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Abstract

Nano sized zero valent iron (nZVI) has been studied for *in-situ* remediation of contaminated soil and ground water. However, little is known about its effects on organisms in soil and aquatic ecosystems. In this study, the effect of nZVI on degradation of DDT and its ecotoxicological effects on collembola (*Folsomia candida*) and ostracods (*Heterocypris incongruens*) were investigated. Two soils were used in suspension incubation experiments lasting for 7 and 30 days; a spiked (20 mg DDT kg^{-1}) sandy soil and an aged (>50 yrs) DDT-polluted soil (24 mg DDT kg^{-1}). These were incubated with 1 or 10 g nZVI kg^{-1}, and residual toxicity in soil and the aqueous phase tested using ecotoxicological tests with collembola or ostracods. Generally, addition of either concentration of nZVI to soil led to about 50 % degradation of DDT in spiked soil at the end of 7 and 30 d incubation, while the degradation of DDT was less in aged DDT-polluted soil (24 %). Severe negative effects of nZVI were observed on both test organisms after 7 d incubation, but prolonged incubation led to oxidation of nZVI which reduced its toxic effects on the tested organisms. On the other hand, DDT had significant negative effects on collembolan reproduction and ostracod development. We conclude that 1g nZVI kg^{-1} was efficient for significant DDT degradation in spiked soil, while a higher concentration was necessary for treating aged pollutants in soil. The adverse effects of nZVI on tested organisms seem temporary and reduced after oxidation.

Key words: Nano-remediation, DDT, Nano-ecotoxicity, aged-polluted soil, chlorinated organic pollutants, nanoparticles, nZVI, ostracods, collembola.

1. Introduction

Organo-chlorine insecticides such as DDT [1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl) ethane] have been extensively used throughout the world since the 1940-ies to control pests in agriculture and vectors of diseases such as malaria mosquitos (WHO, 1979). DDT has been recognized as potentially toxic to humans and animals because of its persistence, bioaccumulation, and biomagnification in food chains (Behrooz et al., 2009). Therefore, during the 1970-ies the production of DDT was banned in most Western countries and the usage of DDT as insecticides was restricted in many developing countries (Yang et al., 2008). With regard to its persistence, the half-life of DDT in nature has been estimated to be between 4 to 30 years (Tomlin, 2005). Even though it was banned three decades ago, its residues and metabolites can still be detected in the environment (Guo et al., 2009).

Several techniques or approaches have been developed for remediation of DDT, including biodegradation treatments (Li et al., 2010), soil excavation and incineration or thermal degradation at high temperatures (Rodante et al., 1992), washing soil with surfactants (Smith et al., 2004), and advanced oxidation technologies, such as photochemical reactions using nano-sized TiO_2/UV (Lin and Lin, 2007) and metal-catalyzed reactions (Pd/C catalysts) (Zinovyev et al., 2005). Both the latter have been shown to be effective for DDT degradation, but they are also expensive treatment methods. As a powerful, inexpensive and environmentally friendly reducing agent, zero-valent iron has been used for DDT degradation in water and soil (Sayles et al., 1997; Eggen and Majcherczyk, 2006; Yang et al., 2010).

Recently, nanotechnology has offered a new generation of environmental remediation technologies that can provide cost effective solutions to some of the most challenging environmental cleanup problems. Nanoscale zero-valent iron (nZVI) has smaller particle size than traditional ZVI and a very high reactivity, well suited for injection and transport in porous media.

nZVI has been tested for remediation of several contaminant groups, including chlorinated organic contaminants (Wang and Zhang, 1997; Karn et al., 2009). Most published studies targeting dechlorination of chlorinated organics have however used bimetallic nZVI, containing small amounts of Palladium (Pd) or Nickel (Ni). These include organochlorine pesticides (Zhang, 2003), polychlorinated biphenyls (PCBs), trichloroethylene (TCE) (Schrick et al., 2002), pentachlorophenol (PCP) (Zhang and Elliott, 2006), atrazine (Zhang et al., 2011) and DDT degradation in water (Tian et al., 2009). Even though bimetallic nZVI is effective for pollutant degradation, it has drawbacks regarding cost efficiency and environmental compatibility due to spreading of other metals than Fe (Mueller et al., 2012).

So far, *in situ* nZVI applications have mainly targeted contaminants in aqueous systems and groundwater. For instance, most of field applications carried out in Europe, and about 80% of the sites treated in USA until now, have targeted contaminated groundwater only (Karn et al., 2009; Mueller et al., 2012). Few studies have reported on the use of nZVI in soil. Nevertheless, the application of nZVI in soil is important because the residues of the contaminants mostly remain within the soils above the groundwater (Reddy, 2010). Usually, nZVI reactivity and degradation efficiency is less in soils than in aqueous solutions due to limited desorption or solubilization of the contaminants in soil (Wang and Zhang, 1997; Varanasi et al., 2007). Further, degradation of chlorinated compounds which have aged in soil for many years is far slower than for recently polluted and spiked soil due to lower bioavailability of the former.

