EHT Environmental Health and Toxicology

Open Access

Volume: 29, Article ID: e2014022, 9 pages
http://dx.doi.org/10.5620/eht.e2014022

• Special Topic

eISSN: 2233-6567

Potential environmental implications of nanoscale zero-valent iron particles for environmental remediation

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Objectives Nanoscale zero-valent iron (nZVI) particles are widely used in the field of various environmental contaminant remediation. Although the potential benefits of nZVI are considerable, there is a distinct need to identify any potential risks after environmental exposure. In this respect, we review recent studies on the environmental applications and implications of nZVI, highlighting research gaps and suggesting future research directions.

Methods Environmental application of nZVI is briefly summarized, focusing on its unique properties. Ecotoxicity of nZVI is reviewed according to type of organism, including bacteria, terrestrial organisms, and aquatic organisms. The environmental fate and transport of nZVI are also summarized with regards to exposure scenarios. Finally, the current limitations of risk determination are thoroughly provided.

Results The ecotoxicity of nZVI depends on the composition, concentration, size and surface properties of the nanoparticles and the experimental method used, including the species investigated. In addition, the environmental fate and transport of nZVI appear to be complex and depend on the exposure duration and the exposure conditions. To date, field-scale data are limited and only short-term studies using simple exposure methods have been conducted.

Conclusions In this regard, the primary focus of future study should be on 1) the development of an appropriate and valid testing method of the environmental fate and ecotoxicity of reactive nanoparticles used in environmental applications and 2) assessing their potential environmental risks using *in situ* field scale applications.

Keywords Ecotoxicity, Environmental fate, Environmental remediation, Nanoscale zero-valent iron particles

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Received: October 30, 2014
Accepted: November 18, 2014
Published online: December 18, 2014

This article is available from: http://e-eht.org/

Introduction

Nanomaterials have attracted considerable attention due to their excellent electrical, optical, magnetic, and catalytic properties [1]. In particular, efforts to use nanotechnology in environmental applications have been grown steadily. Nanoscale zero-valent iron (nZVI) particles are one of the most widely used nanoparticles for environmental remediation because of their ability to degrade a wide range of contaminants [2-4]. Such an increasingly widespread application of nZVI will lead to its release into the environment, and this release is likely to bring about unexpected hazards in various organisms [5]. A variety of nZVI toxicity mechanisms toward organisms have been investigated and the results indicate that toxicity might be caused by 1) direct nZVI association with biological components [6], 2) oxidative stress compounds generated by nZVI in the aqueous phase [7] or

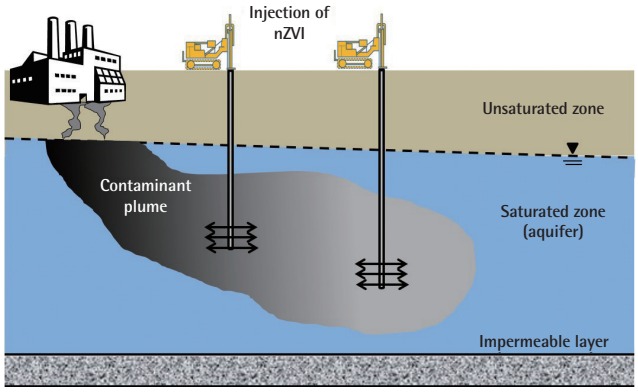

Figure 1. Application of nanoscale zero-valent iron (nZVI) for *in situ* remediation.

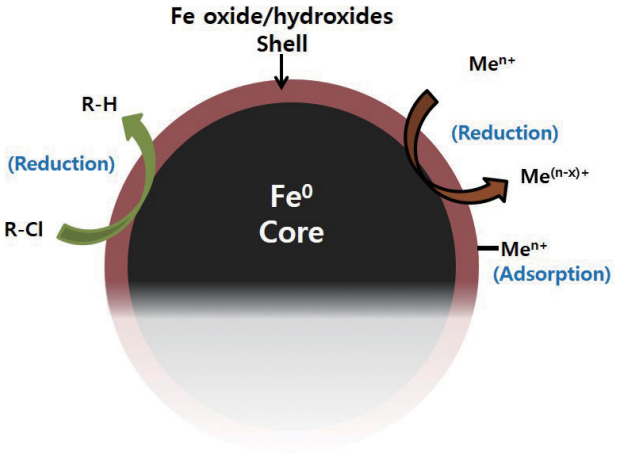

Figure 2. Schematic structure of nanoscale zero-valent iron and the main reaction mechanisms with environmental contaminants. Fe, iron; R-Cl, chloroalkane; R-H, hydrocarbon; Me^{n+}, metal ions.

