Chemosphere 144 (2016) 2221–2228

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

DDT degradation efficiency and ecotoxicological effects of two types of nano-sized zero-valent iron (nZVI) in water and soil

Yehia S. El-Temsah[a,1], Alena Sevcu[b], Katerina Bobcikova[b], Miroslav Cernik[b], Erik J. Joner[a,*]

[a] Norwegian Institute for Bioeconomy Research (NIBIO), Environment and Climate Department, Høyskoleveien 7, NO-1431 Ås, Norway
[b] Institute for Nanomaterials, Advanced Technologies and Innovations & Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Studentska 2, CZ-461 17, Liberec, Czech Republic

HIGHLIGHTS

- Two types of nano-sized zero-valent iron differed in capacity for DDT degradation.
- As much as 25% of aged DDT in soil was degraded within 3 days.
- The nZVI that was most efficient in degradation also showed the highest ecotoxicity.

ARTICLE INFO

Article history:
Received 1 March 2015
Received in revised form 26 October 2015
Accepted 28 October 2015
Available online 18 November 2015

Handling editor: Jun Huang

Keywords:
Aged pollutants
Chlorinated organic pollutants
DDT
Ecotoxicology
Nanoparticles
Nano-remediation

ABSTRACT

Nano-scale zero-valent iron (nZVI) has been conceived for cost-efficient degradation of chlorinated pollutants in soil as an alternative to e.g permeable reactive barriers or excavation. Little is however known about its efficiency in degradation of the ubiquitous environmental pollutant DDT and its secondary effects on organisms. Here, two types of nZVI (type B made using precipitation with borohydride, and type T produced by gas phase reduction of iron oxides under H_2) were compared for efficiency in degradation of DDT in water and in a historically (>45 years) contaminated soil (24 mg kg^{-1} DDT). Further, the ecotoxicity of soil and water was tested on plants (barley and flax), earthworms (*Eisenia fetida*), ostracods (*Heterocypris incongruens*), and bacteria (*Escherichia coli*). Both types of nZVI effectively degraded DDT in water, but showed lower degradation of aged DDT in soil. Both types of nZVI had negative impact on the tested organisms, with nZVI-T giving least adverse effects. Negative effects were mostly due to oxidation of nZVI, resulting in O_2 consumption and excess Fe(II) in water and soil.

1. Introduction

Until 1972, the organochlorine insecticide DDT [1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl) ethane] was used massively worldwide, whereupon it was banned internationally due to its persistence and biomagnification in the food chains (Yang et al., 2008). Still, DDT is detected in soils all over the world (Daly et al., 2007) and continues to pose serious risks to human health and the environment. For this reason, many methods for degrading DDT in the environment have been tested, including bioremediation treatments (Li et al., 2010a), soil excavation and incineration or thermal degradation (Rodante et al., 1992), photocatalytic techniques using TiO_2/UV (Lin and Lin, 2007), soil washing (Smith et al., 2004) and metal-catalyzed reactions (Zinovyev et al., 2005). More recently, macro-scale zero-valent iron (ZVI) has been used for DDT degradation in water and soil with some success (Eggen and Majcherczyk, 2006; Yang et al., 2010).

Nanomaterials offer a new generation of environmental remediation technologies that can provide cost effective solutions to challenging environmental clean-up problems. Nano-scale zero-valent iron (nZVI) has a small particle size and high reactivity, making it suitable for injection and transport in porous media, though mobility is a continuous challenge. Electrosteric stabilization can increase nZVI mobility in porous media (He et al., 2009; Phenrat et al., 2009a; Tiraferri and Sethi, 2009) and use of particle coat-

* Corresponding author.
E-mail address: Erik.Joner@nibio.no (E.J. Joner).
[1] Current address: GenØk, Center for Biosafety, Forskningsparken, P. O. Box 6418, NO-9294, Tromsø, Norway.

http://dx.doi.org/10.1016/j.chemosphere.2015.10.122

ings like carboxymethyl cellulose can make nZVI more mobile at the field scale, traveling at least 1 m from the point of injection (Kocur et al., 2014).

The use of nZVI for remediation of several contaminants has been developed and is particularly promising for chlorinated compounds (Wang and Zhang, 1997; Karn et al., 2009). Some studies show that nZVI degradation capacity is usually lower in soils than in aqueous solutions due to lower availability of the contaminants. For example, Wang and Zhang (1997) observed 90% degradation of PCB in aqueous solution, while Varanasi et al. (2007) detected significantly lower degradation (ca. 38%) in soils. They assumed that the difference in reaction rate was due to lower diffusion of PCB from soil particles to the surface of nZVI particles. Increasing binding strength during aging of organic pollutants in soil is likely to result in even lower availability for degradation. Thus, in historically contaminated soil, aged DDT is likely to be poorly bioavailable and less prone to diffusion from soil particles to nZVI (Yang et al., 2010), and thus constitutes a major challenge for remediation.

Treating soil with nZVI is considered the single largest stream of engineered nanoparticles into the environment (Mueller et al., 2012). Despite the environmental concerns, the potential negative effects of nZVI on organisms and the environment are poorly known (Chen et al., 2011; Fajardo et al., 2012), and most studies have merely investigated short term toxic effects of nZVI in bacteria (Auffan et al., 2008; Marsalek et al., 2012; Fajardo et al., 2013). This uncertainty currently limits nZVI applications in many European countries (Mueller et al., 2012). The reason is that the same properties which makes nZVI useful for environmental remediation, such as their small size and high redox reactivity, also make them potentially harmful to living organisms (Sevcu et al., 2011; Crane and Scott, 2012). Few studies on the adverse effects of nZVI toward terrestrial organisms have been conducted, but negative effects have been observed during exposure of microbial assemblages, plants and higher organisms like earthworms and collembola (Wang et al., 2011; Miralles et al., 2012). These organisms can be important contributors to remediation processes, and it is therefore important to understand how they are affected by nZVI.

