Title Page

Yehia S. El-Temsah[1], Deborah H. Oughton[2] and Erik J. Joner[1*]

Effects of nano-sized zero-valent iron on DDT degradation and residual toxicity in soil: A column experiment

[1]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Soil and Environment Department, Fredrik A. Dahls vei 20, NO-1432 Ås, Norway
[2]Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

*Corresponding authors e-mail address: Erik.Joner@bioforsk.no
Telephone number: +47 928 33 168
Fax number: +47 63 00 94 10

Abstract

Background and aims: Nanoscale zero-valent iron (nZVI) application is a promising technology for degradation of chlorinated contaminants in soil. Plants also play an important role in soil remediation and nZVI should not adversely affect plants growing on treated soils. Large amounts of DDT are still found in certain soils and means to remediate these soils are limited. Our aims were to investigate the effect of nZVI on DDT degradation and evaluate possible negative effects of nZVI on plants.

Methods: Columns with spiked (20 mg DDT kg^{-1}) soil was percolated with nZVI (1 g nZVI L^{-1}) and leached with five pore volumes of water to assess leaching of nZVI and residual toxicity of leachates and soil to plants using seed germination and plant growth tests (barley, flax).

Results: Addition of nZVI led to degradation of 45 % of the added DDT. Percolation with water significantly oxidized and transported iron through the columns. The first leachates had negative effects on plant development, but after leaching with 4 pore volumes, neither soil nor leachates affected plant negatively.

Conclusions: nZVI is efficient for degradation of DDT and adverse effects of nZVI on plants seem ephemeral and are alleviated after oxidation mediated by percolating water.

Key words: Chlorinated organics, DDT, Ecotoxicity, nanoparticles, pesticides, polluted soil, remediation.

Effects of nano-sized zero-valent iron on DDT degradation and residual toxicity in soil: A column experiment

Yehia S. El-Temsah[1], Deborah H. Oughton[2] and Erik J. Joner[1]

[1]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Soil and Environment Department, Fredrik A. Dahls vei 20, NO-1432 Ås, Norway

[2]Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

Abstract

Background and aims: Nanoscale zero-valent iron (nZVI) application is a promising technology for degradation of chlorinated contaminants in soil. Plants also play an important role in soil remediation and nZVI should not adversely affect plants growing on treated soils. Large amounts of DDT are still found in certain soils and means to remediate these soils are limited. Our aims were to investigate the effect of nZVI on DDT degradation and evaluate possible negative effects of nZVI on plants.

Methods: Columns with spiked (20 mg DDT kg^{-1}) soil was percolated with nZVI (1 g nZVI L^{-1}) and leached with five pore volumes of water to assess leaching of nZVI and residual toxicity of leachates and soil to plants using seed germination and plant growth tests (barley, flax).

Results: Addition of nZVI led to degradation of 45 % of the added DDT. Percolation with water significantly oxidized and transported iron through the columns. The first leachates had negative effects on plant development, but after leaching with 4 pore volumes, neither soil nor leachates affected plant negatively.

Conclusions: nZVI is efficient for degradation of DDT and adverse effects of nZVI on plants seem ephemeral and are alleviated after oxidation mediated by percolating water.

Key words: Chlorinated organics, DDT, Ecotoxicity, nanoparticles, pesticides, polluted soil, remediation.

66
67
68 **Introduction**

69 Chlorinated organic pollutants are among the most persistent and toxic contaminants in
70 soil, and pose serious risks to human health and the environment throughout the world. Among
71 these, organochlorine pesticides like DDT [1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl) ethane],
72 used massively worldwide for three decades after World War 2 to control agricultural pests and
73 malaria bearing mosquitos, are well known (Li et al. 2010; Wong et al. 2005). DDT was subject
74 to an international ban in 1972, but is still used in smaller amounts under strict regulations, even
75 in Europe. One example is Kelthane (Dicofol) (containing 14% DDT isomers) which is used to
76 control acaridae pests in agriculture, and which currently contributes to environmental
77 contamination (Yang et al. 2008). Due to its persistence, DDT residues and its metabolites are
78 thus widely distributed and can be found at polluted sites all over the world (Hitch and Day
79 1992), and is frequently detected in air, water, soil, sediments, fish, birds and humans. DDT has
80 received a great environmental concern because of its persistence, bioaccumulation and
81 biomagnification in food chains, and its potential toxicity to humans and wildlife (Daly et al.
82 2007; Eggen and Majcherczyk 2006; Guo et al. 2009; Hinck et al. 2009; Li et al. 2010; Yang et
83 al. 2008).

84 During the past two decades, several methods have been developed for degradation of
85 DDT, including bioremediation treatments (Li et al. 2010), soil excavation and incineration or
86 thermal degradation at high temperatures (Rodante et al. 1992), photocatalytic techniques using
87 photochemical reactions with TiO_2/UV (Lin and Lin 2007), washing soil with surfactants
88 (Smith et al. 2004) and metal-catalyzed reactions (Pd/C catalysts) (Zinovyev et al. 2005). Bulk
89 sized zero-valent iron has been used for DDT degradation in water and soil with some success
90 (Eggen and Majcherczyk 2006; Sayles et al. 1997; Yang et al. 2010).