Nanoecotoxicology is a recent branch within toxicology which has focused on measuring toxicity of nanoparticles entering in contact with organisms like plants, bacteria, fish and invertebrates (Handy et al., 2008). Nanoscale ZVI is considered the single largest source of engineered nanoparticles entering the environment (Nowack and Bucheli, 2007). Further, the same properties which make nZVI potentially useful for environmental remediation, such as its small size and high reactivity, may also make it potentially harmful to living organisms (Sevcu et al., 2011; Crane and Scott, 2012). Yet, its ecotoxicity has evoked little research interest until recently. A few studies have been conducted using terrestrial species (earthworms, microorganisms and plants) (Sevcu et al., 2011; El-Temsah and Joner, 2012a), but the lack of ecotoxicological data and unknown potential effects of nZVI on organisms and the environment is currently hampering the use of the nZVI technology in Europe.

The aims of this study were a) to test the efficiency of nZVI on DDT degradation in a spiked sandy loam soil compared to that in a historically contaminated soil and b) to test the toxicity of nZVI in DDT-containing soil on ostracods and collembola. The selected test organisms were chosen because they represent key organism groups in their respective environment. [collembola are among the most abundant soil arthropods, they feed on soil microorganisms (Crouau et al., 1999), they play an important role in soil organic matter degradation, and they even contribute to remediation processes in soil. Ostracods are crustaceans that feed on settled organic materials either as particles or as larger fragments (Baun et al., 2008), and which are considered one of the important food sources for fish larvae].

2. Materials and methods

2.1. Nanosized zero-valent iron preparation

Nanosized zero-valent iron was prepared using a modified borohydride method according to He et al. (2010). Briefly, nZVI was prepared by dissolving 50 g of $FeSO_4 \cdot 7H_2O$ in 450 mL water immediately before use and mixing with 450 mL of an aqueous solution of 1 % carboxymethyl cellulose (CMC; non-toxic according to Chen et al. 2011). ZVI nanoparticles were formed by reducing the ferrous sulphate using a 1.9 M borohydride solution (30 mL, introduced at 5 ml min^{-1}) and adjust the volume to 1L. The size of the resulting nZVI particles, measured using high resolution transmission electron microscopy (JEM-2011; Jeol, Japan, operating at 200 keV), was in the range 20–100 nm. The hydrodynamic diameter and zeta potential, measured by dynamic light scattering (DLS) and phase analysis light scattering (PALS), respectively, using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., England) showed particle size between 178 and 424 nm and a zeta potential of −42.8 mV (previously described in El-Temsah and Joner, 2012a).

2.2. DDT degradation experiment

Fifty grams (dry weight) of a sandy loam soil (sieved <2 mm, sand 85 %, silt 11%, clay 4 %, organic matter 1.1% and pH_{water} 5.8) amended with 20 mg DDT kg^{-1} (PS-74, Chem Service Inc., West Chester, PA, USA; containing 18 % o,p' DDT and 77 % p,p' DDT) was incubated with 100 ml of an nZVI suspension at 1 or 10 g kg^{-1} soil in 250 ml glass bottles at room temperature. Controls without nZVI were included, and all treatments prepared in triplicate. Bottles were shaken at 175 rpm on a horizontal shaker for 7 or 30 days, and during the incubation bottles were opened for 1 minute once per day for aeration. At the end of each shaking period, the slurry samples were separated into a solid and a liquid phase by centrifugation (3622 × g) and the water phase filtered using Whatman No. 5 paper filters. Samples of the solid and water phases were taken for DDT analysis. The same procedure was used with a historically polluted soil rich in organic matter (organic silty clay soil, clay 11 %, silt 49 %, sand 40 %, organic matter 8.8 %, pH_{water} 5.2) containing 23.1 mg DDT kg^{-1} (sampled at a fruit farm at the west coast of Norway, approx. 50 years since contamination).