3) ferrous ion released from nZVI followed by the fenton reaction [8]. Despite the numerous studies that have been conducted on nZVI toxicity, there is a critical gap in our knowledge on the environmental risks of nZVI, including ecotoxicity as well as the fate and transport of nZVI in natural and engineered environments. For this reason, we present an overview of current research findings related to the environmental implications of nZVI particles. We also provide insight into current knowledge gaps and give pointers for future research directions.

Environmental Applications of Nanoscale Zero-valent Iron Particles

Macro-scale zero-valent iron (ZVI) has been recognized as a good electron donor with a property to release electrons in aquatic environments [2]. ZVI has been used as a reactive material in subsurface permeable reactive barriers to degrade groundwater pollutants since the early 1990s [3]. ZVI is very active in transforming of halogenated compounds, polychlorinated hydrocarbon pesticides and dyes [4].

The nZVI has significantly increased available reactive surface areas compared to larger sized iron particles, which consequently enhances contaminant degradation reactions [9,10]. Moreover, one benefit of nZVI is the ability to inject it directly into a contaminated aquifer [11]. Therefore, the use of nZVI to remediate soil and groundwater has increased within the last decade. Figure 1 illustrates a schematic diagram of the applications of nZVI for site remediation.

Figure 2 presents a schematic structure of nZVI and the major reaction mechanisms with environmental contaminants. In aqueous solutions, nZVI can react with dissolved oxygen and water to form an outer iron oxide/hydroxide layer, which results in a typical core-shell structure [4,6]. The high reactivity and surface area of nZVI particles lead to strong reducing capacity and bring about the degradation of chlorinated compounds, heavy metals, radionuclides, organic dyes, pesticides, inorganic anions, polychlorinated biphenyls and pharmaceutical products [4,12-15]. The most common process identified in reductive dehalogenation on the iron surface are hydrogenolysis and reductive elimination (α or β) [6]. The reaction of nZVI with inorganic contaminants involves reduction and adsorption/precipitation [6]. Recently, surface modification and reuse of nZVI to increase durability, reactivity and mobility were also investigated [11,15]. Table 1 shows the representative results of the remediation of organic and inorganic materials by nZVI.

Environmental Implications of Nanoscale Zero-valent Iron Particles

Ecotoxicity

Despite the increasing use of nZVI particles and concerns for their potential toxic effects on both water and soil organisms, only a limited number of studies have investigated the ecotoxicity of nZVI. In addition, convincing evidence of the ecotoxicity of nZVI has not yet been obtained, because this likely depends on the organism species, composition, concentration, size and surface properties of the nanoparticles and the experimental method used. Table 2 summarizes the currently published ecotoxicological studies on nZVI.

Bacteria

Compared with other organisms, the potential toxic impacts of nZVI on microorganisms have been considerably studied. Several studies have shown that nZVI is toxic to microorganisms, such as *Bacillus cereus, Pseudomonas stutzeri,* and *Escherich-*

Table 1. Summary of current studies on the environmental applications of nanoscale zero-valent iron (nZVI)