Recently, a new technology using nano-sized zero-valent metals for remediation has been developed, being particularly promising for chlorinated organics when employing nanosized zero valent iron (nZVI). The advantages of using nZVI for treatment of contaminated water and soil include: 1) Ability to treat contaminants *in situ*, avoiding costly transportation of soil to remote treatments sites or waste disposals (Karn et al. 2009; Otto et al. 2008). 2) On site, contaminated groundwater need not be pumped out for above-ground treatment (as in "pump and treat"-remediation). 3) Due to their small size, high surface area and special surface coatings, nanoparticles may penetrate and move even within very small soil pores. They may also remain suspended in groundwater for a sufficiently long time to interact with pollutants. Nanoparticles can thus travel farther than larger, macro-sized particles, which facilitates distribution within a contaminated matrix and reduce work and costs in connection with injections. Further, nanoparticle suspensions can be injected from the surface to any location and depth (e.g. underneath buildings). However, nZVI could be less efficient for degradation of contaminants in water and soil compared with larger sized ZVI due to high reactivity and short lifetime (Comba et al. 2011). Several methods do however exist to modify nZVI reactivity, lifetime and mobility. Coating with surfactants, such as polyacrylic acid (PAA) or caboxymethyl cellulose (CMC), has been proven useful in this respect (He et al. 2010; Schrick et al. 2004). Another modification involves incorporation of noble metals like palladium (Pd) and nickel (Ni) that enhance the catalytic properties of nZVI. However, the high cost and environmental concern for spreading heavy metals has limited a widespread use of such bimetallic nZVIs in field applications (Comba et al. 2011; Jiang et al. 2011; Mueller et al. 2012). Comba et al. (2011) and Li et al. (2010) also found that there were no significant difference between mono and bimetallic nZVI for efficient degradation of DDT and other contaminants in soil and water. Still, several studies have shown that bimetallic nZVI is efficient in dechlorination of many chlorinated compound such as trichloroethylene (TCE),

116 pentachlorophenol (PCP), carbon tetrachloride (CCl_4) (Elliott and Zhang 2001; He et al. 2010;
117 Lien and Zhang 2007; Xu and Zhang 2000; Zhang et al. 1998). Field applications of both types
118 have also been conducted with good results on degradation of compounds like PCB, PCE, TCE,
119 DCE and VC (Karn et al 2009; result presentations on www.nanoiron.cz and
120 www.nanotechproject.org).
121 Although this technology may be efficient in degrading chlorinated pollutants in soil, it is also
122 important that such remediation preserves or restores soil quality to permit reuse of soil for a
123 wide range of purposes. The lack of knowledge about possible negative effects of nZVI on
124 plants and soil organisms following its application to soil is therefore an aspect that currently
125 hampers a wider use and large scale implementation of nZVI technology. Toxic effects on
126 plants have been shown during exposure both in the presence and absence of soil (El-Temsah
127 and Joner 2012b; Phenrat et al. 2009). These authors also suggested that oxidation and aging
128 could reduce the adverse effects of nZVI related to the induction of unfavorable red-ox
129 conditions. Leaching of water through treated soil may move nZVI away from an injection
130 point and lead to dilution. Also, the oxygen contained in leaching water may oxidize nZVI and
131 raise Eh to a level where O_2 availability to aerobic organisms is no longer critical. To our
132 knowledge, these aspects have not been examined in an ecotoxicological context. The
133 objectives of the present work were thus; 1) to investigate the effect of monometallic nZVI
134 coated with CMC on the degradation of DDT in soil columns, 2) to assess the impact of leaching
135 water on movement of nZVI and other Fe species, and 3) to measure possible negative effects
136 on plants of nZVI in leaching water and leached soil. The possible contribution of boron and
137 Fe^{2+} to the observed toxic effects was also examined.
138
139 **Materials and methods**
140 **Synthesis of nano-sized zero valent iron**

141 Nano-particles of zero-valent iron stabilized with carboxymethyl cellulose (CMC) was
142 prepared by the borohydride method with ferrous ion, as described by He et al. (He et al. 2010),
143 without using Pd. Briefly, 5 g of $FeSO_4 \cdot 7H_2O$ was dissolved in 450 mL water immediately
144 before use and mixed with 5 g CMC in 450 mL water. The mixture was then shaken for about
145 15 min to ensure formation of Fe^{2+}-CMC complexes. ZVI nanoparticles were then formed by
146 reducing Fe^{2+} ions using a borohydride solution (30 mL of a 1.9 M, introduced at 5 mL min^{-1}).
147 The resulting suspension was adjusted to 1 L and contained 1 g Fe L^{-1}. The suspension was
148 shaken until hydrogen evolution ceased to ensure efficient use of BH_4^-. The size of the resulting
149 nZVI particles, measured using high resolution transmission electron microscopy (JEM-2011;
150 Jeol, Japan, operating at 200 keV), was in the range 20-100 nm. The hydrodynamic diameter
151 and zeta potential, measured by dynamic light scattering (DLS) and phase analysis light
152 scattering (PALS), respectively, using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd.,
153 England) showed particle size between 178 and 424 nm and a zeta potential of -42.8 mV
154 (previously described in El-Temsah and Joner, 2012b).
155
156 **Column experiment**
157 Triplicate PVC tubes (40 cm long, 2.5 cm diam.), cut longitudinally and joined with
158 silicon glue to facilitate separation at harvest, were filled with 250 g d.wt. sandy loam soil (85%
159 sand, 11% silt, 4% clay, 1.1% organic matter, pH 5.8, sieved < 2 mm). One day before filling
160 the columns and starting the nZVI treatment, the upper 50 g of soil in each column were
161 amended with 20 mg DDT kg^{-1} (PS-74, Chem Service Inc., West Chester, PA, USA; containing
162 18 % o,p' DDT and 77 % p,p' DDT). DDT was dissolved in hexane (1 mg mL^{-1}) and added to
163 10 % of the soil volume (dried soil), evaporated over-night and mixed with humid soil (90 %
164 on a dry weight basis). This soil was placed on top of each column, and separated from the soil
165 below with disks of medical cotton cloth to facilitate the separation of spiked and non-spiked
166 soil at harvest. Columns were saturated with deionized water, left to equilibrate for 6 h and then