2.3. DDT extraction and analysis

Soil samples (3 g dry weight) were extracted in 50 ml glass bottles by using 10 ml hexane and 10 ml acetone. The suspension was shaken at 175 rpm on a horizontal shaker for 1h (adapted from Tian et al 2009). After shaking, 15 ml of deionized water were added and the emulsion shaken for another 5 min. The emulsion was centrifuged (671× g, 5 min) to obtain phase separation, and

1.5 ml of the hexane phase was transferred to GC glass vials and analyzed by GC-MS (GC 6890N and MS 5973N, Agilent, USA) using a 0.2 mm x 50 m (0.25 μm film thickness) Varian CP7482 capillary column and 1 ml/min He as carrier gas. A 2 µl sample was injected into a split/split less injector (Agilent) at an initial temperature of oven 80 ˚C, injector temperature of 250˚C and column temperature of 325˚C. Partially due to difficulties in separating isomers, and partly because differences in concentrations of the separated isomers were never significantly different between treatments, we present the combined isomers only; the o,p′-DDT+ p′,p′ DDT as DDT, the o,p′-DDD+ p′,p′ DDD as DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane), and the o,p′-DDE+ p′,p′ DDE as DDE ([1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene). The recovery of total DDT from soil was 93.6±4.8 %. DDD and DDE were quantified as DDT metabolites, but not subjected to individual toxicity measurements, as their toxicity are inherently lower than that of DDT (Richardson and Gangolli, 1994)

2.4. Toxicity to ostracods

A 6-day direct contact ostracod (*Hetrocypris. incongruens*) toxicity test was performed according to the standard operational procedure of the Ostracodtoxkit F (Micro-BioTests, Nazareth, Belgium). The test was carried out in twenty-four well trays. Three replicates were prepared from each treatment for both soil and filtrated aqueous samples taken immediately after separation. For soil samples, 0.4 g dry weight soil was added to each well and mixed gently with 1 ml of medium-hard EPA water (Ostracodtoxkit F) and left for the soil to settle. One ml of algae suspension and 5 neonate ostracods were added to each well. The same procedure was made with water samples where 0.2 ml sample per well was added instead of soil. The test plate was sealed with parafilm, covered by a lid and incubated in darkness at 25 ˚C for 6 d. The contents of each well were then microsieved to retain the ostracods, which were transferred to small Petri dishes. Mortality and growth of the surviving ostracods were determined. The measurement of length was carried out by using of a micrometric strip placed under the well. The growth inhibition test was considered valid when the mean death of ostracods concurrently exposed to a reference sediment was less than 30 %. Growth inhibition (GI) of *H. incongruens* was calculated as: GI= 100-(A/B*100), where A is growth increment of ostracods in the reference sediment, B is increment of the ostracods in the treatment.

2.5.Collembolan tests

Collembola (*Folsomnia candida*) were synchronized to 11 to 13 days of age according a standard protocol (OECD, 2008), and ten collembola were exposed to treated and untreated soil immediately after the 7 or 30 d incubations. Approx. 29 g soil was transferred into plastic cylinders measuring 5.5 cm height and 4 cm inner diameter with small a space in the plastic lids for collembolan respiration. Dried baker's yeast (15 mg) was spread onto the soil surface to serve as food source. The tests were carried out at about 50 % of the soil's water holding capacity. The test containers were kept at 20 °C with a light–dark cycle of 16:8 h at 400–800 lux. The reproduction of the test species took 4 weeks to complete, and at the end of the incubation period, adults and juveniles were counted after flotation (Skovlund et al., 2006).

2.6. Fe (II) extract from soil

Immediately after the 7 and 30 d incubation periods, approx. 0.5 g of soil was transferred to 5 ml of 0.5 M HCl in a glass vial and mixed gently for 30 s. After 1 h at room temperature, a 0.1 ml sample of the extract was added to 5 ml of ferrozine (1 g l^{-1}) in 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffered to pH 7 using NaOH. The amount of Fe(II) was determined spectrophotometrically by measuring the absorbance of the supernatant at 562 nm. Fe(II) was not oxidized and FeIII was not reduced during the extraction. Another sample of the soil was extracted by the same procedure as that described above with the exception that the extractant was 5 ml of 0.25 M hydroxylamine hydrochloride in 0.25 M HCl. Under acidic conditions, hydroxylamine reduces Fe(III) to Fe(II). The amount of hydroxylamine-reducible Fe(III) was calculated as the difference between the Fe(II) measured in the hydroxylamine and HCl extractions (Lovley and Phillips, 1986).

Standard toxicity curves were established for ostracods to determine LC_{50} and EC_{50} by using a wide range of concentrations of Fe(II) ($FeSO_4$), Fe(III) ($FeCl_3$), DDT and nZVI. Ostracods were exposed to DDT added to soil and Fe(II), Fe(III) and nZVI were tested in water. pH and Eh for all water samples were determined and Fe(II) and Fe(III) determined in treatment samples and in nZVI suspensions.

For statistical analysis, one way analysis of variance (ANOVA) followed by Student t-tests were used for comparisons of toxic effects between nZVI treatments and controls. Probit regression analysis (EPA Probit analysis, v. 1.5) was used to determine EC_{50} and LC_{50} values (50 % effect or lethal concentration) using % mortality or growth inhibition at the different exposure concentrations.