Two types of nZVI, corresponding to two different production techniques, are commonly used for remediation, i.e. nZVI synthesized in aqueous suspensions of ferric or ferrous salts using borohydride as a reductant, and gas phase reduction of iron oxides under H_2. The former often contains residual reductants and high amounts of boron, while the latter may be a more environmentally sound alternative. The degradation efficiency of these two nZVI types is also likely to be different, and needs to be compared for different pollutants and under different conditions.

Soil pollution with DDT is most problematic in topsoils where interactions with soil organisms and plant roots are likely. Such topsoils are largely aerobic, so that nZVI reactivity and DDT degradation potential face exhaustion through reactions with dissolved oxygen, oxide minerals and organic matter. The lack of a targeted mode of action of nZVI would then have to be compensated with increasing amounts of applied nZVI, the cost of which may easily be justified by both higher value of arable or habitable soils, and the higher efficiency in reducing exposure and risks from surface soils as compared to sub-soils. As we have previously shown, this approach has a considerable potential to degrade DDT under aerobic conditions (El-Temsah and Joner, 2013; El-Temsah et al., 2013).

The objectives of this study were to compare and evaluate two contrasting types of nZVI with regard to DDT degradation efficiency in water and historically contaminated soil, and to examine the ecotoxicity of the two types of nZVI on non-target organisms following DDT degradation treatment in soil and water.

2. Materials and methods

2.1. Synthesis of nZVI

nZVI type B (nZVI-B) was prepared using a modified borohydride method according to He et al. (2010) by reducing a solution of $FeSO_4$ and carboxymethyl cellulose using $NaBH_4$ to a final concentration of 1.0 or 4.0 g Fe(0) L^{-1}. These concentrations were chosen and used for degradation experiments with soil, and represent a compromise that both provide a capacity for DDT degradation under aerobic conditions and a limited toxicity potential towards soil organisms after initial oxidation, according to our previous results. The produced particles were within the size range 20–100 nm. Details on nZVI preparation, analyses of particle size and zeta potential can be found in El-Temsah and Joner (2012a). nZVI-T (average particle size <100 nm) was obtained as commercial NANOFER 25S (NANOIRON, Czech Republic). An LD05 dispersing unit (NANOIRON) was used to prepare suspensions with the desired concentration of nZVI-T (1.0 or 4.0 g Fe(0) L^{-1}) from a 20% aqueous nZVI stock solution. The mean diameter of both nZVI-B and nZVI-T particles was verified immediately after preparation using a Nano ZS Zetasizer (Malvern Instruments, UK) and TEM (Figs. S4 and S5).

2.2. Degradation of DDT in water

A DDT stock solution (500 mg L^{-1} containing 18% o,p′ DDT and 77% p,p′ DDT) was prepared in acetone and diluted with acetone-deionized water (1:50, v/v). A diluted solution (10 mg L^{-1}, 50 ml) was incubated in glass bottles on a vertical shaker at 20 °C with nZVI-B or nZVI-T at 1 g L^{-1}, or without nZVI. Samples were prepared individually for reaction times of 1 h, 6 h and 24 h. At the end of the experiment, the liquid phase and particulate material were separated by centrifugation (3622× *g*) (nZVI-B only) and filtration (Whatman no. 5), and analyzed for DDT, Fe(II) and Fe(III).

DDT was extracted with hexane and acetone (5 + 5 ml) added to 50 ml of the water phase, or 2 ml hexane + 5 ml acetone to the solid phase (shaken for 1 h). After separation, a sample of the hexane phase was analyzed for DDT using a gas chromatograph-mass spectrometer (Varian PC-3800) with a 0.25 mm × 30 m (0.25 μm film thickness) 5 m capillary column and 1 ml min^{-1} He as carrier.

2.3. DDT degradation in soil slurry

Similar experiments with historically (>45 years) contaminated soil (24.7 mg DDT kg^{-1}, organic silty clay soil: clay 11%, silt 49%, sand 40% of mineral fraction, organic matter 9%, pH_{water} 5.2, from a fruit farm at the west coast of Norway) was carried out in 1 L glass bottles. Soil suspensions (300 g dry weight and 300 ml nZVI at 1 g L^{-1}, n = 3) were shaken on a vertical shaker for 48 h at 20 °C with 1.0 g L^{-1} of nZVI-B or nZVI-T. The water phase was then filtered as above, extracted (3 g soil, 10 ml hexane and 10 ml of acetone) and samples analyzed for DDT. pH, Eh, Fe(II), and Fe(III) were measured in the same liquid phase samples. Remaining soil and water were used for ecotoxicity tests.

2.4. DDT degradation in soil columns

Triplicate glass columns (10 cm long, 7 cm diam.) were filled with 200 g d.wt. historically contaminated soil and percolated with 50 ml aqueous suspensions of nZVI-B or nZVI-T (4 g nZVI L^{-1}). The volume corresponded to 50% of the pore volume of the soil to avoid losing the nZVI from the column before leaching treatments started. Triplicate columns without nZVI were also established. Two days after nZVI treatment, 100 ml of distilled water

was added at 1 ml min^{-1} to the top of each column and leaching water collected. The leaching and sampling were repeated for three days. Triplicate samples were collected and leached water and soil were analyzed separately for pH, Eh, Fe(II), Fe(III) and DDT. Remaining soil and water samples were used for ecotoxicity tests.

2.5. Fe, pH and Eh analyses

Fe(II) and Fe(III) in soil and aqueous samples were determined using ferrozine and absorbance at 562 nm, prior to or after reduction with acid hydroxylamine (Lovley and Phillips, 1986). pH and Eh were determined in the liquid phase before and after treatment with nZVI using a pH/ORP (Oxidation Reduction Potential) sensor (Memosens CPS16D).

2.6. Germination tests

Leached water and soil from slurries and columns were used for seed germination tests (OECD, 2006). Five ml of leachate were added to triplicate Petri-dishes with paper filters and 10 pre-soaked barley (*Hordeum vulgare* L.) or flax (*Linum usitatissimum* L.) seedsand incubated for 4 d at 25 °C, recording root and shoot length and percentage seed germination. Germination in soil (50 g in plastic beakers) used five pre-soaked seeds of barley or flax placed 1 cm below the surface, incubated for 5 d under light, recording percent germination and root and shoot lengths.