167 received 50 mL of a freshly made and continuously agitated nZVI suspension (described above)
168 added drop-wise from the top with a pipette. Triplicate columns without nZVI were also
169 prepared as controls. During the next 5 days, and after leaving the nZVI to react with the DDT-
170 spiked soil at room temperature for 24h, 48h, 72h etc., 50 mL per day of de-ionized water was
171 added to the top of the columns at 2 mL min^{-1} and leaching water collected in vials placed below
172 the columns. Five days after adding nZVI, the columns were split longitudinally and the soil
173 divided into three sections (the top 50 g of spiked soil and upper and lower half of the underlying
174 soil). These portions of soil were homogenized by mixing and 3 g of soil from each section
175 were taken for DDT analysis and 1 g used for measurements of Fe^{+2} and Fe^{+3}. The remaining
176 soil from each section was used for seed germination tests.

177 **Seed germination tests**

178 Seed germination was used to test whether leached water or soil could have adverse
179 effects on plants. Soil from each section and leached water from all samplings were used in
180 seed germination tests, and compared to non-treated controls, as described in El-Temsah and
181 Joner (2012). Briefly, ten seeds of barley or flax, representative of monocots and dicots,
182 respectively and previously verified as dose-response sensitive to nZVI, were placed either in
183 the sampled soil at field capacity (in triplicate petri dishes), or on Whatman no. 5 filter paper
184 (in triplicate petri-dishes) amended with 6 mL freshly leached water. Seeds were incubated at
185 25 ºC in the dark (seeds in soil were moved to a growth chamber with 16h/8h light-dark cycle
186 after 24h). Percent germinating and length of roots and shoots were recorded after 5 days in soil
187 or 4 days on filter paper (OECD 2006).

188 To evaluate which component of nZVI leachates that may cause toxicity, we separated
189 a freshly made nZVI suspension into a particulate fraction and an aqueous fraction by
190 centrifugation (9433 × g, 15 min). Serial dilutions from 0 to 100 % of the supernatant were used
191 in seed germination tests with two species × ten seeds × three replicates, as above: Five mL of

the supernatant was added to 50 g untreated sandy loam soil in 6 cm polypropylene pots, or 6 mL supernatant was added to petri dishes lined with Whatman no. 5 filter paper, and germination percentage, and root and shoot length recorded as above. The effects of boron (as boric acid) and Fe^{2+} (as $FeSO_4$) on seed germination were also tested using this scheme to establish thresholds for no observed effect concentrations (NOEC) for these components individually.

DDT extraction

Soil samples were analyzed for DDT after extracting 3 g of air dry soil with 10 mL of cyclohexane and 10 mL acetone in glass flasks at 150 rpm on a horizontal shaker (adapted from Tian et al. 2009). After 1 h, 15 mL of deionized water were added and the resulting emulsion shaken for another 5 min. The emulsion was centrifuged at 671 × g for 5 min for phase separation. The cyclohexane phase was then sampled for analysis on GC-MS (GC 6890N and MS 5973N, Agilent, USA) using a 0.2 mm × 50 m (0.25 µm film thickness) Varian CP7482 capillary column and 1 mL min^{-1} 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: 250 ˚C and column: 325 ˚C. DDT, DDD and DDE were separated by retention times and selective ion mass. The recovery of total DDT from soil was 93.6±4.8 %.

Fe extraction from soil:

Fe^{2+} was measured in fresh leachates using the ortho-phenathroline method (Christian 2004). Fe^{2+} and Fe^{3+} was measured in soil using HCl extraction and a ferrozine regent (Lovley and Phillips 1986). Approx. 0.5 g of soil was transferred to 5 mL of 0.5 M HCI in a glass vial. The soil and acid were mixed by gentle swirling for 30 s and left for 1 h at room temperature, after which a 0.1 mL sample was extract and 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) is not oxidized and Fe(III) is not reduced during such extraction. Another sample of the soil was extracted by the same procedure as 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 HCI extractions (Lovley and Phillips 1986).

Boron measurement in water

The principle of the spectrophotometric method for determination of boron is its reaction with azomethine-H, which is the product of 8-aminonaftyl-1-ol-3,6-pyrosulfuric acid and salicylic aldehyde. In the presence of dissolved forms of borates, at pH=6, formation of a yellow complex takes place, which can be measured spectrophotometrically as described by Edwards (1980). Briefly, 1 mL sample is mixed thoroughly with 2 mL of a buffer-masking solution and mixed with 2 mL of azomethine-H solution. After 30 min, absorbance is measured at 420 nm.

