubation period and exposure condition	Effects	References
<i>Klebsiella planticola</i> (Gram ⁻)	nZVI (NANOFER 25S ^a); Size < 50 nm	1000–10,000 mg/L	Incubated for 0.2, 2 and 24 h, without shaking at room temperature	No significant changes were observed in viability and activity. Attachment of nZVI to cell surface. No significant cell damage. No change in gene expression levels.	(Fajardo et al., 2012)
<i>Bacillus nealsonii</i> (Gram ⁺)	nZVI (NANOFER 25S ^a); Size < 50 nm	1000–10,000 mg/L	Incubated for 0.2, 2 and 24 h, without shaking at room temperature	Slight and high toxicity at 5000 and 10,000 mg/L, respectively. Reduced metabolic activity. Cell content leakage.	(Fajardo et al., 2012)
<i>Bacillus cereus</i> (Gram ⁺)	nZVI (NANOFER 25S ^a); Size < 50 nm	1000, 5000 and 10,000 mg/L	Incubated at 30 °C for 0.2, 2, 24 and 48 h in the dark	Significant toxicity on cell. Viability decrease after 10 min and 2 h exposure, but no effect after 48 h exposure. No change in gene expression levels.	(Fajardo et al., 2013)
<i>Pseudomonas stutzeri</i> (Gram ⁻)	nZVI (NANOFER 25S ^a); Size < 50 nm	1000, 5000 and 10,000 mg/L	Incubated at 37 °C for 0.2, 2, 24 and 48 h in the dark	No significant change was observed in cellular viability. A well-defined cell wall. Upregulation of proteins in response to oxidative stress. Downregulation of membrane proteins.	(Saccà et al., 2014b)
<i>Klebsiella oxytoca</i> (Gram ⁻)	nZVI (NANOFER 25S ^a); Size: 80–120 nm	1000, 5000 and 10,000 mg/L	Incubated at 28 °C for 0.2, 2, 24 and 48 h in the dark	No bactericidal and negligible bactericidal effects. Around some bacterial cells, but no apparent morphological changes. Attachment to the cell surface and no significant cell damage. Overproduction of tryptophanase.	(Saccà et al., 2013)
<i>Escherichia coli</i> (Gram ⁻)	nZVI-B (made using precipitation with borohydride); Size: 20–100 nm nZVI-T (produced by gas phase reduction of iron oxides under H ₂); Size: <100 nm	1000 mg/L, 4000 mg/L	Mixed with 4.5 mL samples and incubated for 24 h at 37 °C, then transferred into Plate Count Agar (Biorad) and incubated for 48 h at 37 °C	Significant negative effects on bacteria were only found in the first two leachates. The highest nZVI-Toxicity towards <i>E. coli</i> was detected in the aqueous phase of the slurry treated with nZVI-B. nZVI-T showed no negative effects.	(El-Temseh et al., 2016)
<i>Pseudomonas putida</i> (Gram ⁻)	Home-made bare nZVI; Size: 50–100 nm	10 µg/L–1.0 g/L	Incubated at 30 °C for 20, 46, 72 h in aerated conditions	A significant mortality of <i>P. putida</i> cells occurred when they were exposed to nZVIs at concentrations as low as 100 µg/L. The negative responses were not detectable after sustained exposure of the nanoparticles to oxygen.	(Ortegacalvo et al., 2016)
<i>Arbuscular mycorrhizal fungi</i>	Home-made bare nZVI; Size: 10 ± 5.2 nm	270 µg Fe/g dry weight soil	Incubated at 25 °C for 4 h.	A potential reduction of <i>arbuscular mycorrhizal</i> fungi were presented.	(Pawlett et al., 2013)
<i>Aspergillus versicolor</i> (Ascomycetous Fungi)	Home-made bare nZVI; Size: 20–30 nm	100, 1000, 10,000 mg/L	5 min exposure, shaking at room temperature	No toxic effect. Yellow-brown coating observed on cell surface.	(Diao and Yao, 2009)
<i>Trametes versicolor</i> (Basidiomycetous fungi)	nZVI (Sun innovation, Inc., USA); Size: 2–60 nm	0.1 mM Fe ⁰	Incubation at 25 °C for 42 days, shaking at 100 rpm under daylight	Decrease of cellulolytic enzymes production significantly. No effect on growth.	(Shah et al., 2010)

^a NANOFER 25S: nZVI coated with sodium polyacrylic acid with a percentage Fe⁰ of 70–90% (NANOIRON s.r.o., Czech Republic).

