Environmental Pollution 216 (2016) 419–427

Contents lists available at ScienceDirect

Environmental Pollution

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

The ecotoxic potential of a new zero-valent iron nanomaterial, designed for the elimination of halogenated pollutants, and its effect on reductive dechlorinating microbial communities☆

Andreas Schiwy [a], Hanna M. Maes [a], Daniel Koske [a], Mirkko Flecken [a], Kathrin R. Schmidt [b], Heico Schell [b], Andreas Tiehm [b], Andre Kamptner [c], Silke Thümmler [c], Helge Stanjek [d], Marc Heggen [e], Rafal E. Dunin-Borkowski [e], Jürgen Braun [f], Andreas Schäffer [a, g, h], Henner Hollert [a, g, h, i, *]

[a] Institute for Environmental Research, ABBt – Aachen Biology and Biotechnology, RWTH Aachen University, Worringerweg 1, Aachen 52074, Germany
[b] Department of Environmental Biotechnology, Water Technology Center, Karlsruher Str. 84, Karlsruhe 76139, Germany
[c] UVR-FIA GmbH, Chemnitzer Straße 40, 09599 Freiberg, Germany
[d] Clay and Interface Mineralogy, RWTH Aachen University, Bunsenstrasse 8, 52072 Aachen, Germany
[e] Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons, Forschungszentrum Juelich GmbH, 52425 Juelich, Germany
[f] VEGAS-Research Facility for Subsurface Remediation, University of Stuttgart, Pfaffenwaldring 61, 70569 Stuttgart, Germany
[g] College of Resources and Environmental Science, Chongqing University, 1 Tiansheng Road Beibei, Chongqing 400715, China
[h] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, 163 Qixia District Xianlin Avenue, Nanjing 210023, China
[i] College of Environmental Science and Engineering and State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, 1239 Siping Road, Shanghai 200092, China

ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form
16 May 2016
Accepted 19 May 2016
Available online 14 June 2016

Keywords:
nZVI
Nanoremediation
Ecotoxicology

ABSTRACT

The purpose of this study was to assess the ecotoxic potential of a new zero-valent iron nanomaterial produced for the elimination of chlorinated pollutants at contaminated sites. Abiotic dechlorination through the newly developed nanoscale zero-valent iron material and its effects on dechlorinating bacteria were investigated in anaerobic batch and column experiments. The aged, i.e. oxidized, iron material was characterization with dynamic light scattering, transmission electron microscopy and energy dispersive x-ray analysis, x-ray diffractometry and cell-free reactive oxygen measurements. Furthermore, it was evaluated in aerobic ecotoxicological test systems with algae, crustacean, and fish, and also applied in a mechanism specific test for mutagenicity. The anaerobic column experiments showed co-occurrence of abiotic and biological dechlorination of the common groundwater contaminant perchloroethene. No prolonged toxicity of the nanomaterial (measured for up to 300 days) towards the investigated dechlorinating microorganism was observed. The nanomaterial has a flake like appearance and an inhomogeneous size distribution. The toxicity to crustacean and fish was calculated and the obtained EC50 values were 163 mg/L and 458 mg/L, respectively. The nanomaterial showed no mutagenicity. It physically interacted with algae, which had implications for further testing and the evaluation of the results. Thus, the newly developed iron nanomaterial was slightly toxic in its reduced state but no prolonged toxicity was recorded. The aquatic tests revealed a low toxicity with EC50 values $\geq$ 163 mg/L. These concentrations are unlikely to be reached in the aquatic environment. Hence, this nanomaterial is probably of no environmental concern not prohibiting its application for groundwater remediation.

© 2016 Published by Elsevier Ltd.

1. Introduction

In environmental technology, engineered nanomaterials (ENMs) are increasingly applied in water treatment and remediation technologies (Bhawana and Fulekar, 2012; Karn et al., 2009;

☆ This paper has been recommended for acceptance by B. Nowack.

* Corresponding author. Institute for Environmental Research, ABBt – Aachen Biology and Biotechnology, RWTH Aachen University, Worringerweg 1, Aachen 52074, Germany.
E-mail address: henner.hollert@bio5.rwth-aachen.de (H. Hollert).

http://dx.doi.org/10.1016/j.envpol.2016.05.051
0269-7491/© 2016 Published by Elsevier Ltd.

O'Carroll et al., 2013; Patil et al., 2016). For example, nanoscaled zero-valent iron (NZVI) is used (Chang et al., 2005; Köber et al., 2014; Mueller and Nowack, 2010; Tratnyek and Johnson, 2006; Wang and Zhang, 1997; Zhang, 2003). Like its micro scale counterparts, NZVI is applied for remediation purposes to transform various environmental contaminants (Cantrell et al., 1995; Gillham and O'Hannesin, 1994; Wang and Zhang, 1997). Two example applications are the transformation of heavy metals (e.g. chromium) or organic compounds (e.g. chlorinated hydrocarbons (CHCs)) to non-mobile or non-toxic compounds in aquifers (Ponder et al., 2000; Zhang, 2003). Due to their huge surface nanomaterials possess an increased reactivity compared to their micro scale counterparts (Comba et al., 2011; Lowry and Johnson, 2004; Wang and Zhang, 1997; Zhang, 2003).