2.7. Earthworm tests

Standard OECD toxicity tests were performed using *Eisenia fetida* (OECD, 1984). Aerated containers with 200 g soil (d.wt.) were prepared from each soil column using three weighed worms (0.8–1.5 g) per container. Freshly moistened horse manure (1.5 g) was added weekly to the soil surface in each container. Mortality was recorded after 14 and 28 d. At 28 d, produced cocoons and juveniles were counted and adult worms weighed. Cocoons were left on moist paper for four weeks to hatch, counting the appearing juveniles.

2.8. Ostracod tests

A 6-day ostracod (*Heterocypris incongruens*) toxicity test was performed according to the operational procedure of Micro-BioTests (Nazareth, Belgium), using 24-well trays. For soil, 0.5 g (d.wt.) was added to each well and mixed with 1 ml medium-hard EPA water (Ostracodtoxkit F) and left to settle. One ml of algae suspension and five neonate ostracods were added to each well. The same procedure was used for the aqueous phase samples, using 0.5 ml sample per well instead of soil. Mortality and growth were determined after 6 d at 25 °C using a microscope. Growth inhibition (GI) was calculated as: $GI = 100-(A/B*100)$, where A = growth increment in the reference sediment and B = growth increment in the treatment.

2.9. Tests with Escherichia coli

Toxicity tests with *E. coli* (strain CCM3954, Czech Collection of Microorganisms) were performed four months after nZVI treatment of historically contaminated soil on leachate from column soil and the slurry water phase stored at −18 °C in air-tight glass bottles. Eh, pH, Fe(II) and Fe(III) were measured just before these tests. *E. coli* was spread onto agar plates with Tergitol medium (Modified Tergitol 7 Agar Base, Himedia) and grown for 48 h at 37 °C. For the tests, five bacterial colonies were transferred to 50 ml of Soya extract (Soyabean Casein Digest Medium, Himedia) using plate cross-streaking. Homogenized inoculum (0.5 ml) was mixed with duplicate 4.5 ml samples and incubated for 24 h at 37 °C. The solution was then serially diluted and 1 ml transferred to duplicate Petri dishes, covered with Plate Count Agar (Biorad) and incubated for 48 h at 37 °C before counting colony forming units (CFUs).

2.10. Statistical analyses

One way analysis of variance was used to assess the differences in DDT degradation and ecotoxicity effects between treatments. Student t-tests or Tukey's test were used for comparisons.

3. Results

3.1. DDT degradation in water

Adding nZVI to DDT-contaminated water led to changes in pH, Eh, Fe and DDT concentrations. While pH in the controls did not change during incubation, it increased from 7.0 to 8.6 using nZVI-B, and to 7.8 using nZVI-T (Table 1). Eh of the control (180 mV) did not change, but decreased to −498 mV after 1 h in the presence of nZVI-B, increasing again to −238 mV after 6 h, and further to +122 mV after 24 h. nZVI-T decreased Eh to +154, +159 mV and +83 mV after 1 h, 6 h and 24 h incubation, respectively. Fe concentrations increased slowly during incubation with nZVI-T, while in the presence of nZVI-B, Fe(II) increased abruptly reaching 9.2 mg L^{-1} after 1 h, where after it decreased to 3.6–5.0 mg L^{-1}. This difference between the two types of nZVIs was caused by differing rates of oxidation, most likely due to differences in surface modification.

Compared to controls, both nZVI-B and nZVI-T had significant effects ($p < 0.05$) on degradation of DDT and its metabolites (DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] and DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane]) in water (Fig. 1). DDT degradation after 24 h was 92% with nZVI-B and 78% with nZVI-T (percentage of sum of residual DDT and its degradation products). After 24 h, DDT was reduced from 7.27 mg L^{-1} to 0.58 and to 1.54 mg L^{-1} in the presence of nZVI-B and nZVI-T, respectively.

Degradation increased with time. There were significant differences ($p < 0.05$) between the amount of DDT remaining in the liquid and iron phases using either nZVI-B or nZVI-T (Table 2). Treatment with nZVI-B resulted in a maximum remaining percentage of DDE + DDD of 1.0% in water and 6.1% in the Fe phase after 24 h. For nZVI-T, the corresponding values were 7.6 and 14%, respectively.

3.2. DDT degradation in soil

The pH of the slurry filtrates increased from 5.2 to 6.1 both for nZVI-T and nZVI-B (Table 3). Simultaneously, Eh decreased from +190 to −120 mV (nZVI-B) and +81 mV (nZVI-T). Fe concentrations increased in filtrates, most so with nZVI-B. Fe concentrations in column leachates were low and the Eh remained positive in all leachates.

Partial DDT degradation in soil slurries was obtained with nZVI-B (22.4%) and nZVI-T (9.2%), compared to controls (Fig. 2). For soil columns, only nZVI-B resulted in significant DDT degradation (25.4%). In the solid fraction of slurries and in soil columns, the Fe(II) concentrations were significantly higher (427 and 160 mg kg^{-1} in slurry and 63 and 144 mg kg^{-1} in soil treated with nZVI-T and nZVI-B, respectively) than in controls (28 and 31 mg kg^{-1}) (Table 4). Fe(III) concentrations decreased in slurry for nZVI-B and increased in soil for both types of nZVI.

Table 1
pH, Eh and iron in DDT-water mixtures during 24 h treatment with nZVI-B or nZVI-T.