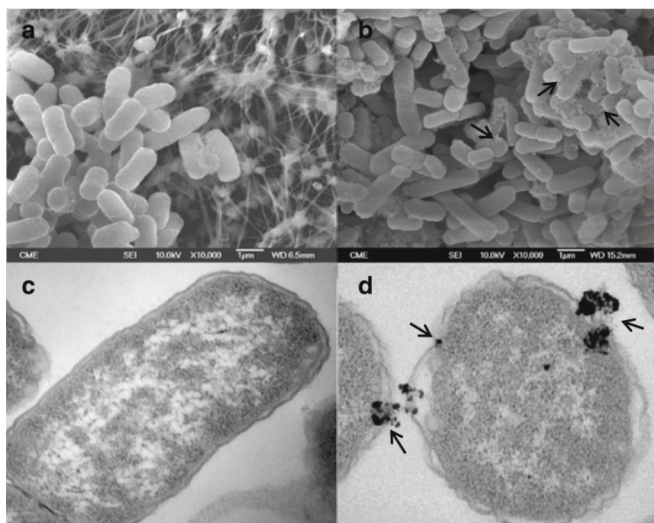


Fig. 5. a). SEM micrograph of untreated *K. oxytoca* cells. b). SEM micrograph of *K. oxytoca* bacteria treated with 5 mg/mL nZVI for 3 h. c). TEM micrograph of non-treated *K. oxytoca* cells. d). TEM micrograph of *K. oxytoca* bacteria treated with 5 mg/mL nZVI for 3 h. nZVI are pointed out by black arrows (Adopted from (Saccà et al., 2013)).

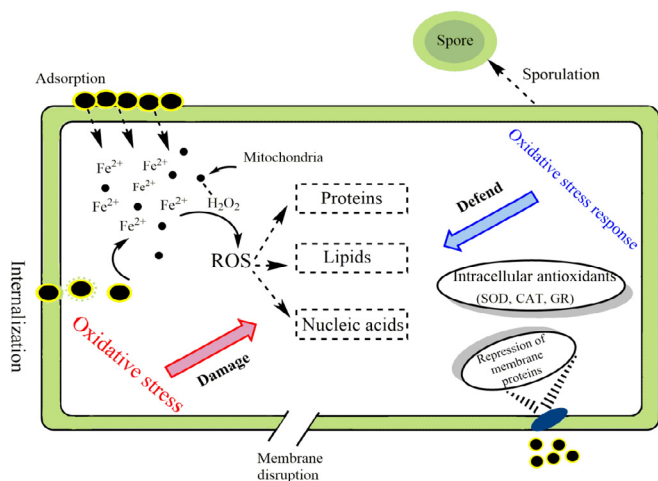


Fig. 6. Simplified diagram highlighting the potential toxicity mechanisms of nZVI particles and the cellular defenses behaviors.

the increase of nZVI concentration (Fajardo et al., 2012). Some researchers have found that high concentration of nZVI was less toxic to microorganisms (Fajardo et al., 2013; Saccà et al., 2014b). Fajardo et al. (2013) investigated the effect of nZVI toxicity on microorganism *Bacillus cereus*. nZVI concentrations were 1,000, 5000 and 10,000 mg/L and the environmental medium was the sandy soil. Results showed that 10,000 mg/L nZVI was less toxic to *Bacillus cereus* than 1000 mg/L after 2 h, and the toxic effects of nZVI could be observed only at low concentrations. In addition, Saccà et al. (2014b) reported that nZVI was toxic to soil bacterium *Pseudomonas stutzeri* only at concentrations from 1000 to 5000 mg/L compared with 10,000 mg/L. These observations could be interpreted by the reason that higher nZVI concentrations may result in more aggregation of nZVI and increased the particle size, thus, decreasing its reactivity and anti-bacterial.