3. Results

3.1. DDT degradation

DDT degradation in soil with 1 or 10 g kg soil^{-1} of nZVI after incubation for 7 or 30 days is shown in Table 1. Significant amounts of DDT were degraded in the nZVI treatments compared with controls without nZVI. Generally, the lowest concentration of DDT was found in treatments with the lowest concentration of added nZVI for the first incubation period of 7 days. Adding 1g nZVI kg^{-1} soil thus resulted in 56 % degradation of DDT as the sum of DDT and its degradation products after 7 d, while it was only 5 % with 10 g nZVI kg^{-1} soil (not significantly different from the control). Longer incubation time (30 days) led to continued DDT degradation at the highest dose of nZVI, whereas the lowest dose of nZVI gave no additional degradation during the period from 7 to 30 d. After 30 d, the concentrations of remaining DDT also differed significantly between the 1g and the 10 g nZVI kg^{-1} soil treatments, but the difference was smaller than after 7 days. The nZVI treatment did not reduce DDE levels significantly after 7 d, whereas DDD levels were enhanced in the 10 g nZVI kg^{-1} at both incubation times compared to controls.

The effect of nZVI on DDT degradation in historically contaminated soil is shown in Table 1. The untreated controls contained 23.1 mg kg^{-1} of total DDT after 7 d incubation, whereas the soils treated with 1 and 10 g nZVI kg^{-1} soil contained 17.6 and 16.7 mg kg^{-1} of total DDT, respectively, corresponding to 24 % and 28 % reduction of total DDT. No significant changes in DDD or DDE were found for any of the treatments for the historically contaminated soil. The pH did not change significantly in either of the soils due to nZVI additions.

3.2.Toxicity effects on collembola

Toxicity of nZVI at 1 and 10 g kg^{-1} to collembola measured as mortality and production of juveniles after 21 days test exposure are shown in the Table 2. There were significant ($p<0.05$) negative effects on both adult and juvenile collembola compared with control soil without nZVI and DDT. Toxicity effects of nZVI at 1 and 10 g kg^{-1} concentration on collembola after 30 days incubation was significantly lower than after 7 days incubation. Mortality reached 100 % when adults were exposed to either nZVI concentration in soil after 7 days incubation, while after 30 days incubation toxicity was reduced and resulted in about 60 % and 80 % mortality for 1 and 10 g nZVI kg^{-1}, respectively. Additionally, pristine nZVI suspensions had highly negative effect on adult and juvenile collembola. On the other hand, DDT alone had no significant negative effect on adults, whereas no juveniles were observed in soil spiked with DDT alone. Generally, there were no observed juveniles in either of the soils treated with the two nZVI concentrations, irrespective of the length of the nZVI incubation period.

3.3. Toxicity effects on ostracods

The toxicity effects of nZVI residues in solid and aqueous soil fractions from treated soil after 7 and 30 days incubation on ostracod mortality and growth inhibition are shown in Table 3 and 4. Toxicity effects of nZVI in soil and leachates decreased with increased incubation time. There was a highly negative effect of nZVI in soil and its leachate on mortality and growth of ostracods compared to controls consisting of untreated soil and a standard sediment included in the Ostracod tox kit. The liquid phase of slurries from soil contaminated with DDT alone had negative effects on ostracod mortality and growth inhibition which resulted in 27 % mortality and 56 % growth inhibition after 7 days incubation. Similarly, 33 % mortality and 72 % growth inhibition were observed in the solid phase of the DDT-treated soil without nZVI. Both nZVI treatments had strong negative effects on ostracod mortality after 7 d incubation, and no ostracod survival was observed with any concentrations in soil or its leachate (Table 3). After 30 days incubation with nZVI there was less negative effects of nZVI on ostracod development. There was a similar weak toxicity of 1 g nZVI kg^{-1} soil and soil treated with DDT alone. Soil treated with 10 g nZVI kg^{-1} soil still caused 100 % mortality after 30 d. Leachates from DDT-spiked soil enhanced the ostracod growth about 12 %. On the other hand, 100% mortality was observed in the soil and leachates of 10 g kg^{-1} of nZVI treatment. EC_{50} and LC_{50} values for mortality and growth inhibition of ostracods after exposure to serial dilutions of DDT, Fe(II) and nZVI in soil and water are presented in Table 5. EC_{50} calculated from the data on growth inhibition was 11.5 mg kg^{-1} soil for DDT, while in water it was 36 and 19 mg l^{-1} for nZVI and Fe(II), respectively. LC_{50} was 77 and 13 mg l^{-1} for nZVI and Fe(II), respectively.