Besides the intended pollutant transformation, anaerobic ZVI corrosion leads to the formation of hydrogen, which may result in gas clogging and consequently limited hydraulic permeability of the aquifer. On the other hand, hydrogen is consumed by hydrogenotrophic bacteria, acting as a sink for hydrogen and mitigating the problem of gas clogging. Moreover, hydrogen is an excellent electron donor for supporting biological reductive dechlorination (Bruton et al., 2015; Lohner et al., 2011; Lohner and Tiehm, 2009; Velimirovic et al., 2015). Biological reductive dechlorination represents an environmental friendly, sustainable and cost-efficient option for removal of e.g. chlorinated compounds (Tiehm and Schmidt, 2011). The formulation ingredients (coatings, suspending agents, stabilization materials) of NZVI preparations can also serve as a source of electron donors supporting biological dechlorination (He et al., 2010; Kirschling et al., 2010; Su et al., 2012; Wei et al., 2012). Despite toxic effects of NZVI on natural microflora and dechlorinating microorganisms (Barnes et al., 2010; Bruton et al., 2015; Kumar et al., 2014b; Velimirovic et al., 2015; Xiu et al., 2010a, 2010b), the combined use of abiotic dechlorination with NZVI and biological dechlorination can result in synergetic effects, positive for the remediation process (Bruton et al., 2015; He et al., 2010; Kocur et al., 2015; Su et al., 2012). High pH conditions developing during anaerobic ZVI corrosion as well as acidification due to fermentation of formulation ingredients being unfavourable for biological processes have to be taken into account during field applications (Bruton et al., 2015; Velimirovic et al., 2015).

The innovative aspect of the application of NZVI is the remediation of contaminants in areas where classic remediation technologies cannot be applied due to difficulties to reach the subsurface contamination (Li et al., 2006). It is possible to inject the NZVI *in situ* directly into the source of contamination (Kanel et al., 2005). This approach can reduce the remediation duration as well as its costs (Li et al., 2006; Mueller et al., 2012).

There are two main problems concerning the possible use of NZVI for remediation purposes: 1) this material is not sufficiently tested for its safety, and 2) varying forms of NZVI are produced and their environmental behaviour and effects cannot directly be compared. The aim of this study was to assess the ecotoxic potential of a new NZVI and to evaluate its efficiency in eliminating chlorinated pollutants at contaminated sites. The newly developed NZVI used in this study were produced in a cost effective top down process by milling micro scale iron raw material to micrometre and nanometre scale (Köber et al., 2014). It has been applied at a pilot site and injected as a slurry with 10 g/L (Köber et al., 2014).

In this study, both reduced and oxidized (referred to as "aged";**SI** 1) materials were tested. A commercial product of reduced NZVI was tested in anaerobic batch experiments and the newly developed reduced NZVI was investigated in column studies to assess its toxic potential onto selected dechlorinating microorganisms as well as synergies between abiotic and biological dechlorination of the model contaminant perchloroethene (PCE). Newly developed aged NZVI was tested in an ecotoxical test battery including methods to investigate its safety at different biological levels (cell tests & organisms).

2. Material and methods

2.1. Effect on anaerobic-reductive biological dechlorination

Batch and column studies were performed under anaerobic conditions to study the interplay between abiotic dechlorination through reduced NZVI and anaerobic-reductive biological dechlorination. The response of the most important anaerobic-reductively dechlorinating microbial groups *Dehalococcoides* sp., *Desulfitobacterium* sp., *Desulfomonile tiedjei*, *Dehalobacter* sp., and *Desulfuromonas* sp. was monitored by nested polymerase chain reaction (PCR) with the method described in Kranzioch et al. (2013). The occurrence of these organohalide-respiring microorganisms can be taken as an indicator for the existence of biological dechlorination (Schmidt et al., 2006). Furthermore, the presence of the gene sequences coding for the four reductively dechlorinating enzymes (dehalogenases) pceA, tceA, bvcA and vcrA from *Dehalococcoides* sp. was assessed by PCR using the primers described in Behrens et al. (2008).

Batch experiments (with commercial NZVI) were conducted under anaerobic conditions in 2L bottles filled with a carbonate buffered mineral medium with 10 mg/L PCE. As reactive agents, the batch assays obtained either 0.5 g/L reduced NZVI Nanofer 25 (NANO IRON, Rajhrad, Czech Republic) or an actively dechlorinating laboratory microbial culture or NZVI plus microbial culture. Each batch assay was sampled for chloroethene analysis (PCE and its degradation products) via gas chromatography (measured as described in Lohner and Tiehm (2009)) at several points in time as well as for analysis of anaerobic-reductively dechlorinating microorganisms and enzymes by PCR at the start and the end (day 64) of the experiment.

Column experiments (with the newly developed NZVI material) were conducted under anaerobic conditions with sediment and groundwater containing the natural site microflora from a model site in Braunschweig, Germany (Köber et al., 2014). Groundwater with PCE as primary contaminant was obtained at several sampling dates and stored anaerobically at 3 °C. Due to the ongoing NZVI injection at the field site (Köber et al., 2014) the individual groundwater samples differed in their chloroethene composition as well as in their hydrochemical properties (e.g. pH). The column setup consisted of two parallel column systems (A and B) with two sequentially connected sediment columns (0.05 m diameter, 0.4 m height of packed bed), each. The flow-through of 0.34 L/d of groundwater was upwards from the columns A1 and B1 to the columns A2 and B2. The four columns were filled with a 1:1 mixture of field sediment (sandy material also containing fine grains smaller than 0.1 mm) and sand (Dorsilit 0.1–0.5 mm) resulting in a porosity of 0.4. The first columns additionally contained a 10 cm layer of pure sand at the inflow.

Column A1 was supplied with the newly developed NZVI material as anaerobic NZVI suspension prepared as described (Code MMA01 – B5) in Köber et al. (2014). At day 82 10 g NZVI suspended in 1 L groundwater were injected into column A1 at an injection rate of 6.9 L/d. At day 110 20 g NZVI suspended in 1 L groundwater were injected into column A1 at an injection rate of 12 L/d. For the third delivery at day 236 a gravel-packed pre-column filled with 18 g NZVI in suspension was connected to the inflow of column A1. Column B1 was supplied with the same amounts of suspension without NZVI.