Time (h)	Water with nZVI-B				Water with nZVI-T			
	pH	Eh	Fe(II) (mg l^{-1})	Fe(III) (mg l^{-1})	pH	Eh	Fe(II) (mg l^{-1})	Fe(III) (mg l^{-1})
0	7.0	180	0.02	0.1	7.0	180	0.021	0.1
1	8.8	−498	9.2	7.1	6.7	154	1.3	2.9
6	8.6	−238	3.6	8.1	6.6	159	1.9	3.6
24	8.6	122	5.0	8.2	7.8	83	2.6	7.7

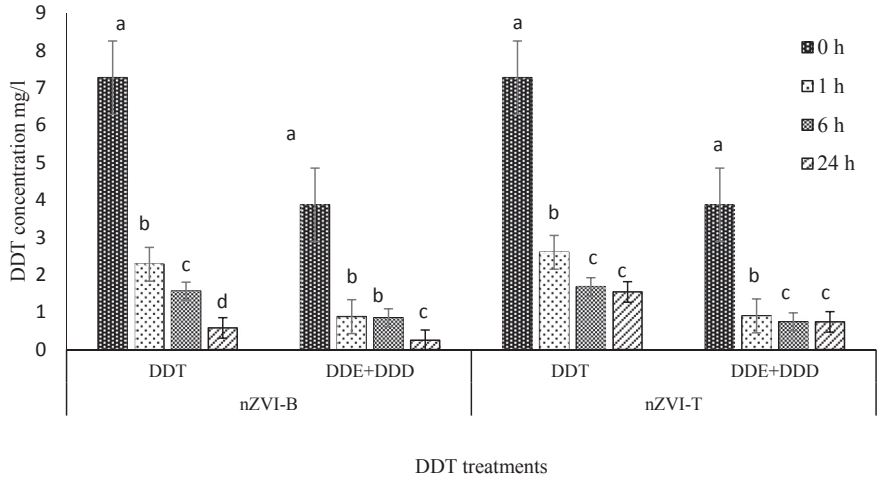

Fig. 1. The cumulative amount of DDT (o + p DDT) and metabolites (both isomers of DDE and DDD) (mg l^{-1}) remaining in water + solid phase after incubation treatments in water with either of the two types of nZVI, and the total percentage of DDT degradation with nZVI-B or nZVI-T. Means associated with the same letter in each column are not significantly different ($p < 0.05$, Student t-test, n = 3).

Table 2
Remaining percentage of DDT and its metabolites in the water and iron phase after filtration and removal of nZVI. Means associated with the same letter in each column are not significantly different ($p < 0.05$, Student t-test, n = 3).

Time (h)	nZVI-B		nZVI-T	
	o and p DDT	DDE + DDD	o and p DDT	DDE + DDD
0	100	100	100	100
DDT in the water phase (% of DDT at time 0)				
1	9.6 c	7.1 d	20.5 a	12.7 b
6	16.5 b	10.2 c	8.2 c	7.2 c
24	3.3 e	1.0 e	5.6 d	7.6 c
DDT in the iron phase (% of DDT at time 0)				
1	21.6 a	15.9 a	14.7 b	11.5 b
6	4.4 d	12.5 b	14.3 b	11.5 b
24	4.9 d	6.1 d	15.5 b	14 a

3.3. Ecotoxicity

No negative effects of nZVI-T were observed on germination of barley or flax for column soil or its leachates. Treatment with nZVI-B, however, resulted in a >50% reduction in seed germination (Table 5, Figs. S1 and S2). Similar trends were observed for nZVI-T in the solid fraction of the slurry and its filtrate, while for nZVI-B both fractions resulted in complete inhibition of seed germination. No significant inhibition was observed on root or shoot growth for nZVI-T in column soil or leachates. For nZVI-B, strong inhibition of germination was observed for both plants in leachate and soil.

Earthworm mortality at 14 d exposure to the solid fraction of the slurry increased from 15% (controls) to 33% and 22% in soil treated with nZVI-T and nZVI-B, respectively (Table 5 and Fig. S1). 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

Table 3
Properties of filtrates from slurry and leachates from soil columns treated with nZVI-B or nZVI-T measured immediately and 4 months after nZVI treatment, at the time of the *E. coli* tests.

	Filtrate from slurry			1. Leachate		2. Leachate		3. Leachate	
	Control	nZVI-B	nZVI-T	nZVI-B	nZVI-T	nZVI-B	nZVI-T	nZVI-B	nZVI-T
pH	5.2	6.1	6.1	5.6	6.4	5.9	6.6	5.9	6.5
Eh (mV)	190	−120	81	149	614	198	152	200	150
Fe(II) (mg kg^{-1})	2.9	17.7	9.2	2.6	0.6	1.4	1.1	1.4	1.1
Fe(III) (mg kg^{-1})	1.4	18.8	7.2	2.8	0.6	2	1.2	2.1	1.2
After 4 months									
pH	5.2	5.4	3.9	5.6	5.9	6.1	6.4	6.1	6.6
Eh (mV)	560	501	639	524	486	470	465	483	455
Fe(II) (mg kg^{-1})	0.06	0.84	0.17	0.47	0.31	1.01	0.36	0.71	0.23
Fe(III) (mg kg^{-1})	0.004	0.12	0.06	0.33	<d.l.	<d.l.	0.22	0.23	0.32

<d.l. = below detection limit.

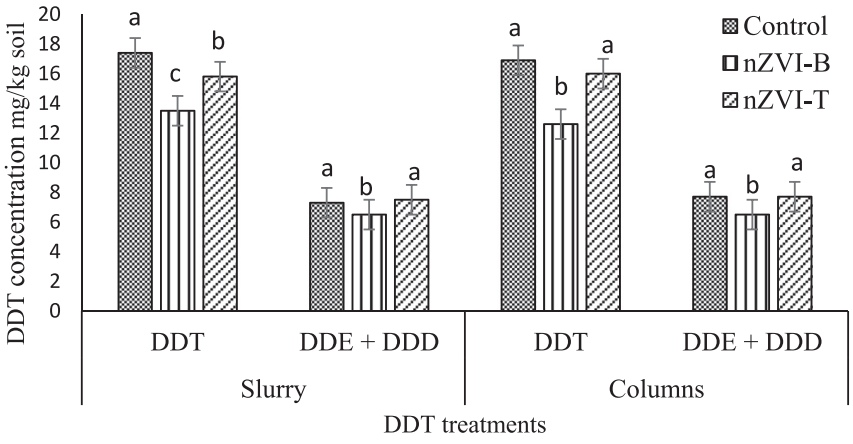

Fig. 2. DDT and its metabolites remaining in soil (mg kg^{-1}) after treatment with nZVI-B or nZVI-T for 2 d in slurry and or 3 d in columns. Means associated with the same letter in each column are not significantly different ($p < 0.05$, Student t-test, n = 3).