Nanoparticle type	Target contaminants	Parameter	Key findings	Reference
nZVI	1,1,1,2-TeCA, Cr(VI)	Aging of nZVI and concentration	Rate constants for 1,1,1,2- TeCA reduction in Cl^-, SO_4^{2-}, and ClO_4^- suspensions decreased by 95% over 1 mo Cr(VI) removal capacities exhibited a pH dependence	[8]
B-nZVI	Cr(VI)	Cr(VI) concentration., pH, temperature, B-nZVI loading, regeneration of B-nZVI	The presence of bentonite led to a decrease in aggregation of iron nanoparticles and a corresponding increase in the specific surface area of the iron particles The removal efficiencies for Cr, Pb, and Cu by B-nZVI were > 90% Reuse of B-nZVI after washing with ethylenediaminetetraacetic acid solution was possible but the capacity of B-nZVI for Cr(VI) removal decreased by approximately 70%	[11]
nZVI	Nitrate	Aging of nZVI and concentration	The freshly synthesized iron reacted at the fastest rate After formation of the oxide shell the rate constants decreased by crica 50% of that of fresh iron, but were still higher than that of commercial grade micro- or milli-sized iron powder	[12]
nZVI	TCE	NOM	Bare nZVI was partly aggregated and precipitated to the bottom of the reactor after 1 hr incubation In contrast, nZVI remained stable in the presence of NOM The presence of NOM reduced the reactivity towards TCE	[13]
nZVI, n-Fe_3O_4	Uranium	Oxic/anoxic, nanoparticle types	Uranium was removed by all nano-Fe^0 systems to < 10 mg/L (> 98% removal) within 2 hr of reaction In contrast, nano-Fe_3O_4 failed to achieve > 20% uranium removal	[14]
nZVI, APGC -coated nZVI	TCE	Coating types	TCE degradation rate with APGC-coated nZVI was higher than that with bare nZVI Chemical reactivity of APGC-coated nZVI remained for 6 mo	[15]

1,1,1,2- TeCA, 1,1,1,2-tetrachloroethane; Cr, chromium; B-nZVI, bentonite-nZVI; TCE, trichloroethylene; NOM, natural organic matter; APGC, amphiphilic polysiloxane graft copolymers.

ia coli [16-18]. The specific mode of action of nZVI appears to be through oxidative stress from reactive oxygen species (ROS) generation that causes oxidative damage to cells via lipid peroxidation and oxidation of thiol groups on proteins and DNA. The toxicity of nZVI under oxic conditions is significantly lower than under anoxic conditions, which is related to the formation of an iron oxide layer resulted from the surface oxidation. This is supported by recent studies [19,20] which showed decreased bactericidal effects through the oxidation of nZVI under aerobic conditions.

Xiu et al. [21] investigated the potential influence of nZVI on the community of indigenous microorganisms that participate in the remediation of trichloroethylene-contaminated sites. They observed that nZVI initially inhibited dechlorinating bacteria, however the populations were able to recover after a lag time. In addition, the authors concluded that nZVI caused the stimulation of H_2 production, which can be used as an electron donor by methanogens and dechlorinating bacteria. These findings are also supported by other studies in which the addition of nZVI created significantly more reduced conditions, and stimulated remediation efficiency of microbes [22,23].

The effects of surface coatings were studied by Li et al. [19] in which surface coated nZVI (e.g., polystyrene sulfonate, polyasparatate, and natural organic matter [NOM]) significantly mitigated the adverse effect to *E. coli* as a result of reduced interaction between particles and organisms. Carboxymethyl cellulose (CMC) stabilized nZVI exerted a minimal oxidative stress response and slowed disruption of cell membrane integrity, resulting in mitigated cytotoxicity towards bacteria *Agrobacterium* sp. PH-08, as compared with the uncoated nZVI [24]. Furthermore, Chen et al. [13] also investigated the role of NOM in the microbial toxicity of nZVI, concluding that surface modification of nZVI might alter physicochemical interactions with organisms and influence toxicity and bioavailability, likely due to electrosteric hindrance effect.

In the same study, the effect of nZVI on different microbial species was investigated. The authors concluded that *B. subtilis* (Gram positive) was more tolerant to nZVI than *E. coli* (Gram negative) due to the thicker Gram positive cell wall. Similarly, Němeček et al. [25] also observed a significant increase in the density of Gram positive bacteria after in situ application of 2 kg/ton of nZVI. In summary, the effect of nZVI on bacteria is variable and depends on not only the species but also on the physicochemical properties of nZVI.