The effects of aged DDT-contaminated soil on ostracods are shown in Table 3. The liquid phase from slurries of soil without nZVI had a low effect on mortality (7 %) and soil treated with 1 g kg^{-1} of nZVI showed three times higher effects on ostracod mortality. Hundred percent mortality was observed in the liquid phase of slurries from soil treated with 10 g kg^{-1} of nZVI. Liquid phase from soil without nZVI and soil treated with 1 g kg^{-1} showed significant negative effects on growth inhibition, which was 42 and 75 %, respectively. Untreated DDT contaminated soil had negative effects on both growth and mortality of ostracods, with 33 % mortality and 51 % growth inhibition. When treated with 1 or 10 g kg^{-1} nZVI, 100 % mortality was observed.

3.4. Effects of individual components on ostracods

From dilution series experiments with iron, Fe(II) (as $FeSO_4$) showed negative effects on ostracods at higher concentrations. Eight concentrations of Fe(II) from 0.1 to 100 µg Fe mL^{-1} water were used. No effects on growth inhibition or mortality were observed at lower concentrations (0.1, 0.5 and 1 µg mL^{-1}). Significant negative effects on ostracod development were observed at 5 and 10 µg Fe mL^{-1}. About 45 % growth inhibition and 40 % mortality was observed at 20 µg Fe ml^{-1}. Increasing the concentration to 50 µg Fe(II) mL^{-1} resulted in 100 % mortality. pH of all samples was between 6.5 and 7.

Freshly prepared nZVI suspensions were used for ostracod test at 4 concentrations (10, 100, 500, and 1000 µg Fe mL^{-1} water). There was a weak negative effect at 10 µg Fe mL^{-1}, whereas 100 µg Fe mL^{-1} had a strong negative effect on ostracod development and mortality and caused 90 % growth inhibition and 45 % mortality. For higher concentrations, 100% mortality was observed.

Effects of DDT on ostracods were studied using 10, 20 and 50 mg kg^{-1} DDT added to soil. DDT at 10 mg kg^{-1} had a negative effect on growth of ostracods (50 % growth inhibition), but no effects on mortality. At 20 mg DDT kg^{-1}, 64 % growth inhibition and 7 % mortality were observed. When 50 mg DDT kg^{-1} was tested, 100 % mortality of ostracods was observed. Growth inhibition of ostracods was a more sensitive end point than mortality.

The concentrations of iron in aqueous and solid phase of spiked and aged DDT soil suspension after 7 and 30 days incubation are shown in Table 5. Both concentrations of nZVI (1 and 10 g kg^{-1}) slightly increased FeII concentrations (7.2 and 10 mg L^{-1}, respectively) in the aqueous phase of treated DDT spiked soil compared to control (6.6 mg L^{-1}), while the addition of 10 g nZVI kg^{-1} to spiked soil significantly increased Fe(II) and Fe(III) concentrations in the solid phase (to 102 and 1042 mg kg^{-1}, respectively). In aged DDT-contaminated soil a similar minor increase in Fe(II) and Fe(III) concentrations were seen in the aqueous phase after addition of 1 or 10 g nZVI kg^{-1} compared to the control. A strong increase in Fe(II) and Fe(III)concentrations (reaching 205 and 1471 mg kg^{-1}, respectively) was observed in the solid phase of soil treated with 10 g nZVI kg^{-1} after 7 d incubation. While soil receiving 1 g nZVI kg^{-1} had increased from 59 to 99 mg Fe(II) kg^{-1} and from 242 to 348 mg Fe(III) kg^{-1}.

4. Discussion

4.1. Effects of nZVI on DDT

In this study, the potential of nano-sized zero-valent iron (nZVI) on degradation of DDT in sandy loam and historically contaminated soil was investigated. Also, the toxicity effects of the complex system (nZVI, DDT and DDT byproducts) on ostracods and collembola was examined. The results showed that nZVI has a potential for degradation of DDT, even in historically contaminated soil where DDT bioavailability for degradation is low. It has been documented that nZVI can be used for remediation of both organic and inorganic pollutants in the aqueous phase, and that it is particularly suited for *in-situ* remediation of contaminated soil and ground water (Wang and Zhang, 1997; Joo and Zhao, 2008). However, very little is known about its efficiency for treating chlorinated pollutants in topsoil or about effects on ecosystems and organisms in soil and freshwater. DDT is recalcitrant and persistent in soils, and our control treatments showed no significant changes in DDT concentrations even in spiked soil without nZVI additions during incubated for up to 30 days. The DDT degradation in treated soils was thus clearly caused by nZVI. The data also showed that after 7 d incubation, nZVI at 1 g kg^{-1} reduced DDT levels faster than at 10 g kg^{-1}. This could be due to extensive and more complete oxidation and enhanced electron release from nZVI which is required for reduction reactions of chlorinated compounds. On the other hand, the degradation capacity with 1 g kg^{-1} of nZVI did not change much from 7 to 30 d incubation. This may be caused by the oxidation process and production of hydroxide substance on the nZVI particles surface and a general depletion of reactive nZVI due to such oxidation. In contrast, for the soil with aged DDT having a lower bioavailability in soil, adding either 1 or 10 g nZVI kg^{-1} resulted in similar rates of DDT degradation after 7 d incubation. Eggen and Majcherczyk (2006) found that DDT degradation in aged sediment was difficult using macro-sized zero-valent iron, even when they added high concentrations of ZVI (1.7 g ZVI g^{-1} sediment for 10 and 40 weeks resulting in 64 and 93 % degradation of DDT, respectively). Adsorption of organic matter such as humic acid onto the nZVI surface is known to decrease its activity due to accumulation of humic acid on the active surface sites of the nZVI interface with water and soil (Giasuddin et al., 2007).