The transport of the NZVI through the column was assessed visually. During the injection with higher flow rate an uneven

distribution of the NZVI was clearly visible in column A1 (Fig. **SI** 2). No further spreading of NZVI was observed during the column experiment with the normal flow rate. At a later stage of the experiment the column was blackened due to the precipitation of iron sulphide thus masking the location of the NZVI. During 300 days test duration the influent of the whole column system as well as the effluents of each sediment column were regularly analysed for the concentrations of chloroethenes (PCE and its degradation products) and for the presence of anaerobic-reductively dechlorinating microorganisms and enzymes. Results are shown for the influent as well as for the effluents of the second sediment columns (A2 and B2).

2.2. Sample preparation for characterization and ecotoxicity testing

To prepare oxidized referred to as "aged" NZVI, an aliquot of the reduced newly developed NZVI nanomaterial in its original suspension prepared as described (Code MMA01 – B5) in Köber et al. (2014) was transferred into a glass vessel and dried in an oxygen atmosphere at 80 °C in an oven over night. For the characterization and the aquatic toxicity tests, the air dried iron powder was ground as the material covered the glass surface and formed a homogenous layer. Each dilution of the aged NZVI in 100 mL ultrapure water or corresponding test medium was weighed individually on an ultrabalance XP6U (Mettler-Toledo GmbH, Gießen, Germany). Then, the samples were sonicated with a HD 2200 ultrasonic probe with a VS70T tip (Bandelin, Berlin, Germany; 200 W; 0.8″/0.2″ pulse/pause; 5 min; 100% power) inserted into the liquid approx. 1 cm above the bottom of the beaker (250 mL, short form). After sonication, the suspension was introduced in the subsequent test without delay.

2.3. Characterization of aged NZVI

2.3.1. Transmission electron microscopy (TEM) and energy dispersive x-ray analysis (EDX)

For the TEM investigations a suspension in ultrapure water with a concentration of 100 mg/L was prepared and 3 μL of this suspension were transferred onto a carbon coated copper TEM grid (Plano, Wetzlar, Germany). Following, it was air dried for storage and analysis. Subsequently, the nanomaterial was investigated with a Philips CM 20 FEG (Philips electronics, Eindhoven, Netherlands) transmission electron microscope operated at 200 kV using a Philips double-tilt sample holder. The images were collected with a GATAN CCD camera (Gatan Inc., Pleasanton, CA, USA). Selected objects were tilted with respect to the electron beam and a series of images was collected to estimate the three-dimensional shape and thickness of these objects. Additionally, for selected objects an EDX analysis was conducted using a connected EDAX spectrometer (EDAX Inc., Mahwah, NJ, USA).

2.3.2. X-ray diffractometry (XRD)

The XRD investigations were conducted with the aged NZVI with a MC 9300 (HUBER Diffraktionstechnik GmbH & Co. KG, Rimsting, Germany) using Co Kα-radiation produced at 45 kV and 35 mA. The angles were from 20° to 135°. Quantitative phase analysis was performed by Rietveld refinement. The resulting data were analysed with BGMN software 5.1.3.

2.4. Cell free reactive oxygen species (ROS) detection

A cell free reactive oxygen species (ROS) detection was conducted according to the procedure by Simon et al. (2014) and Rushton et al. (2010). This assay quantifies the amount of ROS in the medium whereas other assays measure the ROS within animal cells. The preparation is detailed in **SI** 3. The reaction mix was incubated with sonicated aged NZVI suspensions at 10, 100, 450 and 1000 mg/L and sonicated medium as a process control. As a positive control H_2O_2 was applied at concentrations of 1, 2, 5, 10, 20 and 40 μM. Unsonicated medium was used as blank. The samples were incubated in darkness at 37 °C for 15 min in technical triplicates in 6-well plates (TPP Techno Plastic Products AG, Trasadingen, Switzerland). The formation of the fluorescent product was detected by excitation at 485 nm and emission at 530 nm with a plate reader Infinite M200 (Tecan Group Ltd., Männedorf, Switzerland).

2.5. Ecotoxical test battery of aged NZVI

2.5.1. Algae growth inhibition test

The algae growth inhibition test with *Desmodesmus subspicatus* was conducted based on the OECD guideline 201 (OECD, 2011), Hafner et al. (2015) and Altenburger et al. (2008). The triplicate test suspensions (0, 6, 11, 23, 45, 90 mg/L) were prepared in a 24-well plate by sonicating separately weighed aged NZVI in ultrapure water before the addition of the algae. Afterwards, 10-fold concentrated growth medium was added to obtain a 1-fold concentrated growth medium with the nutrients necessary for the algae. Before the start of the test, the chlorophyll fluorescence of the preculture was determined. The excitation of light with a wavelength of 465 nm and the emission of light with a wavelength of 685 nm were measured with a plate reader Infinite M200 (Tecan Group Ltd., Männedorf, Switzerland). The fluorescence is correlated to algal cell number which was calibrated in advance. The initial cell density in all wells was calculated to be 5000 cells/mL in a volume of 2 mL. Every 24 h, the chlorophyll fluorescence was determined with the plate reader, the cell number was derived, and the growth rate was calculated and compared to the control. Additionally, a microscopic viability evaluation of the algae cells was conducted for selected samples after 72 h. Therefore, the algae were transferred from the selected wells onto a cover glass and microscopically evaluated with a 1000-fold magnification by oil immersion microscopy. A fluorescent light illumination in combination with filters for 485 nm excitation and 685 nm emission were applied.