Table 4
Concentrations of Fe(II) and Fe(III) in soil after treatment with nZVI. Values with different letters within a column are significantly different ($p < 0.05$, Student t-test, n = 3).

	Soil from slurry		Soil from columns	
	Fe(II) (mg kg^{-1})	Fe(III) (mg kg^{-1})	Fe(II) (mg kg^{-1})	Fe(III) (mg kg^{-1})
Control	28 c	230 a	31 c	236 c
nZVI-B	427 a	161 b	63 b	413 a
nZVI-T	160 b	286 a	144 a	335 b

Table 5
Summary of the outcome of ecotoxicity tests with five species testing adverse effects of aqueous phase or solids containing residues of either of two types of nZVI. Negative effects are marked with "–", no effects with "0" and positive effects with "+". Full data sets are presented as supplementary information.

Organism	Endpoint	Soil slurry				Soil columns							
		Aqueous phase		Solid phase		1. Leachate		2. Leachate		3. Leachate		Soil	
		nZVI-B	nZVI-T	nZVI-B	nZVI-T	nZVI-B	nZVI-T	nZVI-B	nZVI-T	nZVI-B	nZVI-T	nZVI-B	nZVI-T
Earthworms	Mortality	n.a.	n.a.	–	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0	0
	Growth	n.a.	n.a.	–	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+	0
	Reproduction	n.a.	n.a.	–	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	–	0
Ostracods	Mortality	–	0	–	0	–	0	–	0	–	0	–	–
	Growth	–	0	–	+	–	–	–	0	–	0	–	–
Bacteria	Growth	–	0	n.a.	n.a.	–	–	–	–	0	0	n.a.	n.a.
Barley	Germination	+	0	–	–	–	+	0	0	0	+	–	–
	Root growth	–	+	–	+	–	+	–	0	–	0	–	+
Flax	Germination	–	0	–	0	–	0	–	0	0	0	–	0
	Root growth	–	+	–	0	–	0	–	0	–	0	0	+

n.a. = Not assessed.

controls. Significant reduction in body weight of earthworms was seen only for soil of the slurry treatment and only for nZVI-B. Other treatments showed higher body weights compared to controls. Reproduction was completely suppressed in both column soil and slurry soil after treatment with nZVI-B. No reduction in production of juveniles or cocoon was observed with nZVI-T, except a 50% reduction of cocoons in column soil.

The first and second leachates from columns treated with nZVI-B caused 100% mortality of ostracods, with <80% mortality observed in the third leachate and in the column soil (Table 5 and Fig. S2). No mortality, but ≤35% growth inhibition was observed in leachates from columns with nZVI-T. In column soil, 20% mortality and 52% growth inhibition were observed. Only growth inhibition was observed for filtrates from the soil slurry treated with nZVI-T, while, 100% mortality was observed with nZVI-B. In the solid fraction of the soil slurry, 33, 27 and 100% mortality was observed in the control, nZVI-T and nZVI-B treatments, respectively.

Sample storage prior to the *E. coli* tests had decreased the content of Fe(II) and Fe(III) and increased Eh (Table 3). The highest nZVI-Toxicity towards *E. coli* was detected in the aqueous phase of the slurry treated with nZVI-B (Fig. S3). Here, nZVI-T showed no negative effects. Significant negative effects on bacteria were only found in the first two leachates, the third having bacterial CFUs similar to the controls.

4. Discussion

4.1. Effects on degradation

Degradation efficiency of the two types of nZVI we used were clearly different, both regarding degradation of DDT dissolved in

water, and aged DDT in a surface soil rich in organic matter. As expected, the capacity of nZVI to degrade DDT in water and aqueous suspension was much higher than in soil due to interactions between DDT and soil particles and subsequent low desorption and solubilization of DDT. In addition, interactions between soil constituents and nZVI may also have led to reduction of other molecules than DDT (Comfort et al., 2001; Satapanajaru et al., 2009). Most studies investigating nZVI degradation efficiency have been carried out in aqueous systems and been designed to understand the reactivity of nZVI with contaminants in groundwater and anaerobic aquifers (Reddy, 2010). Few studies have investigated the degradation efficiency and reactivity of nZVI in surface soil due to its high content of organic matter and the presence of O_2 that render nZVI less efficient (Yang et al., 2010). Our results indicate, however, that nZVI may still represent a feasible application, similar to macro-scale ZVI (Comfort et al., 2001).

In this study, nZVI was applied at a concentration of 1 g L^{-1}, compared to concentrations ranging from 1.5 to 40 g L^{-1} nZVI employed in previous studies (Barnes et al., 2010a). With this concentration, we achieved a degradation efficiency of >90% and 78% of DDT in aqueous solution using nZVI-B and nZVI-T, respectively. These results are comparable to those of Tian et al. (2009), who observed >80% degradation of DDT in water after 8 h incubation using 50 g L^{-1} nZVI. In our slurry experiments, 1 g L^{-1} nZVI added to an aged contaminated soil resulted in only 6% and 19% degradation of DDT and DDD + DDE, respectively, after 48 h. This is rather low, even for a soil, but such low degradation efficiencies may be expected when targeting aged pollutants (Eggen and Majcherczyk, 2006). The contaminated soil we used had been polluted by DDT up to fifty years ago, rendering DDT strongly sequestered and the diffusion of DDT from the surface of the soil particles to nZVI surfaces a likely limiting factor. Yang et al. (2010) conducted a similar study using ZVI to degrade DDT in soil from a pesticide plant. They observed that DDT degradation was very slow due to aging and low DDT diffusion rates. Further, the organic matter and clay content of the soil used in our study was quite high (9% and 11%, respectively), which also contributes to strong DDT sequestration and reduced nZVI degradation efficiency (Phenrat et al., 2009b).