Statistical analysis

For the statistical analysis, a one way analysis of variance (ANOVA) was used to assess the differences in toxic effects between nZVI treatments and controls. Student T-tests were used for comparing differences between means. Probit regression analysis (EPA Probit analysis, v. 1.5, US EPA) was used to determine EC50 and LC50 values (50 % effect concentration or lethal concentration, respectively) using % plant growth inhibition at the different exposure concentrations.

Results

DDT degradation

241 Addition of nZVI and subsequent leaching with water led to a reduction in DDT
242 concentrations in soil of almost 50 % compared to controls without nZVI (Table 1). DDT in
243 leachates were below the detection limit (<0.01 mg L^{-1}; data no shown). DDT distribution
244 within the different sections of the soil columns showed low mobility of DDT and limited
245 transport of the metabolites DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene], and DDD
246 [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane]). Compared to controls, the reduction in DDT
247 concentration in the treated soil was 44.8 %, while extractible concentrations of DDT from the
248 control treatment was 19 % lower than the initial nominal concentration, presumably due loss
249 by adsorption to pipettes during spiking and to the PVC columns during the experiment. DDT
250 degradation was followed by significant increase of DDD and DDE in soil treated with nZVI.
251 These metabolites were also recovered in higher amounts in the soil below the spiked/nZVI
252 treated soil compared to the soil below spiked/non-treated soil. The recovered metabolites of
253 DDT (DDD and DDE) constituted 1.3 % of the initial concentration of DDT in nZVI-treated
254 soil compared to 0.4 % of the initial DDT concentration in the control soil.

255 Concentrations of Fe^{2+} and Fe^{3+} in the different soil sections after five leaching episodes
256 are shown in Table 2. Fe^{2+} concentrations in soil significantly increased after nZVI treatment
257 and leaching, while there were only small differences in Fe^{3+} concentrations between soil
258 amended with nZVI and controls, and between spiked soil and the sections underlying it.
259 Concentrations of Fe^{2+} measured in leachates from the soil columns during the 5 days are
260 presented Table 3. There was a small difference between Fe^{2+} in leachates from control and
261 nZVI treated soil, and there was only an increase in Fe^{2+} from 18 to 25 mg L^{-1} from the control
262 to the highest value recorded (which was found for the 2nd and 3rd leaching event).

263 *Soil-less germination tests*

264 The effects of water leachates on germination of barley and flax in the absence of soil
265 are shown in Table 3. While leachates from control soil permitted a high germination rate, the

266 first leachate from nZVI-treated columns reduced germination of barely from 93 to 67 % and
267 only reached a germination rate not significantly different from controls after the 3rd leaching.
268 For flax, 100% germination was observed for controls and the first leachate, and only a slight
269 reduction to 93 % for leachates from the 2nd and 3rd day of leaching, after which germination
270 rates increased to 100 % again.

271 Inhibition of shoot and root development in barely and flax seedlings responded
272 differently to leachates with higher relative inhibition of root growth than for shoot growth
273 (Figure 1). Also, shoot and root growth was a more sensitive indicator of the negative effects
274 of water leached from the soil column than germination percentage. While water from the first
275 leaching had only a modest effect on seedling growth, water from the second and third leaching
276 had severe negative effects on both root and shoot development. Water from the 4th leaching
277 had only weakly negative effects on development of barley and no significant (p<0.05) effects
278 on flax, while the 5th leaching had no adverse effects on either plant species.

279 *Seed germinated in soil*

280 The effects of nZVI remaining in soil on germination of barley and flax are shown in
281 Figure 2. As for germination on filter paper, root development was affected to a higher extent
282 than shoot development. Strong negative effects of nZVI addition and leaching were observed
283 in soil from all sections of the soil column with respect to root development of both species.
284 The strongest negative effects were observed in the top layer also containing DDT. Less
285 negative effects on root development were observed for the bottom section of the soil column
286 compared to the soil section closer to the point of nZVI introduction. Shoot development of
287 germinating barley was unaffected for all soil sections, and only moderately affected for flax.
288 Attempts to germinate seeds in soil freshly amended with nZVI (with no leaching) resulted in
289 complete inhibition for both plant species.