DDE is known as the DDT metabolite that degrades slowest among DDE and DDD, and it is therefore often recovered in higher concentrations during degradation studies (Sayles et al., 1997). Thus, DDE was recovered in higher concentrations than DDD in both soils after incubation for 7 days. The control treatment also contained significant amounts of DDE, indicating that it was already present in the DDT added to soil at the start of the experiment. DDE is an aerobic dehalogenation product of DDT, and DDE has been reported as difficult to decompose further (Wang et al., 2006). The fact that no DDE accumulated still shows that nZVI lead to proper dechlorination and that its efficiency towards DDE dechlorination is comparable to that towards DDT.

Generally, the degradation efficiency of nZVI in spiked soil was higher than in historically contaminated soil, and degradation of DDT depended on both nZVI concentration and time. This may be due to complex relationships between DDT and soil, such as desorption, solubilization and dissolution of DDT. Dombek et al. (2001) showed in their study that the dechlorination reaction between iron and organochlorine compounds in water solution was as follows:

$Fe^0 + R\text{-}Cl + H^+ \rightarrow RH + Fe^{2+} + Cl^-$

This is consistent with reduction reactions occurring by electron transfer at the iron surface to degrade these chlorinated compounds. Usually the reaction between pollutants and nZVI is carried out in the water phase; therefore the DDT solubility in water has significant impact on the efficiency of the degradation. Water solubility of DDT, DDD and DDE are 25 µg L^{-1}, 120 µg L^{-1} and 90 µg L^{-1} (25 $^{\circ}$C), respectively (ATSDR, 2012). The low solubility of DDT makes transfer of DDT into an aqueous solution, where it can react with iron, a rate limiting factor. This may explain why spiked DDT was easier to reduce than aged DDT when either of these reacted with limited amounts of iron (low dose of nZVI) during a short incubation period.

4.2. Toxicity effects on collembola

Nanosized ZVI is considered the largest stream of engineered nanoparticles entering the environment, and existing ecotoxicological data are not conclusive. NZVI application mainly targets treatment of pollutants in the subsoil under saturated conditions (Mueller et al., 2012). Supposedly, the beneficial effects of nZVI degrading pollutants should largely outweigh any potential harmful effects (Karn et al., 2009; Grieger et al., 2010). However, mobility aspects are not resolved, and there is still a lack of knowledge in most soil remediation approaches regarding the impact of soil treatment processes on soil biota.

Collembola, or springtails, are one of the most abundant groups of soil arthropods. Ecotoxicity tests using the collembola *Folsomia candida*, which assess its population development, has been standardized for use in Europe (ISO 11267), and was originally designed to test the effects of individual chemicals on a soil arthropod (Crouau et al., 2002). There is a risk that when nZVI is applied to subsurface soil, it will come in contact with organisms of terrestrial ecosystems. It is therefore useful to know if the ecotoxicity of nZVI can be measured by standardized tests based on for example soil arthropods. The results of the present study indicate that the collembola test can be applied also for the evaluation of the toxicity of nZVI in soil, even in combination with a complex insecticide like DDT.

Our results showed severe toxic effects on collembola, particularly short time (7 days) after addition to soil with a high mortality of adults at both nZVI application rates. After 30 days, adults could survive in treated soils, while no juveniles were produced. Thus, collembolan reproduction was a more sensitive parameter than survival, and supplies more detailed information on toxicity, as recognized for other environmental toxicants (Krogh and Petersen, 1995; Crouau et al., 2002). DDT alone showed negative effects only on reproduction, but contrary to nZVI, the toxicity of DDT in untreated soil is not likely to decline nearly as fast.