We believe that the difference in degradation efficiency between the two types of nZVI is related to differences in reactivity, suspension stability, particle size and lifetime of nZVI in water. Further, nZVI-B has a higher specific surface area that may contribute to higher reactivity (30 m^2 g^{-1} compared to 20 m^2 g^{-1} for nZVI-T) (Sun et al., 2006). After 24 h incubation with nZVI-B in water (with dissolved oxygen), we observed that part of the nZVI-B turned from black to dark brown or orange, whilst nZVI-T remained black. According to Reinsch et al. (2010), rapid oxidization of nZVI leads to formation of a brown iron oxide phase (maghemite) within the oxide layer, which occurs at close to complete nZVI oxidation. Based on both the color change and DDT analyses, it appears that reactivity and degradation of the DDT fraction in contact with nZVI was higher for nZVI-B than nZVI-T.

pH is important for the reactivity and life-time of nZVI in water, with low pH increasing oxidation and higher pH resulting in passivation of nZVI (Liu and Lowry, 2006; Song and Carraway, 2006). Liu and Lowry (2006) studied the reactivity and life-time of two nZVI products that were very similar to our nZVI-T and nZVI-B (nZVI from Toda America Inc. and nZVI made using borohydride). Their results showed that nZVI from Toda remained reactive for several months at pH 8.9 but for only a few weeks at pH 6.5, while the nZVI made using BH_4 was fully oxidized in less than two weeks at pH 8.9. In our study, pH increased after adding nZVI-B, reaching 8.6, while pH only increased to 7.6 with nZVI-T. This increase was expected due to oxidation of Fe(0). The reaction with oxygen and water increases pH, presumably most so for nZVI-B due to their smaller particle size. Low Eh also reflected the highly reducing environment following the consumption of O_2 and production of H_2 (Zhang, 2003).

4.2. Ecotoxicological effects

Toxicity effects of the two types of nZVIs were compared using several organisms and showed that there can be significant differences between different types of nZVI regarding toxicity. For instance, soil from slurry treated with nZVI-T showed little toxicity towards earthworms, while nZVI-B caused severe lethal effects. On the other hand, nZVI-B in soil columns after three leaching episodes had no lethal effects on earthworms, but a negative effect on reproduction was observed. This confirms our previous results on toxicity of nZVI-B where aging reduced toxicity to earthworms, though adverse effects on reproduction occurred at 0.1 g L^{-1} nZVI (El-Temsah and Joner, 2012a). The high toxicity found for nZVI-B in soil columns could be due to their rapid oxidation, production of Fe(II) and consumption of O_2. After oxidation and removal of water with Fe(II), oxidized particles would be far less toxic. Complete oxidation of nZVI has been shown to neutralize e.g. its bactericidal effect and even become beneficial to growth of some bacteria (Lee et al., 2008; Li et al., 2010b). This was confirmed by our measurements. Fajardo et al. (2012) assessed the impact of nZVI at 34 g kg^{-1} on microorganisms in soil, and even at these high concentrations observed only weak negative effects on viability and activity. The rapid decline in observed toxicity may be attributed to the inherent short lifetime of nZVI-B when even small amounts of reducible molecules are present, and the consequent declining reactivity. Indeed, complete oxidation reduces the negative effect of nZVI on bacteria, and may even enhance bacterial growth (Lee et al., 2008; Li et al., 2010b).

Toxicity to plants also differed strongly between the two types of nZVI, with negative effects observed for nZVI-B and mostly positive effects or no effects for nZVI-T. These results agree with our previous observations for nZVI-B (El-Temsah and Joner, 2012b), and with the findings of Ma et al. (2013) testing effects of nZVI-B on cattail and poplar. The reduction in toxicity observed during leaching of nZVI-B-treated soil again points towards O_2 alleviating toxicity. Lack of toxicity of oxidized iron nanoparticles is also known from soybean (Ghafariyan et al., 2013). The low toxicity of nZVI-T to plants seems to be due to its slower oxidation, lower Fe(II) levels and co-existence with O_2, even in an unaltered state.

While the impact of nZVI on aquatic organisms such as zebra fish (Li et al., 2009; Chen et al., 2011) and aquatic bacteria (Barnes et al., 2010b) has been studied, no information is available regarding the effects of nZVI on sediment feeders. In our tests, ostracods were highly sensitive to changes in water properties such as Fe(II) concentration and O_2 availability. Thus nZVI-B had a strong negative effect on ostracod mortality and development, surely due to the induced anoxic conditions. nZVI-T showed less negative effects, associated with slow oxidation of Fe and lower release of Fe(II). The Fe(II) concentrations we measured were frequently higher than LC_{50} for Fe(II) for ostracods (13 mg L^{-1}), and mostly far higher for nZVI-B than for nZVI-T. Hence, we assume that Fe(II) is playing a significant role in the toxic effects of nZVI to ostracods. This is in agreement with Chen et al. (2011), who studied toxic effects of nZVI on fish larvae. They concluded that nZVI causes hypoxia due to O_2 consumption and that nZVI releases excess Fe(II), which causes production of reactive oxygen species while oxidized Fe nanoparticles containing no Fe(II) were far less toxic to fish larvae. The formation and transport of Fe(II) and itsn consequent off-site concentrations should thus be taken into account for *in-situ* use of nZVI where vulnerable aquatic recipients are found close by.

5. Conclusion

Any overall evaluation of nZVI for DDT remediation must take into account both positive and potentially negative effects. nZVI does appear to be able to reduce levels of persistent organochlorine pollutants like DDT to lower levels, thus reducing DDT exposure for a wide range of organisms. At the same time, adverse effects may affect the same organisms. The type of nZVI used is clearly determining the degree of such negative effects (Table 5), though the crucial point to evaluate is that related to *in situ* exposure. Provided that nZVI is poorly mobile, the negative effects on organisms will last for limited time and be comparatively small or negligible. If nZVI is spilt, or used in situations where mobility and exposure become significant over a prolonged period of time, actions should be taken to avoid negative environmental effects.

Acknowledgments

This study was supported through project OP VaVpI of the Centre for Nanomaterials (CZ.1.05/2.1.00/01.0005) and funding from the Norwegian Research Council (project 186901).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2015.10.122.