290 *Adverse effects of nZVI suspension components*

291 The inhibitory effects of the aqueous phase of the nZVI suspension were evident for
292 both barley and flax when germinated both on filter paper and in soil. The undiluted nZVI
293 aqueous phase caused an approx. 90% reduction in germination on filter paper for both species
294 (Figure 3a). An approx. 50 % reduction was observed when the aqueous phase was diluted to
295 25 % of its original concentration for barley and to 12.5 % for flax. Shoot development was far
296 less sensitive, but showed the same general trend (results not shown).
297 When the aqueous fraction of the nZVI suspension was used for seed germination in soil,
298 inhibition was less evident than when germinated on filter paper (Figure 3b). The undiluted
299 aqueous fraction reduced root development in both barley and flax by approx. 50 %, and no
300 inhibitory effects where seen when the aqueous phase was diluted beyond 50 %. Inhibition of
301 shoot development was less pronounced, but followed the inhibition pattern seen for roots
302 (results not shown).
303 *Toxicity of boron and Fe(II)*
304 Seed germination and root and shoot development were negatively affected when B or
305 Fe^{2+} was added to soil, and the dilution series tests permitted us to establish EC50-values for
306 both ions for comparisons with effects from the aqueous phase of the nZVI suspension (Table
307 4). For B, EC50 values were similar for root inhibition of barley and flax: 13 and 12 mg B kg
308 $soil^{-1}$, respectively. For Fe^{2+}, EC50 for root inhibition differed strongly between the two species,
309 being 140 mg B kg $soil^{-1}$ for barley and 40 mg B kg $soil^{-1}$ for flax. The concentrations of B and
310 Fe^{2+} in the undiluted aqueous phase of the nZVI suspension were 22 and 121 mg L^{-1},
311 respectively, whereas the Fe^{2+} concentration in the leachates was between 20 and 25 mg L^{-1},
312 marginally higher than in the control (18 mg L^{-1}).
313
314 **Discussion**

315 The present study shows that nZVI has a potential for degradation of DDT in surface
316 soil when added in relatively low doses. Effective, inexpensive, rapid and simple methods have
317 been sought for decades to allow remediation and restoration of soils contaminated with
318 recalcitrant chlorinated compounds (Shea et al. 2004; Yang et al. 2010), and nZVI may
319 represent a step-change in remediation this respect. In our study we used 1 g nZVI L^{-1} for
320 treating spiked soil, which is considered a low concentration for use in field applications. The
321 concentrations in field application might be higher due to environmental and soil conditions
322 such as temperature, soil types and structure. Saleh et al. (2007) suggested that field scale
323 application should employ at least 3 g nZVI L^{-1}, and nZVI slurry concentrations used so far for
324 field applications have more commonly varied between 10 to 50 g nZVI L^{-1} (Grieger et al.
325 2010; Phenrat et al. 2009). Increasing doses will however not automatically lead to increased
326 degradation in terms of lower residual concentrations remaining in treated soil, as other factor
327 may become limiting for degradation.
328 Bulk zero-valent iron has been used previously as a reducing agent that mediate
329 degradation of organochlorine compounds such as DDT, lindane, metachlor, alachlor,
330 chloropyrifos and atrazine in water, soils and/or sediments, and even aged DDT (Boussahel et
331 al. 2007; Eggen and Majcherczyk 2006; Kim et al. 2010; Sayles et al. 1997; Shea et al. 2004)
332 e.g. reaching degradation rates of four pesticides (metachlor, alachlor, chloropyrifos and
333 atrazine) of 60 % after incubation for 90 days with 50 g kg^{-1} ZVI in soil (Shea et al. 2004).
334 Similary, adding 50 g kg^{-1} ZVI and 30 % moisture resulted in 91 % and 98 % degradation of
335 metachlor, which has a low solubility (log Kow 3.2) and only one Cl atom, in soil after 3 and
336 40 days incubation, respectively (Kim et al. 2010). Furthermore, 65 and 93 % degradation of
337 DDT in an aged sediment after incubation with ZVI for 10 and 40 weeks, respectively, has been
338 observed (Eggen and Majcherczyk 2006). Nanosized ZVI has later proven even more efficient
339 for dechlorination of pesticides including atrazine for which 64 % degradation was observed

after 2 h incubation with 2 g L^{-1} organobentonite nZVI in water (Zhang et al. 2011). Satapanajaru et al. (2008) observed a degradation rate of atrazine in water and soil that was seven times higher when nZVI (20 g L^{-1}) was used compared to ZVI (50 g L^{-1}) in water, while 100 g kg^{-1} of both nZVI and ZVI was used in soil treatment. Nanosized ZVI was also efficient for DDT degradation in water, with 85 % of DDT degraded in water with nZVI at a concentration of 50 g L^{-1} after 8h incubation, and there was no significant differences between nZVI and nickel-doped nZVI (Ni-nZVI) (Tian et al. 2009). The differences observed between degradation capacity of ZVI and nZVI is due to the fact that nZVI has a larger surface area and more reactive sites, and therefore a higher efficiency in dechlorination of most chlorinated compounds compared to micro-scale zero-valent iron (Wang and Zhang 1997) (Liu et al. 2005; Zhang et al. 2011).

Oxidation of nZVI is the main parameter affecting nZVI reactivity and toxicity. Infiltrating water from the soil surface, as in this experiment and under field conditions during precipitation, is one source of oxygen driving this process leading to reduced concentrations of Fe^0 and temporary increased Fe^{2+} concentrations in soil, seen as a spatial peak in Fe^{2+} in the middle section of the nZVI-treated columns: The upper section having received nZVI and subsequently water with O_2 for 5 days contained less Fe^{2+} and more Fe^{3+} than the underlying section. In the bottom section concentrations of Fe^{2+} and Fe^{3+} were comparable to the soil at the top of the column, perhaps due to O_2 diffusion into the soil from the column outlet. According to Satapanajaru et al. (2003), presence of Fe^{2+} and Fe^{3+} during nZVI oxidation is enhancing the dechlorination of metachlor. It is known that the dechlorination occurs when the chlorine moiety accept electrons released during oxidation of nZVI to Fe^{2+} and Fe^{3+}. Normally, dechlorination produces more biodegradable metabolites, as indicated by temporal increases in the DDT metabolites (DDD and DDE) in soil after incubation with nZVI. There are two common reductive processes degrading DDT: Dechlorination producing DDD and