2.5.2. Daphnia acute immobilisation test

The acute crustacean immobilisation test with *Daphnia magna* was conducted based on OECD guideline 202 (OECD, 2004) and Wyrwoll et al. (2016). The setup consisted of four replicates per concentration with five daphnids in each test beaker (10 mL; 20 °C in darkness). The aged NZVI were evaluated at 10, 25, 50, 100, 500 and 1000 mg/L. Additionally, a negative control (NC) with only the artificial water or a sonication treatment control (TC) without aged NZVI was included. After 24 h and 48 h, the number of immobile animals was noted. The percentage of immobile daphnids was calculated and effect concentrations (EC) were determined using probit analysis of the data using the ToxRat software package (ToxRat Solutions GmbH, Alsdorf, Germany).

2.5.3. Fish embryo toxicity test

The assay with zebrafish (*Danio rerio*) was carried out based on OECD guideline 236 (OECD, 2013) with modifications given by Peddinghaus et al. (2012) and Schiwy et al. (2015). The nanomaterials were tested in duplicates in seven concentrations (16, 31, 63, 125, 250, 500 and 1000 mg/L) prepared with artificial water with a static test design. 3,4-dichloroaniline (Sigma–Aldrich GmbH, Steinheim, Germany; 3.7 mg/L) served as a positive control, artificial water as a negative control, and sonicated artificial water as a treatment control (TC). To each test concentration, ten eggs that reached the 8-cell stadium were added. Then, each egg was

transferred into a volume of 2 mL on a well of a 24-well plate. Each plate was covered with a gas-permeable foil (Renner, Darmstadt, Germany) and incubated at 26 ± 1 °C for the exposure period of 96 h post fertilization (hpf). Evaluation of the test was carried out with an inverted microscope at 40-fold and a 100-fold magnifications. Every 24 hpf, the lethal endpoints (coagulation of the embryo, non-detachment of the tail, non-detection of the heartbeat, and lack of somites) were recorded. As an additional endpoint, the hatching of the embryos was included. The percentage of effect was calculated and an EC50 was determined using Graphpad Prism 6 with least square fit analysis.

2.5.4. Ames fluctuation test

For measurement of the mutagenic potential, a modification of the standard Ames assay was conducted according to ISO guideline 11350 (ISO, 2012), Reifferscheid et al. (2012) and Heger et al. (2012). Mutagenicity was investigated using the *Salmonella typhimurium* strains TA98 and TA100 with the Ames fluctuation test (with and without the metabolic activation S9 mix). Detection of the reverse mutation was realized by using a pH indicator dye in the test media and counting the number of positive wells (wells with revertant growth). Prior to seeding into a 24-well test plate, optical density (OD) was measured photometrically at a wave length of 595 nm. The concentration and absorption relationship of the reference compound formazine called Formazine Attenuation Units (FAU) was used as an estimate for bacterial density. The bacterial density was adjusted to 450 FAU. For exposure, either sample or controls were added to a 24-well plate. Positive controls were inserted according to the guidelines, the negative control consisted of ultrapure water, and validity criteria were checked. The investigated aged NZVI was sonicated in ultrapure water and added to 10× medium. Finally, the bacteria in exposure medium were added. Test concentrations investigated were 5, 10, 20, 40, 50 and 100 mg/L. After 100 min, exposure was terminated by adding the reverse indicator medium to each well. Subsequently, 50 mL of each test concentration with the added reverse indicator medium were added to 48-wells of a 384-well plate and incubated at 30 °C in darkness for 48 h.

3. Results

3.1. Effect on anaerobic-reductive biological dechlorination

Anaerobic batch studies showed a removal of 28%, 7.7% and 24% of the initial PCE concentration after 64 days for the assays with reduced NZVI, with microbial culture and with reduced NZVI plus microbial culture, respectively (Table 1, Fig. **SI** 7). In view of possible variability of the batch assays as well as the unavoidable measuring error the assays with reduced NZVI and with reduced NZVI plus microbial culture performed equally. These results demonstrated that due to the lack of electron donors, the microbial culture alone was hardly able to perform anaerobic-reductive dechlorination despite of the presence of *Dehalococcoides* sp. The other dechlorinating organisms *Desulfitobacterium* sp., *Desulfomonile tiedjei*, *Dehalobacter* sp., and *Desulfuromonas* sp. were not detected. The needed electron donors were provided by the addition of reduced NZVI. From the formation of cDCE (Fig. **SI** 7) and the increase of the number of detected dechlorinating enzymes of *Dehalococcoides* sp. (Table 1) at the end of the experiment in the batch with reduced NZVI plus microbial culture it can be concluded that i) NZVI was not inhibiting *Dehalococcoides* sp. ii) the dechlorinating microorganisms were proliferating and iii) the microorganisms contributed to the reductive dechlorination observed.

During the first 100 days of the anaerobic column experiment the effluents showed only minor dechlorination (**SI** 4) and a lower or equal number of detected groups of dechlorinating microorganisms compared to the influent (Fig. 1). Due to biodegradation of the organic suspending agents pH values at the field site (influent between day 70 and day 100) as well as in the columns (measurable in the effluents after the NZVI injection at day 110 and 236) dropped (Fig. **SI** 5). This acidification and the formation of ethanol and methanol indicated fermentation processes (Fig. **SI** 5 & Fig. **SI** 6). A pH increase caused by anaerobic ZVI corrosion was not measured. After the acidification declined, biological dechlorination was observed (sampling dates 180, 244 and 294 in **SI** 4). A corresponding increase of the number of PCR detected DNA sequences of dechlorinating microorganisms and enzymes in the effluents compared to the influent was then observed as well (Fig. 1).