Terrestrial Organisms

Soil organisms including earthworms and plants also have a chance to be exposed to nZVI, at least around injection areas. However, there are only a very limited number of studies which have investigated the effects of nZVI on terrestrial organisms (i.e.,

Table 2. Summary of current studies on the ecotoxicological effect of nanoscale zero-valent iron (nZVI)

Test organism	Nanoparticle type	Exposure method	Observed effects	Reference
Bacteria				
Paracoccus sp. strain YH1 (denitrification microorganisms) isolated from activated sludge	nZVI: synthesized by liquid phase method, 20-80 nm	Batch experiments of nitrate reduction for 20 hr	Addition of 50 mg/L nZVI promoted the nitrate removal, whereas a hight concentration of nZVI lead to increased production of Fe^{2+}, a toxic ion	[7]
Escherichia coli (ATCC strain 10798) *Bacilllus subtilis* CB310	nZVI: purchased form Toda Kyogo Co., 40% Fe^0, median radius of 50 nm	1 hr exposure of 1 g/L nZVI with/without 10 mg/L SRHA	*Bacilllus subtilis* was more tolerant to nZVI than *Escherichia coli* due to thicker gram positive cell wall1 SRHA significantly mitigated the toxicity of nZVI due to electrostatic repulsion and steric hindrance effect	[13]
Soil microorganisms *Bacillus cereus* *Pseudomonas stutzeri*	Sodium polyacrylic acid coated nZVI (Nanofer 25S): supplied by NANO IRON s.r.o., 70-90% iron, 10-30% iron oxide,<50 nm	7 d exposure of 17 mg/g nZVI	Changes in microbial biodiversity and biomarker gene expression (*pyk*A, *kat*B) were significantly changed but the changes were related not only to the nZVI treatment but also to the soil characteristics	[16]
Escherichia coli (ATCC strain 8739)	nZVI: synthesized by aqueous-phase reduction of ferrous sulfate using sodium borohydride, 10-80 nm	1.2-110 mg/L nZVI	Significant physical disruption of cell membranes was observed (due to reaction of Fe(II) with intracellular oxygen or hydroperoxide All nanoparticles showed strong adherence onto cell walls, but no evidence of internalization	[17]
Escherichia coli (strain Qc1301 and sodB Qc2472)	nZVI: prepared by adding $NaBH_4$ to $FeCl_3 \cdot 6H_2O$ solution, 50 nm	7, 70, 175, 350, 700 mg/L nZVI	nZVI showed dose-dependent cytotoxicity mainly through oxidative stress, oxygenation of reduced Fe species	[18]
Escherichia coli (ATCC strain 33876)	nZVI,: purchased form Toda Kyogo Co., 40-60 nm PSS or PAP or NOM coatednZVI Aged nZVI	0-60 min exposure of 100 mg/L nZVI	Aerobic condition decreased the toxicity of nZVI compared to anaerobic conditions due to formation of different type of Fe-oxide at surface Surface coating with PSS, PAP, and NOM decreased the toxicity of nZVI Aged nZVI removed the bactericidal effects (due to complete oxidation of Fe^0 in aerobic conditions)	[19]
Luminous bacteria	nZVI dispersed in polyvinylpyrrolidon-K30: synthesized by sodium borohydride reduction method, 20-100 nm	36 d exposure of 0.15 g/L nZVI	Temporary negative effects were observed within 1 hr exposure (due to releasing of iron ions), but long term existence of nZVI was nontoxic	[20]
Dehalococcoides spp. (dechlorinating microorganisms) containing culture	nZVI: purchased form Toda Kyogo Co.	1 g/L nZVI exposure for batch experiments of biodegradation	Methanogens were biostimulated after exposure to nZVI due to H_2 production nZVI initially inhibited dechlorinating organisms, but dechlorination activity and ethane production recovered after a lag period Attachment of nZVI onto bacteria cells but no signs of internalization were observed	[21]
Bacillus fusiformis isolated from activated sludge	nZVI: synthesized by liquid phase method, 30-60 nm	Batch experiments of biodegradation (12 hr)	The growth of *Bacillus fusifomis* was promoted by nZVI (due to H_2 production through nZVI corrosion) Degradation of phenol increased in the presence of nZVI	[22]
Microbial microcosm from TCE contaminated sites	nZVI and polyasparated coated nZVI: purchased form Toda Kyogo Co., 40-60 nm	1.5 g/L nZVI	Addition of nZVI simulated sulfate reducer and methanogen populations and did not decrease total bacterial abundance in microcosms Addition of surface coated nZVI increased bacterial populations	[23]
Agrobacterium sp. PH-08	CMC coated nZVI and bare nZVI: synthesized by borohydride reduction method, 80-120 nm	1, 4 hr exposure of 0.1 g/L nZVI	CMC-stabilized nZVI exerted minimized oxidative stress and slower disruption of cell membrane integrity, resulting in minimized cytotoxicity	[24]
Microbial biota *Vibrio fischeri* (strain NRRL-B-11177)	Sodium polyacrylic acid coated nZVI (Nanofer 25S): supplied by NANO IRON s.r.o., 70-90% iron, 10-30% iron oxide, < 50 nm	Pilot scale *in situ* application of 2 kg nZVI/ton soil (site: Cr(IV)-contaminated site)	No significant changes in cultivable psychrophilic bacteria densities were observed in the groundwater sample, whereas the growth of G+ bacteria was simulated in soil sample Toxicity of *Vibrio fischeri* was not observed	[25]
Digested sludge (two-stage mesophilic digester)	CMC coated nZVI: synthesized by reducing ferrous chloride with sodium borohydride, 55±11 nm	14 d anaerobic digestion experiment (modified biochemical methane potential procedures) nZVI : 0, 1, 10, 30 mM	nZVI reduced methane production due to rapid H_2 production from nZVI (lead to bacterially controlled hydrogentrophic process) At the concentration of 30 mM, nZVI led to a significant increase in soluble chemical oxygen demand (an indication of cell disruption) and volatile fatty acids	[26]