Iron toxicity studies have primarily focused on Fe(II) and its oxides, and little is known about the toxicity specific to nZVI or macroscale ZVI. However, ZVI produces Fe(II) and iron oxides through oxidation, and nZVI can produce free radicals which are highly reactive and cause oxidative stress (Li et al., 2009). This could be one of the mechanisms behind the toxic effects of nZVI on soil organisms. Cullen et al. (2011) assessed the effect of micro- and nano-sized ZVI at 10 g kg^{-1} on soil enzymes under aerobic conditions, and did not observe any significant effects on soil enzyme activity. Fajardo et al. (2012) assessed the impact of nZVI at 34 g kg^{-1} on soil microbial community structure and functionality using a molecular approach. They did however observe little negative effects on microbial cellular viability and biological activity in soil. Recently, we reported that nZVI has a negative impact on plants and earthworms in soil and water (El-Temsah and Joner, 2012b), which support the present results.

4.3. Toxicity effects on ostracods

The majority of the ecotoxicological studies of engineered nanoparticles in water have used the crustacean *Daphnia magna* as test organism (Baun et al., 2008). A few studies have also examined the impact of nZVI on aquatic organisms such as zebra fish (Li et al., 2009; Chen et al., 2011) and river water bacteria (Barnes et al., 2010). To the best of our knowledge, there is no data in the literature on the effects of nZVI or DDT on the ostracod *H. incongruens* as fresh water organism and sediment dweller. The ostracod assay (mortality and growth inhibition) is both rapid, sensitive, relatively inexpensive, and demand small sample volumes compared to e.g. collembolan tests. By using soil as if it was a sediment, the ostracod test makes it possible to measure the combined toxicity of DDT and nZVI and simultaneously evaluate both acute and chronic endpoints (mortality and growth inhibition). It thereby gives a reflection of the toxicity of the whole sample taking into account bioavailability of the contaminants present in the matrix. Ostracods (*H. incongruens)* have previously been used successfully as test organism for soil toxicity assessment in this way (Joner et al., 2004; Manzo et al., 2011). Manzo et al. (2011) has used *H. incongruens* to evaluate the toxicity effects of ZnO nanoparticles in soil, and the results indicated that ostracods were the most sensitive organisms to ZnO nanoparticles in soil. In our study, ostracods were also very sensitive to nZVI in water and soil, which was probably due to low oxygen levels resulting from initial oxidation of nZVI. This assumption is supported by the fact that mortality declined strongly with prolonged slurry incubation prior to the test which allowed oxygenation, and that mortality was higher in treatments receiving higher doses of nZVI where more oxygen would be needed to reach aerobic conditions. EC50 thresholds established for the individual components showed that nZVI is less toxic than either DDT or Fe(II). The negative effects of nZVI on ostracod mortality could thus also be indirect, due to release of Fe(II). This is in agreement with the findings of Chen et al. (2011) who studied the toxic effects of nZVI and its oxidation products in medaka fish larvae. They concluded that nZVI causes hypoxia due to O_2 consumption, and that nZVI released excess Fe(II) which caused toxicity due to production of reactive oxygen species (ROS). Indirect effects on food depletion has also been used to explain nanoparticle toxicity (Manzo et al., 2011), but this seem less relevant in the case of ostracods under the test conditions used here.

5. Conclusion

In this study, nanosized zero-valent iron was used to degrade DDT in spiked and aged contaminated soil. Toxicity of aqueous and solid phases of soil slurries after incubation with nZVI were measured on collembola and ostracods. The results showed that the degradation rates of DDT in spiked soil were higher than in historically contaminated soil. nZVI had severe effects on collembola and ostracods, while DDT had weaker negative effects on the reproduction of collembola and development of ostracods. We also observed that increasing the incubation time or reaction period alleviate the toxicity effects of nZVI on collembola and ostracods. The addition of nZVI increased the concentration of Fe(II) and Fe(III) after incubation in soil, and particularly Fe(II)was more toxic to ostracods than nZVI. Further studies are needed to optimize the use of nZVI in different types of soils to ensure high degradation of DDT, and at the same time take into account the extent and duration of negative effects on soil biota.