Due to the changing chloroethene concentrations within the groundwater sampled at different days all effluent results need to be compared to the corresponding influent concentrations as it is done in Fig. SI 4. In both column systems, the metabolites trichloroethene and *cis*-1,2-dichloroethene were formed. Further degradation to vinyl chloride and ethene was not observed. Except for the last sampling date, showing a higher degree of dechlorination for the column A with NZVI, there was no significant difference in dechlorination between the columns with and without NZVI. There was no significant difference in the amount of PCR detected DNA sequences of dechlorinating microorganisms and enzymes between the column system with and without NZVI, neither. Corresponding to the results of the batch studies, the column studies again demonstrated that NZVI did not exert a measurable inhibition of the microbial activity being relevant for biological dechlorination in the subsurface environment.

Table 1
Anaerobic batch studies - PCR detection of DNA sequences of *Dehalococcoides* sp. and its four enzymes and percent of perchloroethene (PCE) degraded at the start and the end (64 days) of the batch studies (-: not detected; +: detected).

Batch		*Dehalococcoides* sp.	pceA	tceA	bvcA	vcrA	PCE degraded [%]
NZVI	start	-	-	-	-	-	-
	end	-	-	-	-	-	28
microbial culture	start	+	-	-	-	+	-
	end	+	-	-	-	+	7.7
NZVI + microbial culture	start	-	-	-	-	+	-
	end	+	+	+	-	+	24

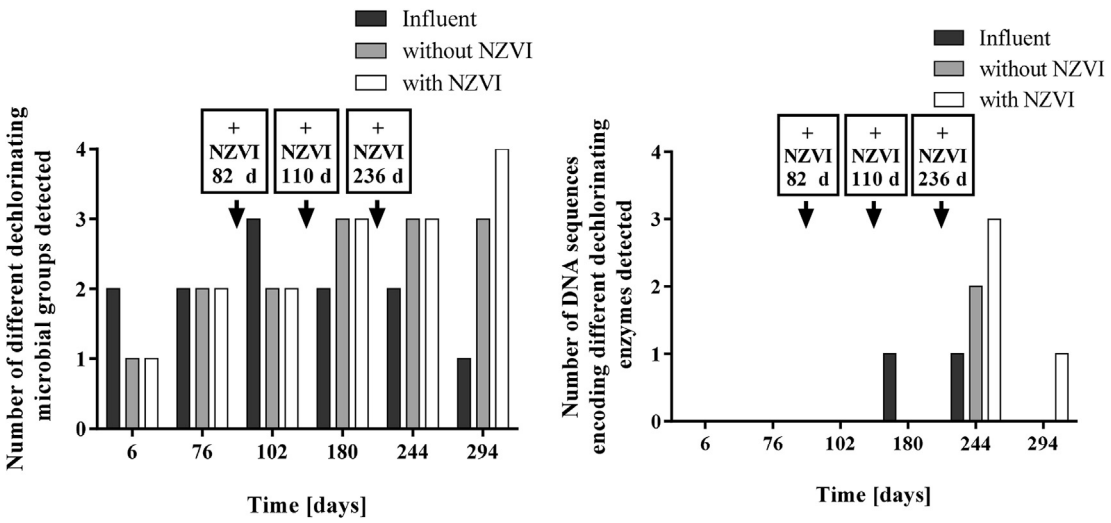

Fig. 1. Anaerobic column experiments - Number of different anaerobic-reductively dechlorinating microbial groups detected with PCR (five in total tested) in the influent and in the effluents of columns without and with NZVI treatment (left). Number of DNA sequences encoding different anaerobic-reductively dechlorinating enzymes of *Dehalococcoides* sp. detected with PCR (four in total tested) in the influent and in the effluents of columns without and with NZVI treatment (right).

3.2. Characterization of aged NZVI

3.2.1. Transmission electron microscopy (TEM) and energy dispersive x-ray analysis (EDX)

The results show various particle sizes between some tens of nanometres and some micrometres for the aged nanomaterial (**SI** 8). No statistical analysis was conducted due to the heterogeneity and no uniform morphology of the aged NZVI. To estimate the thickness of a selected particle, the sample was tilted stepwise and a series of micrographs was subsequently taken (**SI** 9). The analysis revealed a sample thickness below 100 nm. The composition of the particle was analysed by EDX and the presence of iron was verified (**SI** 9).

3.2.2. X-ray diffractometry (XRD)

The results of the XRD measurement indicate that the aged nanomaterial consist mainly of the allotrope α-Fe (ferrite) with a percentage of over 81% and the remaining part of 19% γ-Fe (austenite). Besides these both modifications no additional phases were detected. The broad peaks indicated both size and strain contributions. From the size term mean coherence lengths of 62 ± 22 nm were calculated, which corresponds well with the particle dimensions determined by TEM.

3.3. Cell free reactive oxygen species (ROS) detection

The results of the cell free ROS measurement showed a dose response for the positive control H_2O_2 and the aged NZVI (Fig. 2). The results indicate that high concentrations of the aged NZVI are correlated with the formation of ROS. The treatment control with sonicated medium as well as the aged NZVI concentrations of 10 mg/L and 100 mg/L showed a response comparable to the blank. The response of 450 mg/L and 1000 mg/L NZVI was approximately 4-fold higher.