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Table 2. Continued

Test organism	Nanoparticle type	Exposure method	Observed effects	Reference
Soil bacterium (*Bacillus cereus*)	Sodium polyacrylic acid coated nZVI (Nanofer 25S): supplied by NANO IRON s.r.o., 70-90% iron, 10-30% iron oxide, < 50 nm	48 hr exposure of 1, 5, 10 g/L nZVI	Significant toxicity was observed after 2 hr exposure, whereas after 48 hr no toxicity was observed due to likely the particle aggregation during the exposure period Exposure to nZVI did not change the expression rate of the assayed genes (*nar*G, *nir*S, *pyk*A, *kat*B, *gyr*A) Proteomic analysis highlighted the *Bacillus cereus* molecular stress-response to nZVI (GlmM depression, Fla depression, Trx upregulation, Ald and Gdh upregulation)	[27]
Klebsiella planticola DSZ *Bacillus nealsoni* Soil microbes	Sodium polyacrylic acid coated nZVI (Nanofer 25S): supplied by NANO IRON s. r. o., 70-90% iron, 10-30% iron oxide, < 50 nm	0.2, 2, 24 hr exposure of 1-10 mg/L nZVI	nZVI was nontoxic to G- strain (*Klebsiella planticola*), whereas was toxic to G+ strain (*Bacillus nealsoni*) Exposure of soil microbes to nZVI did not change the expression rate of the assayed genes (*nar*G, *nir*S, *gyr*A) Significant changes in the structure and composition of soil bacteria population were detected	[28]
Soil bacteria (from PCB contaminated soil)	nZVI dispersed in 0.18% PAA: supplied by Golder Associates Inc., 12.5 nm	28 d exposure of 10 g/kg nZVI	nZVI has the potential to inhibit microbial functions important for PCB remediation strategies nZVI altered the microbial composition	[29]
Activated sludge	nZVI: synthesized by sodium borohydride reduction method	0, 20, 50, 200 mg/L nZVI	Addition of nZVI improved the phosphorous removal Addition of 200 mg/L nZVI inhibited NH_4^+-N removal Microbial activities were inhibited (ROS increase, lactate dehydrogenase increase, adenosine triphosphate decrease)	[30]
Soil microbial	nZVI dispersed in 0.18% PAA: supplied by Golder Associates Inc.	14 d exposure of 10 mg/g nZVI	nZVI inhibited soil ammonia oxidation potential nZVI stimulated dehydrogenease activity but had minimal influence on hydrolase activity	[31]
Earthworms				
Eisenia fetida *Lmbbricus rubellus*	CMC coated nZVI: synthesized by borohydride method, 20-100 nm	100, 250, 500, 750, 1,000 mg/kg nZVI exposure (avoidance test-ISO, acute chronic test –OECD method	Avoidance, weight changes and mortality were significantly affected by nZVI concentrations above 500 mg/Kg Reproduction was affected at 100 mg/kg nZVI Toxicity effects of nZVI were reduced after aging *Eisenia fetida* Avoidance effect - EC_{50} = 511-563 mg/kg Mortality - LC_{50} = 399 mg/kg *Lmbbricus rubellus*: Avoidance effect - EC_{50} = 532-582 mg/kg Mortality - LC_{50} = 447-866 mg/kg	[32]
C. *elegans* (strain N2), first juvenile stage	Sodium polyacrylic acid coated nZVI (Nanofer 25S): supplied by NANO IRON s.r.o., 70-90% iron, 10-30% iron oxide, < 50 nm	96 hr exposure of 17 mg/g nZVI (ISO 10872 method)	Growth and reproduction were not inhibited and survival was also not modified with nZVI *In vitro* assay, nZVI significantly inhibited *Caenorhabditis elegans* growth, survival and reproduction in a concentration dependent manner (LC_{50} = 3.24×10^3 mg/L)	[33]
Soil invertebrates				
Ostracods (*Heterocypris incongruens*) Collembolan (*Folsomnia candida*)	CMC coated nZVI: synthesized by borohydride method	1, 10 g/kg nZVI (direct contact toxicity – SOP of Oxtracodtoxkit F, reproduction test – OECD method)	Several negative effects of nZVI were observed on both test organisms after 7 d incubation, but prolonged incubation (30 d) reduced its toxicity effects Ostracods: Growth inhibition - EC_{50} = 36 mg/kg Mortality - LC_{50} = 77 mg/kg Collembolan: Reproduction inhibition - not observed Mortality - 100% above 1 g/kg nZVI	[34]
Plants				
Cattai (*Typha latifolia*) Hybrid poplars (*between Populous deltoids and Populos nitra*)	nZVI: synthesized by reductive precipitation method	4 wk exposure of 0-1,000 mg/L nZVI	Enhanced growth of *Typha* at lower concentration (< 50 mg/L) was observed, but strong toxic effect (growth inhibition, dry leaves, lower biomass) was observed at higher concentrations (> 200 mg/L) nZVI reduced the transpiration and growth of hybrid poplars at higher concentrations (> 200 mg/L) Internalization of nZVI by polar root cells was observed, but not by *Typha* root cells	[35]