Exposure time has an important relationship with the structure of nZVI particles (Yan et al., 2013). The increase of reaction time of

nZVI, promotes the formation of iron oxides, and the iron oxide layer has a great effect on the particle activity which consequentially may decrease agglomeration and biological toxicity (El-Temsah and Joner, 2013). For example, toxic effects of nZVI were observed only within 10 min on *Pseudomonas stutzeri* and reduce after extended react times (Saccà et al., 2014b). *Bacillus cereus* cells exposed to nZVI particles showed significant cytotoxicity after 10 min or 2 h treatment at all concentrations. Instead, after 24 h exposure, only lower concentration induced a viability decrease, and no toxicity was observed after 48 h (Fajardo et al., 2013). With above study, nZVI oxidation is likely to induce the reduce or loss of the toxicity of nZVI than aggregation.

3.4.2. Surface modification and soil organic matter

Although most toxicity studies are carried out on bare nZVI particles, nZVI are usually modified by surface stabilizers in situ remediation, and the surface stabilizers used to enhance the stability of nZVI have also been investigated to regulate nZVI toxicity. The surface stabilizers coated on nZVI surface can afford electrostatic, steric, or electrosteric repulsion (Dong et al., 2016a; Li et al., 2010). Electrostatic and steric repulsion, called as electrosteric repulsion are supported by large or low molecular weight polyelectrolytes. Large uncharged polymers can produce a steric repulsion by restraining the close contact between nanoparticles and cells (Fig. 7) (Li et al., 2010). Electrosteric repulsions offered by the negatively charged polymer coatings can reduce the adhesion between the bacteria cell wall and nZVI, thus reducing the toxicity of nZVI particles (Kirschling et al., 2010; Xie et al., 2016). Kirschling et al. (2010) investigated the microbial population changes in soils when the presence of bare and polyaspartate modified nZVI after 250 days. They demonstrated that nZVI had no negative effects on microbial population, however, polyaspartate coated nZVI stimulated the native microorganisms, causing a positive impact on the application of nZVI in the environment. CMC, a typical polyelectrolyte, is generally used as stabilizer to modify nZVI and able to reduce the damages of cell membrane integrity or decrease the oxidative stress response (Pawlett et al., 2013). This may be due to the CMC posed as hydroxyl radical scavenger, preventing the cell from oxidative stress (Zhou et al., 2014). In addition, the modification of nZVI by other metals (such as Ni, Ag) can increase its toxicity. It is reported that Ni may lead to the decline in cell growth. For Fe/Ag nanoparticles, each of them has antimicrobial activity at least to a certain extent (Xie et al., 2016). Soil organic matter has also been confirmed to reduce the toxicity of nZVI (Navarro et al.,

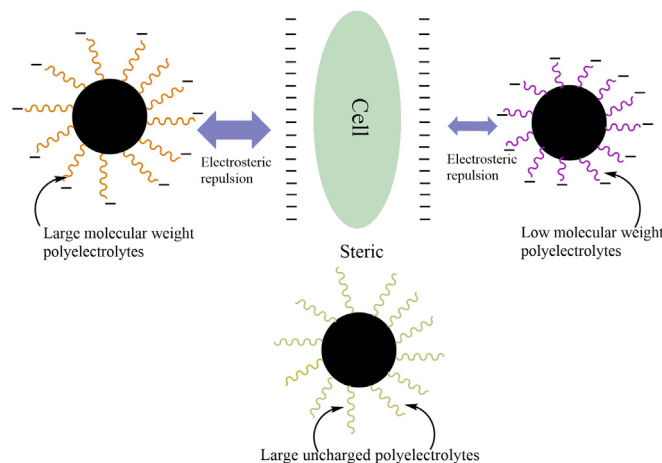


Fig. 7. The inhibited physical contact of cell with nZVI modified with different surface stabilizers.