3.4. Ecotoxicity testing of aged NZVI

3.4.1. Algae growth inhibition test

The algae growth inhibition test did not show any evaluable results (data not shown). No growth could be recorded for the aged nanomaterial dilutions by measuring the chlorophyll fluorescence

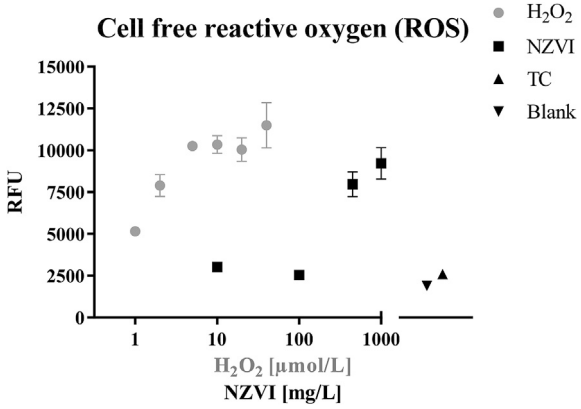

Fig. 2. ROS detection - Fluorescence response of the cell free ROS 2′,7′-dichlorodihydrofluorescin diacetate (DCFDA) assay with the aged NZVI and H_2O_2 as reference. The whiskers represent the standard deviation on the mean of three technical replicates. (RFU: Relative fluorescence units; TC: Treatment control; Blank: Untreated control; NZVI: Nanoscale zero-valent iron).

with a plate reader. The nanomaterial associated to algae and sedimented within 20 min forming a settled layer in the middle of the well plate. A microscopic investigation of the sample showed that the algae were viable (Fig. 3) and incorporated in a cluster of aged NZVI.

3.4.2. Daphnia acute immobilisation test

The results of the *Daphnia* tests showed a dose response relationship between exposure to the aged NZVI and daphnid immobility. The EC50 value of the pooled tests was determined and amounted to 163 mg/L (Fig. 4). The dissolved oxygen levels at the beginning and end of the tests showed a trend dependent on the aged NZVI concentration (**SI** 10). The higher the nanomaterial concentration the lower the oxygen levels recorded indicating that the nanomaterial was not fully oxidized. Similarly to the algae test, the nanomaterial sedimented visibly within 20 min. Some of the *Daphnia* were coated with the material and their movement was impaired. After molting, some of the *Daphnia* shed their carapax free of the associated nanomaterial and could move again without any impairment.

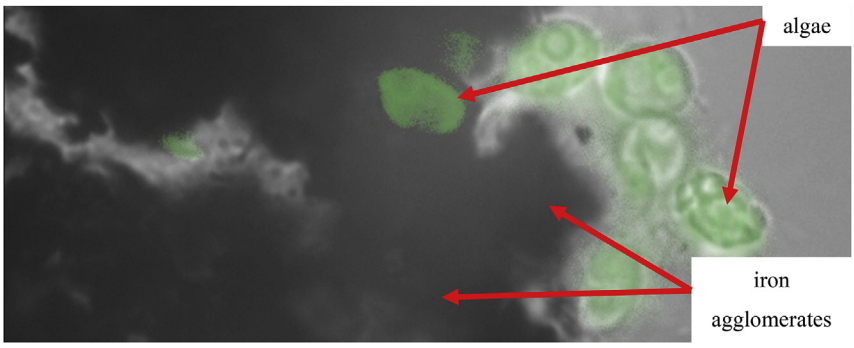

Fig. 3. Algae growth inhibition - Algae cells associated with clusters of aged NZVI made visible with oil immersion microscopy (1000-fold magnification) with fluorescence filters. Viable algae cells are indicated in green.

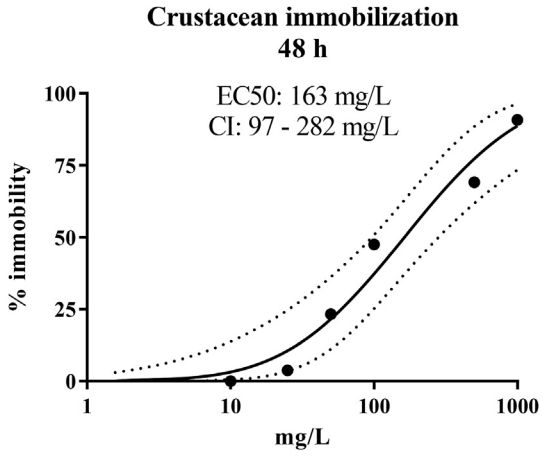

Fig. 4. Crustacean immobilisation - Percentage immobility of *Daphnia magna* after exposure to aged iron nanomaterial at concentrations of 10, 25, 50, 100, 500 and 1000 mg/L for 48 h. The 95% confidence interval is indicated (dashed lines).

3.4.3. Fish embryo toxicity test

A dose dependent effect on the fish embryos was observed upon exposure to the aged NZVI. An EC50 of 458 mg/L 96 hpf was calculated (Fig. 5). Again, the nanomaterial sedimented quickly and covered the fish eggs (Fig. 6). The eggs showed an increasing association with the aged iron nanomaterial throughout the experiment. At concentrations higher than 62 mg/L the bottom of the wells was completely covered and hindered microscopic evaluation of the heart beat and blood circulation during the first 48 hpf. With the help of an additional light source the investigation could be partially conducted (Fig. 6).

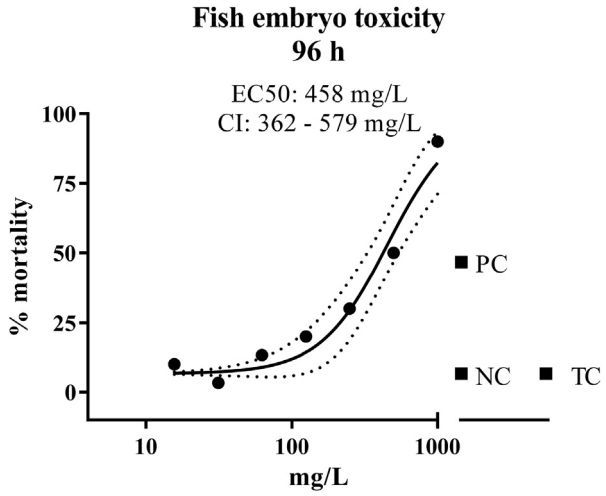

Fig. 5. Fish embryo toxicity - Percentage mortality of zebrafish (*Danio rerio*) embryos after exposure to aged iron nanomaterial at concentrations of 16, 31, 63, 125, 250, 500 and 1000 mg/L at 96 hpf. The 95% confidence interval is indicated (dashed lines). TC: sonication treatment control; PC: positive control treatment with 3.7 mg/L 3,4-Dichloranilin; NC: negative control untreated control.