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Table 1. Residual concentrations of DDT in sandy loam soil spiked with 20 mg DDT kg^{-1} and soil contaminated with DDT >50 years ago incubated with 1 or 10 g nZVI kg^{-1} for 7 and 30 days in soil slurries. Means associated with the same letter in each column are not significantly different (one way ANOVA, $p<0.05$, n=3)

	o+p DDT (mg kg^{-1})	DDD (mg kg^{-1})	DDE (mg kg^{-1})	Total DDT (mg kg^{-1})
7 days incubation of spiked soil				
Control	15.8 a	0.2 c	2.3 a	18.3 a
1 g nZVI	5.1 d	0.2 c	2.8 a	8.1 c
10 g nZVI	15.2 a	0.4 b	1.9 a	17.4 a
30 days incubation of spiked soil				
Control	16.1 a	0.4 b	0.04 c	17 a
1 g nZVI	7.6 c	0.2 c	0.02 c	7.9 c
10 g nZVI	10 b	0.9 a	0.04 c	10.9 b
7 days incubation of aged DDT-contaminated soil				
Control	16.3 x	0.2 x	6.6 x	23.1 x
1 g nZVI	11.3 y	0.2 x	6.0 x	17.6 y
10 g nZVI	10.7 y	0.3 x	5.7 x	16.7 y

Table 2. Effects of DDT and/or nZVI on collembolan mortality (adult survival) and reproduction (juvenile numbers) in spiked soil after 7 and 30 days of incubations with 1 or 10 g nZVI kg^{-1}.

	7 days		30 days	
	Adults	Juveniles	Adults	Juveniles
Soil without DDT (control)	7±1.4	68±12	8± 0.9	138±13
Soil with DDT	9±0.7	0	8±1.2	3±0.7
Soil with 1g nZVI, no DDT	0	0	4±0	0
Soil with 10 g nZVI, no DDT	0	0	2±0.7	0
Soil with 1g nZVI and DDT	0	0	4±0.7	0
Soil with 10g nZVI and DDT	0	0	2±0.7	0

Table 3. Effects of exposure to the aqueous phase or solid phase of soil suspension made of spiked or historically DDT-contaminated soil treated with nZVI for 7 or 30 d on ostracod mortality and growth inhibition (GI), (n=3).

	Water phase		Solid phase	
	Mortality %	G I %	Mortality %	G I %
Spiked soil after 7 days slurry incubation				
Soil without DDT	0	0	7 c	39 c
Soil with DDT	27 c	56 b	33 b	72 b
Soil with 1g nZVI kg^{-1}	67 b	**	100 a	100 a
Soil with 10 g nZVI kg^{-1}	100 a	100 a	100 a	100 a
Soil with 1g nZVI kg^{-1} and DDT	100 a	100 a	100 a	100 a
Soil with 10 g nZVI kg^{-1} and DDT	100 a	100 a	100 a	100 a
Spiked soil after 30 days slurry incubation				
Soil without DDT	7 c	17 c	7 c	7 b
Soil with DDT	27 b	-12 c	27 b	28 c
Soil with 1g nZVI	27 b	58 b	27 b	27 c
Soil with 10g nZVI	100 a	100 a	100 a	100 a
Soil with 1g nZVI kg^{-1} and DDT	20 b	60 b	20 b	57 b
Soil 10g nZVI kg^{-1} and DDT	100 a	100 a	100 a	100 a
Aged DDT-contaminated soil after 7 days slurry incubation				
Soil without nZVI	7 c	42 c	33 b	51 b
Soil with 1 g nZVI kg^{-1}	23 b	75 b	100 a	100 a
Soil with10 g nZVI kg^{-1}	100 a	100 a	100 a	100 a

** High mortality invalid for inhibition measurement

Table 4. EC_{50} and LC_{50}–values of components from nZVI contributing to ostracod toxicity.

	EC_{50}	LC_{50}
DDT (mg kg^{-1})	11.5	-
nZVI (mg L^{-1})	36	77
Fe^{2+} (mg L^{-1})	19	13

Table 5. Iron in the aqueous and solid phases of slurries from spiked or historically DDT-contaminated soils after 7 and 30 days incubation with nZVI (mean ± SD, n=3).

	Water phase		Solid phase	
	Fe^{II} (mg L^{-1})	Fe^{III} (mg L^{-1})	Fe^{II} (mg kg^{-1})	Fe^{III} (mg kg^{-1})
	7 days			
Control	6.6±0.5	0.33±0.6	31±2.5	82±30
1 g nZVI kg^{-1}	7.2±0.1	1.4±0.2	34±4.4	136±39
10 g nZVI kg^{-1}	10 ±0.2	2.3±0.1	102±7	1042±61
	30 days			
Control	4.9±0.2	0,18±0.3	37±2	175±28
1 g nZVI kg^{-1}	6.1±0.1	3.1±0.7	45±8	193±46
10 g nZVI kg^{-1}	7±0.8	2.8±0.03	148±24	1102±72
	DDT-contaminated soil after 7 days			
Control	6.8±0.3	0.26±0.2	59±1.3	242±15
1 g nZVI kg^{-1}	6.9±0.1	0.62±0.15	99±2	348±5
10 g nZVI kg^{-1}	8.1±0.4	2.2±0.2	205±11	1471±244