365 dehydrochlorination producing DDE (Quensen et al. 1998). DDT and its metabolites have very
366 low solubility in water. DDT, DDD and DDE water solubility is 3.1-3.4 μg L^{-1}, 160 μg L^{-1} and
367 40 μg L^{-1}, respectively (Royal Society of Chemistry 1996). The amounts of DDT transported
368 down through the column (>20 μg) are far higher than what can be accounted for by DDT
369 solubilized in percolating water (<1 μg). It is therefore likely that some DDT has been adsorbed
370 onto nZVI and transported further down the column on these particles. These amounts still
371 represent only approx. 0.1 % of the initial DDT added to the system, and DDD+DDE even less,
372 and therefore should not represent any danger for enhanced mobility and transport to
373 uncontaminated soil or aquifers.

374 **Effects of nZVI on plants**

375 We have previously shown that nZVI can affect seed germination and plant growth
376 negatively at concentrations below those commonly used in field treatments (El-Temsah and
377 Joner 2012). The present study shows that ecotoxicity tests with plants are also suited for testing
378 potential negative effects in water leaching through nZVI treated soil. Further, we have also
379 shown that oxidation during ageing of nZVI in non-saturated soil partially alleviate this toxicity
380 (El-Temsah and Joner 2012b). These findings are in agreement with those of El-Temsah and
381 Joner (2012a) and Phenrat et al. (2009) who observed that oxidization rendered nZVI non-toxic
382 in cyto- and neurotoxicological tests. Further, partial oxidation of nZVI was shown to reduce
383 the toxic effects on bacteria (*Escherichia coli*) (Li et al. 2010). Changes in a microbial
384 community caused by nZVI could even be reversed after the complete oxidation of nZVI
385 (ageing for 250 d) (Kirschling et al. 2010). In this case, restoration occurred within a long time-
386 span, whereas our experiment showed that a certain functional restoration can be achieved
387 within a far shorter time if oxidation is enhanced e.g. by leaching water.

388 In our study we tested the effects of two secondary components of nZVI in an attempt
389 to reveal if either of them was causing the observed effects on plant development. Apparently,

390 the contribution of Fe^{2+} to the observed phytotoxicity of nZVI treated soil or its leachates was
391 low. Even though Fe^{2+} concentrations in soil were 300-450 mg kg^{-1} higher in nZVI-treated soil
392 and underlying soil at the end of the experiment, compared to controls, the reduced growth of
393 seedlings (Fig 2) did not reflect the measured Fe^{2+} concentrations (Table 2). Neither was seed
394 germination of flax (the most Fe^{2+}-sensitive plant we tested) affected to any higher extent than
395 the more Fe^{2+}-tolerant plant, barley, in germination tests on treated soil (Fig 2). On the other
396 hand, the residual boron may contribute to the phytotoxicity of nZVI, as it had EC50 values
397 (12-13 mg B kg^{-1}) that were well below that of the B concentration in nZVI suspensions (22
398 mg B kg^{-1}) and 4-10 times lower than the EC50 values for Fe^{2+}. However, B is easily leached
399 out of coarse textured soils (e.g. Mertens et al. 2011 and references therein). To avoid negative
400 effects of B altogether, it would be relatively easy to remove excess B by washing nZVI prior
401 to application. This would remove both residual BH_4 and its oxidation product (boric acid).
402 Using washed nZVI or nZVI produced by other methods will thus not cause this type of
403 negative secondary problems and may be preferable in situations where enhanced levels of B
404 are undesirable. Boron is fairly mobile in soil, but has a far lower bioavailability than in water
405 (Butterwick et al. 1989). In the present experiment this led to both elution of added B during
406 leaching and a lower toxicity response when comparing toxicity towards germinating seeds in
407 soil with seed germination on filter paper. The former showed no effect of B, even for the most
408 sensitive plant species used (barley), even though root development was affected at lower
409 concentrations. Our EC-values are in agreement with those of Mertens et al. (2011) who tested
410 boron toxicity on barley in different soils and found EC10 for added B in the range of 3-27 mg
411 kg^{-1}.

412 The use of nZVI for degradation of chlorinated organics is designed for treating
413 contaminants in ground water and anaerobic subsoil. In surface soils, the presence of oxygen
414 and organic matter will compete with chlorinated substances and react with Fe0 as to render

dechlorination less effective. In this way, treating surface soils may be less efficient, but if oxygen levels are reduced by prior saturation with water, plus a certain incubation time to allow microbial consumption of dissolved O_2, the efficiency may still be sufficiently high to obtain a significant reduction of the targeted pollutants. The lack of alternative sustainable methods to treat chlorinated organics in nan-saturated soils makes further testing of the nZVI technology important. Our own studies on nZVI-induced DDT degradation in soil polluted in the 1960-ies indicate that even aged DDT may be attained (El-Temsah and Joner, unpublished results). Future experiments should focus on the feasibility to treat such soils and continue to include tests on possible negative effects on plants and soil biota as they are likely to be exposed during and after treatment of surface soils. To the extent that boron from nZVI synthesis using BH_4 creates negative side effects, washing of crude nZVI suspensions or different synthesis methods should be considered.