3.4.4. Ames fluctuation test

The results of the Ames fluctuation assays showed a bacterial toxicity in the TA 100 strain at aged NZVI concentrations higher than 50 mg/L. The assay did not indicate any mutagenic potential of the aged NZVI with or without the S9 mix with both tester strains (TA 98 and TA 100).

4. Discussion

4.1. Effect on anaerobic-reductive biological dechlorination

The anaerobic laboratory studies showed that biological dechlorination of chloroethenes was stimulated due to the microbial degradation of the suspending agents of NZVI. Long term inhibitory or toxic effects of NZVI on dechlorinating microorganisms were not observed. These findings were corroborated by the results of a field test published by Köber et al. (2014): PCR results as well as metabolite formation pointed to the occurrence of biological dechlorination in the aquifer after NZVI injection despite transient unfavourable pH values due to the fermentation of the suspending agents.

Toxic effects of NZVI on dechlorinating microorganisms were observed in some laboratory studies (Tilston et al., 2013; Zabetakis et al., 2015). Field applications of NZVI did not show any prolonged negative effects on the microbial activity but even a stimulation of biological dechlorination (He et al., 2010; Kocur et al., 2015; Su et al., 2012; Wei et al., 2012) supporting the results of our study. Aging of NZVI as well as interaction with natural organic matter was shown to mitigate the toxicity of the NZVI (Baalousha, 2009; Bruton et al., 2015; Jang et al., 2014; Phenrat et al., 2009).

In order to benefit from synergies between the abiotic and biotic processes, biological processes (biodegradation as well as hydrogen consumption) should be considered during field application of NZVI materials. Transient toxic effects as well as unfavourable pH-effects of abiotic and biotic processes have to be taken into account.

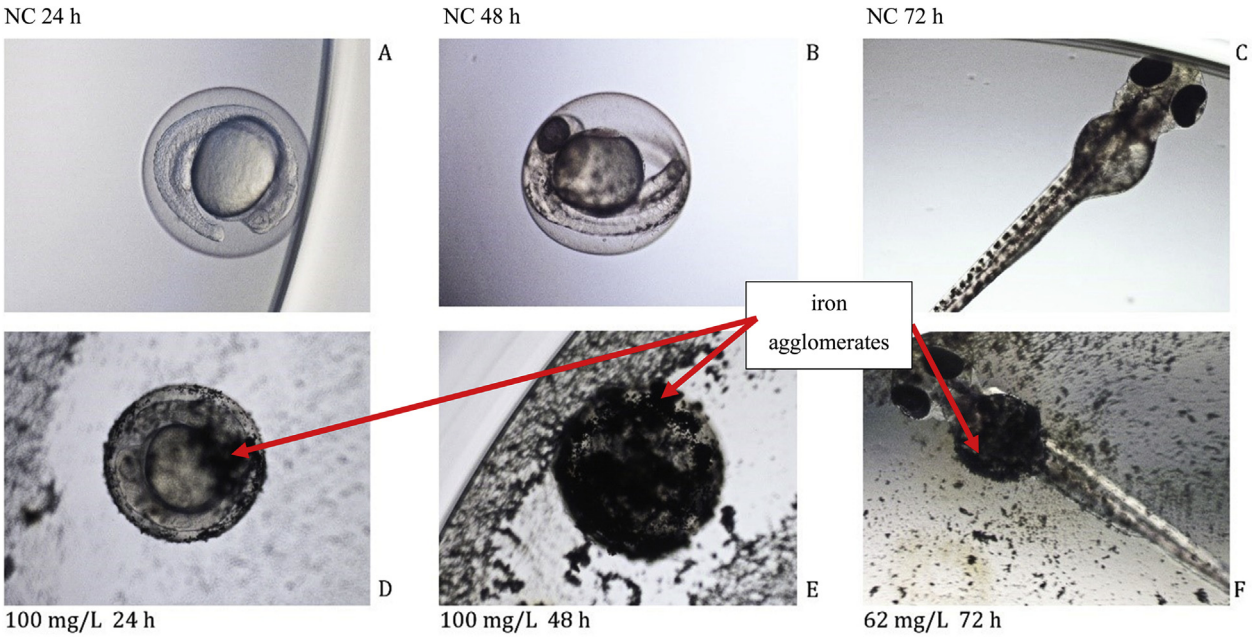

Fig. 6. Fish embryo toxicity - Association of the aged NZVI to the chorion and larvae. The upper row depicts the developmental stages of the negative control (NC) at the time points of 24 h (A), 48 h (B) and 72 h (C). The lower row depicts the association of the aged NZVI to the chorion (D,E) and to the larvae (F) at the same time points.

4.2. Material characterization of aged NZVI

The physical characterization of the aged NZVI was an important aspect of the study, since the physical state of the nanomaterial may have an impact on its behaviour in the aquatic environment. The characterization was conducted with dynamic light scattering (**SI** 11), transmission electron microscopy (TEM – 3.2 & **SI** 8–9) with energy dispersive x-ray analysis (EDX), and X-ray diffractometry (XRD). The investigation by TEM shows an inhomogeneous size distribution of the material consisting of particles and agglomerates in the nanometre unto the micrometre range (**SI** 8). This heterogeneous size distribution is a result of the milling process (Köber et al., 2014). The results of the XRD measurement showed that the aged material consisted of ferrite (81%) and austenite (19%). The aged NZVI investigated were air dried and hence an iron oxide layer was expected. However, none was detected by XRD. A possible explanation is that the iron oxide layer is too thin for detection as it was reported to be as thin as 3 nm–5 nm (Efecan et al., 2009; Kumar et al., 2014a). The aspect of production, composition and the stability in suspension are discussed in detail in **SI** 12. Since we dealt with limitations in the conducted characterization techniques, we used the nominal NZVI concentrations.