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Table 2. Continued

Test organism	Nanoparticle type	Exposure method	Observed effects	Reference
Fishes				
Embryo-larval of medaka fish (*Oryzias latipes*)	CMC coated nZVI: synthesized by borohydride method, 27.1 nm	7 d exposure of 25-200 mg/L nZVI	CMC-nZVI had acute mortality and developmental toxic effects in embryos Acute lethality - LC_{50} = 200 mg/L Oxidative stress response - ROS increase (> 25 mg/L) Developmental toxicity: Reduction in heart rate (> 25 mg/L) Reduction in eye size (> 100 mg/L) Reduction in hatching rate (> 200 mg/L)	[36]
Larvae of medaka fish (*Oryzias latipes*), 7 d old	CMC coated nZVI: synthesized by borohydride method, 49.3 nm	14 d exposure of 1-100 mg/L nZVI, daily renewed	nZVI decreased dissolved oxygen and generated ROS nZVI caused acute lethally and sublethally toxic effects in medaka larvae (intestinal damage, catalase expression inhibition). 75-100 mg/L nZVI = 17-56% mortality	[37]
Medaka fish (*Oryzias latipes*)	nZVI: purchased form 30 nm	0, 0.5, 5, 50 μg/mL	Disturbance of oxidative defense system for embryos and adult fish was observed	[38]
Acuatic invertebrates				
Daphnia magna	nZVI (Nanofer 25, Nanofer 25S, Nanofer STAR): supplied by NANO IRON s.r.o.	96 hr exposure of 0-100 mg/L nZVI	*Daphnia magna* survival was dramatically impacted by both Nanofer 25S and Nanofer STAR at > 0.5 mg/L nZVI	[39]
Algae				
Marine microalgae (*Pavlova lutheri, Isochrysis galbana, Tetraselmis suecica*)	nZVI (Nanofer 25S, Nanofer STAR): supplied by NANO IRON s.r.o., 85% iron purity, 50 nm	28 d exposure of 1.17×10^{-5} M, 1.17×10^{-4} M, 1.17×10^{-6} M nZVI	Growth inhibition effect of *Pavlova lutheri* was observed with Nanofer STAR but *Isochrysis galbana* was not affected by nZVI Growth inhibition of *Tetraselmis suecica* was dose dependent Increased total cellular lipid content in *Tetraselmis suecica* was observed with Nanofer 25 and in *Pavlova lutheri* with Nanofer STAR	[40]
Marine phytoplankton (*Thalassiosira pseudonana, Dunaliella tertiolecta, Isochrysis galbana*) Freshwater phytoplankton (*Pseudokircheneriella subcapitata*)	nZVI (Nanofer 25S, Nanofer STAR): supplied by NANO IRON s.r.o., 70-90% iron, 10-30% iron oxide	0-100 mg/L nZVI	Growth of *Isochrysis galbana* was significantly reduced by Nanofer 25S (> 6 mg/L), but not observed with Nanofer STAR Population growth of *Dunaliella tertiolecta* and *Ilsochrysis galbana* was depressed by Nanofer 25S (1.3 mg/L) Growth of *Pseudokircheneriella subcapitata* was not significantly affected by Nanofer 25S but significantly affected by Nanofer STAR (> 12 mg/L)	[39]