References

Boussahel R, Harik D, Mammar M and Lamara-Mohamedl S 2007 Degradation of obsolete DDT by Fenton oxidation with zero-valent iron. Desalination 206, 369-372.

Butterwick L, Oude N and Raymond K 1989 Safety Assessment of Boron in Aquatic and Terrestrial Environments. Ecotox Environ Safe 17, 339-371.

Christian G D 2004 Analytical Chemistry, 6th Edition. Ed. G D Christian. p. 848. John Willey& Sons, Inc.,Washington.

Comba S, Di Molfetta A and Sethi R 2011 A Comparison Between Field Applications of Nano-, Micro-, and Millimetric Zero-Valent Iron for the Remediation of Contaminated Aquifers. Water Air and Soil Pollution 215, 595-607.

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

Edwards R A 1980 Automatic-determination of boron (0.10-10.0 Mgl-1) in raw and waste-waters. Analyst 105, 139-146.

Eggen T and 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 and 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 and 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 Chem 27, 42-49.

450 Elliott D W and Zhang W X 2001 Field assessment of nanoscale biometallic particles for groundwater
451 treatment. Environmental Science & Technology 35, 4922-4926.
452 Grieger K D, Fjordboge A, Hartmann N B, Eriksson E, Bjerg P L and Baun A 2010 Environmental benefits
453 and risks of zero-valent iron nanoparticles (nZVI) for in situ remediation: Risk mitigation or
454 trade-off? J Contam Hydrol 118, 165-183.
455 Guo Y, Yu H Y and Zeng E Y 2009 Occurrence, source diagnosis, and biological effect assessment of DDT
456 and its metabolites in various environmental compartments of the Pearl River Delta, South
457 China: A review. Environ Pollut 157, 1753-1763.
458 He F, Zhao D Y and Paul C 2010 Field assessment of carboxymethyl cellulose stabilized iron
459 nanoparticles for in situ destruction of chlorinated solvents in source zones. Water Res 44,
460 2360-2370.
461 Hinck J E, Norstrom R J, Orazio C E, Schmitt C J and Tillitt D E 2009 Persistence of organochlorine
462 chemical residues in fish from the Tombigbee River (Alabama, USA): Continuing risk to wildlife
463 from a former DDT manufacturing facility. Environ Pollut 157, 582-591.
464 Hitch R K and Day H R 1992 Unusual persistence of DDT in some Western USA soils. Bullten
465 Environmental Contaminant Toxicology 48, 259-264.
466 Jiang Z M, Lv L, Zhang W M, Du Q O, Pan B C, Yang L and Zhang Q X 2011 Nitrate reduction using
467 nanosized zero-valent iron supported by polystyrene resins: Role of surface functional groups.
468 Water Res 45, 2191-2198.
469 Karn B, Kuiken T and Otto M 2009 Nanotechnology and in situ remediation: A review of the benefits
470 and potential risks. Environ Health Persp 117, 1823-1831.
471 Kim S C, Yang J E, Ok Y S, Skousen J, Kim D G and Joo J H 2010 Accelerated metolachlor degradation in
472 soil by zerovalent iron and compost amendments. B Environ Contam Tox 84, 459-464.
473 Kirschling T L, Gregory K B, Minkley E G, Lowry G V and Tilton R D 2010 Impact of Nanoscale Zero Valent
474 Iron on Geochemistry and Microbial Populations in Trichloroethylene Contaminated Aquifer
475 Materials. Environmental Science & Technology 44, 3474-3480.
476 Li F B, Li X M, Zhou S G, Zhuang L, Cao F, Huang D Y, Xu W, Liu T X and Feng C H 2010 Enhanced reductive
477 dechlorination of DDT in an anaerobic system of dissimilatory iron-reducing bacteria and iron
478 oxide. Environ Pollut 158, 1733-1740.
479 Lien H L and Zhang W X 2007 Nanoscale Pd/Fe bimetallic particles: Catalytic effects of palladium on
480 hydrodechlorination. Appl Catal B-Environ 77, 110-116.
481 Lin C and Lin K S 2007 Photocatalytic oxidation of toxic organohalides with TiO2/UV: The effects of
482 humic substances and organic mixtures. Chemosphere 66, 1872-1877.
483 Liu Y Q, Majetich S A, Tilton R D, Sholl D S and Lowry G V 2005 TCE dechlorination rates, pathways, and
484 efficiency of nanoscale iron particles with different properties. Environ Sci Technol 39, 1338-
485 1345.
486 Lovley D R and Phillips E J P 1986 Availability of ferric iron for microbial reduction in bottom sediments
487 of the fresh-water Tidal Potomac River. Appl Environ Microb 52, 751-757.
488 Mertens J, Van Laer L, Salaets P and Smolders E 2011 Phytotoxic doses of boron in contrasting soils
489 depend on soil water content. Plant Soil 342, 73-82.
490 Mueller N C, Braun J, Bruns J, Černík M, Rissing P, Rickerby D and Nowack B 2012 Application of
491 nanoscale zero valent iron (NZVI) for groundwater remediation in Europe. Environ Sci Pollut R
492 19, 550-558.
493 OECD 2006 OECD Guideline for the testing of chemicals. Proposal for updating guideline 208,
494 Terrestrial Plant Test: 208: Seedling emergence and seedling growth test.
495 Otto M, Floyd M and Bajpai S 2008 Nanotechnology for site remediation. . Remediation 19, 99-108.
496 Phenrat T, Long T C, Lowry G V and Veronesi B 2009 Partial oxidation ("aging") and surface modification
497 decrease the toxicity of nanosized zerovalent iron. Environ Sci Technol 43, 195-200.
498 Quensen J F, Mueller S A, Jain M K and Tiedje J M 1998 Reductive dechlorination of DDE to DDMU in
499 marine sediment microcosms. Science 280, 722-724.
500 Rodante F, Marrosu G and Catalani G 1992 Thermal-analysis and kinetic-study of decomposition
501 processes of some pesticides. J Therm Anal 38, 2669-2682.