References

Auffan, M., Achouak, W., Rose, J., Roncato, M.-A., Chanéac, C., Waite, D.T., Masion, A., Woicik, J.C., Wiesner, M.R., Bottero, J.-Y., 2008. Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward Escherichia coli. Environ. Sci. Technol. 42, 6730–6735.

Barnes, R.J., Riba, O., Gardner, M.N., Scott, T.B., Jackman, S.A., Thompson, I.P., 2010a. Optimization of nano-scale nickel/iron particles for the reduction of high concentration chlorinated aliphatic hydrocarbon solutions. Chemosphere 79, 448–454.

Barnes, R.J., van der Gast, C.J., Riba, O., Lehtovirta, L.E., Prosser, J.I., Dobson, P.J., Thompson, I.P., 2010b. The impact of zero-valent iron nanoparticles on a river water bacterial community. J. Hazard Mater. 184, 73–80.

Chen, P.J., Su, C.H., Tseng, C.Y., Tan, S.W., Cheng, C.H., 2011. Toxicity assessments of nanoscale zerovalent iron and its oxidation products in medaka (*Oryzias latipes*) fish. Mar. Pollut. Bull. 63, 339–346.

Comfort, S.D., Shea, P.J., Machacek, T.A., Gaber, H., Oh, B.T., 2001. Field-scale remediation of a metolachlor-contaminated spill site using zerovalent iron. J. Environ. Qual. 30, 1636–1643.

Crane, R.A., Scott, T.B., 2012. Nanoscale zero-valent iron: future propects for an emerging water treatment technology. J. Hazard Mater. 211, 112–125.

Daly, G.L., Lei, Y.D., Teixeira, C., Muir, D.C.G., Castillo, L.E., Jantunen, L.M.M., Wania, F., 2007. Organochlorine pesticides in the soils and atmosphere of Costa Rica. Environ. Sci. Technol. 41, 1124–1130.

Eggen, T., Majcherczyk, A., 2006. Effects of zero-valent iron (Fe^0) and temperature on the transformation of DDT and its metabolites in lake sediment. Chemosphere 62, 1116–1125.

El-Temsah, Y.S., Joner, E.J., 2012a. Ecotoxicological effects on earthworms of fresh and aged nano-sized zero-valent iron (nZVI) in soil. Chemosphere 89, 76–82.

El-Temsah, Y.S., Joner, E.J., 2012b. Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. Environ. Toxicol. 27, 42–49.

El-Temsah, Y.S., Joner, E.J., 2013. Effects of nano-sized zero-valent iron (nZVI) on DDT degradation in soil and its toxicity to collembola and ostracods. Chemosphere 92, 131–137.

El-Temsah, Y.S., Oughton, D.H., Joner, E.J., 2013. Effects of nano-sized zero-valent iron on DDT degradation and residual toxicity in soil: a column experiment. Plant Soil 368, 189–200.

Fajardo, C., Ortiz, L.T., Rodriguez-Membibre, M.L., Nande, M., Lobo, M.C., Martin, M., 2012. Assessing the impact of zero-valent iron (ZVI) nanotechnology on soil microbial structure and functionality: a molecular approach. Chemosphere 86, 802–808.

Fajardo, C., Saccà, M., Martinez-Gomariz, M., Costa, G., Nande, M., Martin, M., 2013. Transcriptional and proteomic stress responses of a soil bacterium Bacillus cereus to nanosized zero-valent iron (nZVI) particles. Chemosphere 93, 1077–1083.

Ghafariyan, M.H., Malakouti, M.J., Dadpour, M.R., Stroeve, P., Mahmoudi, M., 2013. Effects of magnetite nanoparticles on soybean chlorophyll. Environ. Sci. Technol. 47, 10645–10652.

He, F., Zhang, M., Qian, T., Zhao, D., 2009. Transport of carboxymethyl cellulose stabilized iron nanoparticles in porous media: column experiments and modeling. J. Colloid Interface Sci. 334, 96–102.

He, F., Zhao, D.Y., Paul, C., 2010. Field assessment of carboxymethyl cellulose stabilized iron nanoparticles for in situ destruction of chlorinated solvents in source zones. Water Res. 44, 2360–2370.

Karn, B., Kuiken, T., Otto, M., 2009. Nanotechnology and in situ remediation: a review of the benefits and potential risks. Environ. Health Perspect. 117, 1823–1831.

Kocur, C.M., Chowdhury, A.I., Sakulchaicharoen, N., Boparai, H.K., Weber, K.P., Sharma, P., Krol, M.M., Austrins, L., Peace, C., Sleep, B.E., 2014. Characterization of nZVI mobility in a field scale test. Environ. Sci. Technol. 48, 2862–2869.

Lee, C., Kim, J.Y., Lee, W.I., Nelson, K.L., Yoon, J., Sedlak, D.L., 2008. Bactericidal effect of zero-valent iron nanoparticles on Escherichia coli. Environ. Sci. Technol. 42, 4927–4933.

Li, F.B., Li, X.M., Zhou, S.G., Zhuang, L., Cao, F., Huang, D.Y., Xu, W., Liu, T.X., Feng, C.H., 2010a. Enhanced reductive dechlorination of DDT in an anaerobic system of dissimilatory iron-reducing bacteria and iron oxide. Environ. Pollut. 158, 1733–1740.

Li, H., Zhou, Q., Wu, Y., Fu, J., Wang, T., Jiang, G., 2009. Effects of waterborne nano-iron on medaka (*Oryzias latipes*): antioxidant enzymatic activity, lipid peroxidation and histopathology. Ecotox. Environ. Saf. 72, 684–692.

Li, Z.Q., Greden, K., Alvarez, P.J.J., Gregory, K.B., Lowry, G.V., 2010b. Adsorbed polymer and NOM limits adhesion and toxicity of nano scale zerovalent iron to E. coli. Environ. Sci. Technol. 44, 3462–3467.

Lin, C., Lin, K.S., 2007. Photocatalytic oxidation of toxic organohalides with TiO_2/UV: the effects of humic substances and organic mixtures. Chemosphere 66, 1872–1877.

Liu, Y.Q., Lowry, G.V., 2006. Effect of particle age (Fe-o content) and solution pH on NZVI reactivity: H-2 evolution and TCE dechlorination. Environ. Sci. Technol. 40, 6085–6090.