ATCC, American type culture collection; SRHA, Suwannee River humic acid; PSS, polystyrene sulfonate; PAP, polyaspartate; NOM, natural organic matter; TCE, trichloroethylene; CMC, carboxymethyl cellulose; G+, Gram positive; G-, Gram negative; PCB, polychlorinated biphenyl; ROS, reactive oxygen species; ISO, international organization for standardization; OECD, Organization for Economic Co-operation and Development; EC_{50}, effective concentration 50; LC_{50}, lethal concentration 50; SOP, standard operating procedure.

earthworms, invertebrates, and plants) (Table 2). El-Temsah et al. [32] evaluated the ecotoxicological effects of nZVI on *Eisenia fetida* and *Lumbricus rubellus*. This work demonstrates a negative impact of nZVI on both earthworm species, affecting avoidance, weight changes and mortality (> 500 mg/kg) and leading to a reduced reproduction rate (> 100 mg/kg). In the other published study which investigated the nZVI effects on earthworms, however, the exposure of *C. elegans* (strain N2, first CMC, lethal concentration 50, ROS juvenile stage) to 17 mg/g nZVI did not cause any mortality after 96 hr of exposure [33].

Several negative effects of nZVI on soil invertebrates including ostracods (*Heterocypris incongruens*) and collembola (*Folsomnia candida*) were observed after 7 days of incubation [34]. These few studies indicate that nZVI can significantly affect terrestrial organisms and can even lead to their death. Considering that the use of nZVIs for environmental remediation is mainly focused on contaminated soils and groundwater, these results are very important and reinforce the need for more detailed and structured studies.

Aquatic Organisms

Although aquatic organisms have little chance to directly contact with nZVI during environmental remediation, nZVI could be flowed in surface water via groundwater discharge, resulting in exposure to aquatic organisms. In an aquatic system, Chen et al. [36,37] investigated the effects of nZVI on acute lethality and oxidative stress in medaka fish (*Oryzias latipes*). The results indicated that nZVI caused a disruption in the oxidative defense

system in embryos and larvae, as well as acute lethality in embryos at concentrations above 100 mg/L. Keller et al. [39] investigated the effects of *Daphnia magna* and concluded that *D. magna* survival was dramatically impacted by both bare and surface-coated nZVI, depending on the concentration of nZVI. Great variability in the effects of nZVI on marine and freshwater phytoplankton was observed by Keller et al. [39] and Kadar et al. [40]. For example, the population gowth of the marine phytoplankton species *Isochrysis galbana* was significantly reduced by organic surface coated nZVI (Nanofer 25S supplied by NANO IRON s.r.o., Rajhrad, Czech Republic) and not affected by inorganic surface coated nZVI (Nanofer STAR supplied by NANO IRON s.r.o). In contrast, the growth of freshwater phytoplankton species *Pseudokircheneriella subcapitata* was not significantly affected by organic surface coated nZVI and affected by inorganic surface coated nZVI. These studies indicate that there is a possible impact of nZVI on aquatic organisms that is dose- and species-dependent. Therefore, further detailed studies into the effects of nZVI on aquatic organisms should be made before environmental application.