Royal Society of Chemistry 1996 The Dictionary of Substances and their Effects, volume 3. Ed. M L Richardson. pp 41-50. Athenaeam Press, Gateshead, Tyne & Wear, England.
Saleh N, Sirk K, Liu Y Q, Phenrat T, Dufour B, Matyjaszewski K, Tilton R D and Lowry G V 2007 Surface modifications enhance nanoiron transport and NAPL targeting in saturated porous media. Environmental Engineering Science 24, 45-57.
Satapanajaru T, Anurakpongsatorn P, Pengthamkeerati P and Boparai H 2008 Remediation of atrazine-contaminated soil and water by nano zerovalent iron. Water Air Soil Poll 192, 349-359.
Satapanajaru T, Comfort S D and Shea P J 2003 Enhancing metolachlor destruction rates with aluminum and iron salts during zerovalent iron treatment. J Environ Qual 32, 1726-1734.
Sayles G D, You G R, Wang M X and Kupferle M J 1997 DDT, DDD, and DDE dechlorination by zero-valent iron. Environ Sci Technol 31, 3448-3454.
Schrick B, Hydutsky B W, Blough J L and Mallouk T E 2004 Delivery vehicles for zerovalent metal nanoparticles in soil and groundwater. Chemistry of Materials 16, 2187-2193.
Shea P J, Machacek T A and Comfort S D 2004 Accelerated remediation of pesticide-contaminated soil with zerovalent iron. Environ Pollut 132, 183-188.
Smith E, Smith J, Naidu R and Juhasz A L 2004 Desorption of DDT from a contaminated soil using cosolvent and surfactant washing in batch experiments. Water Air Soil Poll 151, 71-86.
Tian H, Li J J, Mu Z, Li L D and Hao Z P 2009 Effect of pH on DDT degradation in aqueous solution using bimetallic Ni/Fe nanoparticles. Sep Purif Technol 66, 84-89.
Wang C B and Zhang W X 1997 Synthesizing nanoscale iron particles for rapid and complete dechlorination of TCE and PCBs. Environ Sci Technol 31, 2154-2156.
Wong M H, Leung A O W, Chan J K Y and Choi M P K 2005 A review on the usage of POP pesticides in China, with emphasis on DDT loadings in human milk. Chemosphere 60, 740-752.
Xu Y and Zhang W X 2000 Subcolloidal Fe/Ag particles for reductive dehalogenation of chlorinated benzenes. Ind Eng Chem Res 39, 2238-2244.
Yang S C, Lei M, Chen T B, Li X Y, Liang Q and 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 and 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, Wang C B and Lien H L 1998 Treatment of chlorinated organic contaminants with nanoscale bimetallic particles. Catal Today 40, 387-395.
Zhang Y, Li Y M and Zheng X M 2011 Removal of atrazine by nanoscale zero valent iron supported on organobentonite. Sci Total Environ 409, 625-630.
Zinovyev S S, Shinkova N A, Perosa A and Tundo P 2005 Liquid phase hydrodechlorination of dieldrin and DDT over Pd/C and Raney-Ni. Appl Catal B-Environ 55, 39-48.

Figure captions

Fig 1. Effects of nZVI in water (control), a freshly prepared nZVI suspension at 1 g L^{-1}, and from 5 consecutive leaching episodes of nZVI amended soil columns on (a) root and (b) shoot length of barley and flax germinated on filter paper. Means for the same plant species associated with the same letter are not significantly different (Student-t test, $p<0.05$, n=3)

546

547 **Fig 2.** Root and shoot length of (a) barley and (b) flax germinated in unamended soil
548 (control), soil receiving freshly prepared nZVI at 1 g L^{-1}, and in soil from columns treated
549 with nZVI after five leaching episodes. Within roots or shoots, means associated with the
550 same letter are not significantly different (Student-t test, $p<0.05$, n=3)

551

552 **Fig 3** Effects of the aqueous phase of nZVI (100 % supernatant fraction = 1g L^{-1}, and five 2-
553 fold dilutions) on seed germination (percentage noted for individual bars) and root
554 development of barley and flax germinated on filter paper (a) and in soil (b). Within species,
555 means associated with the same letter are not significantly different (Student-t test, $p<0.05$,
556 n=3)