2008). Pawlett et al. (2013) investigated the impacts of nZVI on soil arbuscular mycorrhizal fungi and microbial biomass of Gram-negative bacteria in three different types of soil. It was demonstrated that the inhibitory effect of nZVI on microorganisms was directly dependent on the soil which has more organic matter content. Therefore, the type of stabilizer and the existence of organic matter are also factors considered when assessing nZVI toxicity.

3.4.3. nZVI ageing

Upon entering the environment, the core of nZVI would be oxidized when it reacted with oxygen or water. nZVI could completely or partially convert from Fe⁰ to iron oxides/hydroxides (Dong et al., 2016b). Compared to fresh nZVI particles, aged nZVI has a decreased redox activity and oxidative pressure, and may affect its inherent toxicity (El-Temsah and Joner, 2012). The Fe-oxides formed on nZVI surface restrict adhesion to the cells by decreasing agglomeration. El-Temsah and Joner (2012) reported the toxic effects of fresh and aged (30 day) nZVI at concentrations of 500 mg/kg of soil on two species of earthworms (*Eisenia fetida* and *Lumbricus rubellus*, respectively). Significant reduction of toxic effects in the aged nZVI treated soil was found. Fajardo et al. (2015) investigated the changes of soil microbial populations after exposure to aged nZVI and there was no obvious changes in soil bacterial cell number. In addition, aged nZVI could also maintain cellular metabolism and promote cellular respiration by acting as an electronic conduit for some microbial populations in the soil (Kato et al., 2010). Kato et al. (2010) suggested the soil *Geobacter* species could grow preferentially via utilizing iron oxides for their respiration.

4. Conclusions and future challenges

In this review, the reactions between the nZVI particles and soil contaminants (heavy metals and chlorinated organic compounds) were summarized, and the factors affecting nZVI reactivity were discussed. Furthermore, the toxicity effects of nZVI particles on soil bacteria and fungi were highlighted. The toxicity mechanisms, cellular defenses behaviors and the factors affecting the toxicity of nZVI were also summarized. Although nZVI has a great potential for the remediation of contaminated soil, the studies in soil remediation field are currently in the preliminary stages and further studies need to go forward. There exist at least the following challenges:

- (1) The potential of nZVI particles have been revealed for immobilization or transformation of pollutants in soil, based on numerous data. However, the subsurface mobility has been weakened because of quickly aggregation and precipitation of nZVI, and thus nZVI particles are transported to the pollutants and the reductive function of nZVI becomes ineffective. So, it is important to systematically understand the surface modifications of nanoparticles in boosting the practicability in environmental technology. In addition, it has been demonstrated that polyelectrolytes modification could increase the stability of nZVI particles, but the active sites on nZVI surface may be decreased. Further modifications may need to increase the number of active sites for contaminant removal.
- (2) There are many pollutants existing in the environment, but only a small part of them, mainly including organic pollutants and common heavy metals, can be discovered. However, in the complex system, the simultaneous and selective removal of multi-components and certain contaminants were rarely reported. Moreover, the investigation of the mechanism of the interaction between multi-pollutants and

nZVI particles are basically blank. And although nZVI can effectively stabilize heavy metals, heavy metal will be released with a long time and nZVI still exist in soil.

- (3) Several studies have shown that organic polymers coated nZVI particles reduced the toxicity of nZVI to microorganisms. However, most toxicity studies have been performed *in vitro*, so it is not possible to directly associate the effect of nZVI on microbial populations. Attention should be paid to the effects of organic polymers coated nanoparticles lead to the biostimulation of the native soil microorganisms.
- (4) Numerous studies on nZVI have been undertaken in the past 20 years. However, the potential threats are rather poorly recognized in the application of modified and unmodified nZVI to different matrices. It is necessary to consider not only the toxicity of nZVI during remediation of contaminated soil and the serious consequences of its application when conducting the research.
- (5) The nZVI-based technologies have been developed a lot, many of which are still in an experimental or pilot stage. Practically speaking, it is imperative to demonstrate the practicability of nZVI large-scale applications, rather than under laboratory conditions, and to demonstrate that they are effective, safe and low-cost.

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