Lovley, D.R., Phillips, E.J.P., 1986. Availability of ferric iron for microbial reduction in bottom sediments of the fresh-water Tidal Potomac River. Appl. Environ. Microb. 52, 751–757.

Ma, X., Gurung, A., Deng, Y., 2013. Phytotoxicity and uptake of nanoscale zero-valent iron (nZVI) by two plant species. Sci. Total Environ. 443, 844–849.

Marsalek, B., Jancula, D., Marsalkova, E., Mashlan, M., Safarova, K., Tucek, J., Zboril, R., 2012. Multimodal action and selective toxicity of zerovalent iron nanoparticles against cyanobacteria. Environ. Sci. Technol. 46, 2316–2323.

Miralles, P., Church, T.L., Harris, A.T., 2012. Toxicity, uptake, and translocation of engineered nanomaterials in vascular plants. Environ. Sci. Technol. 46, 9224–9239.

Mueller, N.C., Braun, J., Bruns, J., Cernik, M., Rissing, P., Rickerby, D., Nowack, B., 2012. Application of nanoscale zero valent iron (NZVI) for groundwater remediation in Europe. Environ. Sci. Pollut. Res. 19, 550–558.

OECD, 1984. OECD Guideline for the Testing of Chemicals: Earthworm Acute Toxicity Tests, 207, p. 9 France.

OECD, 2006. OECD Guideline for the Testing of Chemicals Proposal for updating guideline 208, Terrestrial Plant Test: 208: Seedling emergence and seedling growth test.

Phenrat, T., Kim, H.-J., Fagerlund, F., Illangasekare, T., Tilton, R.D., Lowry, G.V., 2009a. Particle size distribution, concentration, and magnetic attraction affect transport of polymer-modified Fe0 nanoparticles in sand columns. Environ. Sci. Technol. 43, 5079–5085.

Phenrat, T., Long, T.C., Lowry, G.V., Veronesi, B., 2009b. Partial oxidation ("aging") and surface modification decrease the toxicity of nanosized zerovalent iron. Environ. Sci. Technol. 43, 195–200.

Reddy, K.R., 2010. Nanotechnology for site remediation: dehalogenation of organic pollutants in soils and groundwater by nanoscale iron particles. 6th International Congress on Environmental Geotechnics New Delhi, India, pp. 165–182.

Reinsch, B.C., Forsberg, B., Penn, R.L., Kim, C.S., Lowry, G.V., 2010. Chemical transformations during aging of zerovalent iron nanoparticles in the presence of common groundwater dissolved constituents. Environ. Sci. Technol. 44, 3455–3461.

Rodante, F., Marrosu, G., Catalani, G., 1992. Thermal-analysis and kinetic-study of decomposition processes of some pesticides. J. Therm. Anal. 38, 2669–2682.

Satapanajaru, T., Onanong, S., Comfort, S.D., Snow, D.D., Cassada, D.A., Harris, C., 2009. Remediating dinoseb-contaminated soil with zerovalent iron. J. Hazard Mater. 168, 930–937.

Sevcu, A., El-Temsah, Y.S., Joner, E.J., Cernik, M., 2011. Oxidative stress induced in microorganisms by zero-valent iron nanoparticles. Microbes Environ. 26, 271–281.

Smith, E., Smith, J., Naidu, R., Juhasz, A.L., 2004. Desorption of DDT from a contaminated soil using cosolvent and surfactant washing in batch experiments. Water Air Soil Pollut. 151, 71–86.

Song, H., Carraway, E.R., 2006. Reduction of chlorinated methanes by nano-sized zero-valent iron. Kinetics, pathways, and effect of reaction conditions. Environ. Eng. Sci. 23, 272–284.

Sun, Y.-P., Li, X.-q., Cao, J., Zhang, W.-x., Wang, H.P., 2006. Characterization of zero-valent iron nanoparticles. Adv. Colloid Interface Sci. 120, 47–56.

Tian, H., Li, J.J., Mu, Z., Li, L.D., Hao, Z.P., 2009. Effect of pH on DDT degradation in aqueous solution using bimetallic Ni/Fe nanoparticles. Sep. Purif. Technol. 66, 84–89.

Tiraferri, A., Sethi, R., 2009. Enhanced transport of zerovalent iron nanoparticles in saturated porous media by guar gum. J. Nanopart Res. 11, 635–645.

Varanasi, P., Fullana, A., Sidhu, S., 2007. Remediation of PCB contaminated soils using iron nano-particles. Chemosphere 66, 1031–1038.

Wang, C.B., Zhang, W.X., 1997. Synthesizing nanoscale iron particles for rapid and complete dechlorination of TCE and PCBs. Environ. Sci. Technol. 31, 2154–2156.
Wang, H.H., Kou, X.M., Pei, Z.G., Xiao, J.Q., Shan, X.Q., Xing, B.S., 2011. Physiological effects of magnetite (Fe_3O_4) nanoparticles on perennial ryegrass (*Lolium perenne* L.) and pumpkin (*Cucurbita mixta*) plants. Nanotoxicology 5, 30–42.
Yang, S.C., Lei, M., Chen, T.B., Li, X.Y., Liang, Q., Ma, C., 2010. Application of zerovalent iron (Fe(0)) to enhance degradation of HCHs and DDX in soil from a former organochlorine pesticides manufacturing plant. Chemosphere 79, 727–732.
Yang, X.L., Wang, S.S., Bian, Y.R., Chen, F., Yu, G.F., Gu, C.G., Jiang, X., 2008. Dicofol application resulted in high DDTs residue in cotton fields from northern Jiangsu province, China. J. Hazard Mater. 150, 92–98.
Zhang, W.X., 2003. Nanoscale iron particles for environmental remediation: an overview. J. Nanopart Res. 5, 323–332.
Zinovyev, S.S., Shinkova, N.A., Perosa, A., Tundo, P., 2005. Liquid phase hydrodechlorination of dieldrin and DDT over Pd/C and Raney-Ni. Appl. Catal. B Environ. 55, 39–48.