Environmental Fate and Transport

In this review, an overview of the ecotoxicological effects at various concentrations of nZVI in laboratory studies has been provided, but the concentrations and physicochemical properties of nZVI during laboratory studies may not be in accordance with the properties of nZVI under real environmental conditions. Because the environmental fate and transport of nZVI is not yet fully understood, it is difficult to determine the environmental risk of nZVI injected into the subsurface.

The mobility of nZVI has been considerably studied for the purpose of their effective applications. The effect of coating materials to enhance the particle mobility has been predominantly investigated through column transport tests at laboratory scale [41-47]. The enhanced mobility of surface coated nZVI is likely caused by the electrosteric stabilization of polymer molecules which prevent the formation of large aggregates and attachment to the surface soil grains. Tiraferri and Sethi [42] studied the effect of surface coating on the transport of nZVI in a column packed with sand, comparing the mobility of bare nZVI and that of surface modified nZVI with guar gum. They found that bare nZVI was basically immobile in sandy porous media. However the particle mobility was significantly enhanced with guar gum at the tested conditions, regardless of the chemistry of the solutions (ionic strength and ionic composition). The enhanced transport of nZVI in saturated porous media was also observed with polyacrylic acid [43], xanthan gum [44], non-ionic surfactants [45], organic matter [46], CMC [47], and other substances.

Transformation (aging) of nZVI has been observed over a prolonged period, i.e., from a few days to years [48,49]. The rate and degree of oxidation and the type of transformation products are dependent on the environmental conditions to which the particles are exposed. Reinsch et al. [50] investigated the transformations of nZVI over 6 months in simulated groundwater and suggested that dissolved oxygen rapidly oxidizes nZVI, causing the formation of both maghemite and magnetite within the oxide layer. In addition, the presence of common groundwater anions (i.e., SO_4^{2-}, HCO^{3-}, HPO_4^{2-}, and Cl^-) does not prevent the oxidation of nZVI in the long term, except for nitrate, which passivates the surface, thereby preventing the oxidation of nZVI. The degree of oxidation and chemical composition could further affect overall the fate, transport, reactivity, and potential toxicity of nZVI. For instance, the oxidation of nZVI can decrease the particle-particle interaction and thus increase the transport, which indicates the increase in the potential for unwanted exposure. However, the adverse effects of nZVI on environmental organisms can decrease with increasing oxidation of the particle. Therefore, understanding the transformation of nZVI upon release to the environment is critical to evaluating the potential efficiency as well as risk of nZVI used for environmental remediation

Conclusion

While nZVI has substantial promise for many remediation applications, the environmental implications are still poorly understood. In particular, standard methods for studying the environmental fate, ecotoxicity, and transport of nanomaterials have not been developed yet. The physical-chemical properties of nanomaterials are substantially different from traditional organic and/or inorganic chemicals. In addition, the properties could be changed by sample preparation, choice of media, dispersant use, presence of environmental ligands, and other factors, and hence these factors easily affect nanoparticle toxicity and behavior. Importantly, the existing test methods (i.e., Organization for Economic Co-operation and Development test guidelines) have been developed based upon general chemical properties and thus do not consider the specific properties of nanomaterials. In this respect, the development of new test methods to assess the environmental fate and ecotoxicity of reactive nanomaterials is critical to conclusively determine the risks associated with nZVI.

The fate, transport, and toxicity of nZVI particles appear to be complex and are dependent on their physicochemical properties (e.g., size, chemical composition, surface charge, and coat-

ing) and environmental conditions (e.g., oxygen level, pH, ionic strength, and organic content). However, the fate and toxicity of nZVI in real site conditions has not yet been studied in detail and current toxicological data are mainly based on short-term tests using simple exposure methods. Therefore, the long-term evaluation of nZVI in pilot- and full-scale systems under realistic operation conditions needs to be addressed in order to prevent any potential environmental risks

Acknowledgements

This work was supported by "The GAIA Project" by the Korea Ministry of Environment (RE 201402059) and "Environmental Risk Assessment of Manufactured Nanomaterial" Project by the Korea Institute of Toxicology (KK-1403-02).

Conflict of Interest

The authors have no conflicts of interest with material prsented in this paper.

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