4.3. Ecotoxicity testing of aged NZVI

4.3.1. Algae test

The algae growth inhibition test was not applicable for testing the iron nanomaterials as the material associated with the algae. The formation of clusters of the material occurred as a result of the particles morphology as well as the salt concentration of the medium. These clusters incorporated the algae and sedimented onto the bottom of the vessel. Thus, the effects on algal growth in our study were rather based on the sedimentation and shading than on a toxic mode of action. In this study, no EC50 values could be calculated. In the study by Keller et al. (2012), the uncoated NZVI Nanofer25 showed a similar agglomerating and sedimenting behaviour and was excluded from their investigation with algae. In other studies e.g. with titanium dioxide nanoparticles, the effect of shading by the nanomaterials or change of light quality could be excluded (Aruoja et al., 2009; Hartmann et al., 2010; Hund-Rinke and Simon, 2006). Hence, a validation of the test system for the nanomaterial of interest is crucial to distinguish between physical interaction and toxic effects.

4.3.2. Daphnia acute immobilisation test

The results of our test with daphnids indicate a toxicity of the aged NZVI with a calculated EC50 of 163 mg/L. The toxicity recorded was higher than the toxicity to *Daphnia magna* reported by Marsalek et al. (2012) who calculated an EC50 for NZVI higher than 1000 mg/L. A study that compared the toxicity of uncoated and coated NZVI to aquatic organism showed that the crustacean *Daphnia magna* was more sensitive to the NZVI that the fish (Keller et al., 2012). These results are in accordance with our study as *Daphnia magna* showed to be approx. 3-fold more sensitive to aged NZVI than the fish *Danio rerio*.

4.3.3. Fish embryo toxicity test

The presented results of the fish embryo toxicity test were comparable to results from former studies with fish and iron nanomaterials. Especially, for oxidized iron nanomaterials Weil et al. (2015) showed no toxicity to the fish *Danio rerio* in a concentration up to 100 mg/L. This results is comparable to our research. In our study, the aged NZVI was more toxic to *Danio rerio* with an EC50 of 458 mg/L than in the study by Marsalek et al. (2012) to the fish *Poecilia reticulate* with an EC50 of 2500 mg/L. In another study with medaka, 40% mortality was reported for an iron nanomaterial at a nominal concentration of 100 mg/L (Chen et al., 2012). Li et al. (2009) reported for the same fish and iron nanomaterial deleterious effects on gills and intestine in histological investigations at a concentration of 5 mg/L. These studies reported a comparably quick agglomerating and sedimenting behaviour of the nanomaterials. Our evaluation of various endpoints in the fish embryo toxicity test indicates that for this nanomaterial the hatching rate after 72 hpf (EC50 337 mg/L, data not shown) is a

more sensitive endpoint than mortality (EC50 458 mg/L). However, more scattering in the hatching data compared to mortality data was observed. This is a consequence of the association of the nanomaterials with the chorion of the embryos. As the embryos were covered, no effects on the embryo could be recorded until the end of the experiment. The average time point of hatching at 72 hpf is 24 h earlier than the end of the test at 96 hpf.

4.3.4. Ames fluctuation test

The Ames fluctuation test did not show any mutagenicity of NZVI, which is consistent with the results of the study from Barzan et al. (2014). The possible mode of action for NZVI to trigger mutagenicity would be due to incorporation in cells and its ROS generating properties (Fu et al., 2014). However, as shown in Fig. 2, the concentrations to produce ROS and elucidate toxicity were higher than 450 mg/L. Hence, any negative ROS effects of the NZVI would act before the onset of mutagenicity.

4.3.5. Modes of NZVI toxicity

The factors influencing NZVI toxicity are size as well as the reactions of the iron nanomaterial including corrosion and transformation processes, ferrous ions release, oxygen consumption and generation of reactive oxygen species (Scown et al., 2010; Zhu et al., 2012)., The production of oxidation enhancing entities such as hydroxides, carbonates and sulfates is facilitated (Liu et al., 2015; Stumm and Morgan, 2012). Next to oxygen depletion, ferrous ions might be responsible for toxicity as was shown in various studies (Chen et al., 2012; Keenan et al., 2009; Keller et al., 2012; Vuori, 1995). Reactive oxygen species (ROS) can also be the cause of toxic effects. It is known that transition metals participating in one-electron oxidation-reduction reactions like iron lead to the formation of ROS (Schrand et al., 2010; Yan et al., 2013; Yin et al., 2012). In our study, acute toxicity was detected at 50 mg/L of aged NZVI exposure. At this concentration, no elevated ROS signals levels were recorded. However, various studies reported deleterious effects to organisms originating in direct contact to NZVI or ROS generated by engineered nanomaterials (Chen et al., 2012, 2013; Li et al., 2009; Ma et al., 2013). We believe that the combination of oxygen depletion, ferrous ion toxicity, ROS-related toxicity and nanomaterial specific toxicity contribute to the observed toxicity.

5. Conclusion

Regarding environmental risk assessment of NZVI applied for groundwater remediation, this nanomaterial will be applied at high (effective) concentrations only in limited areas. Thus, the NZVI will reach a wider environment only at low concentrations that have only transient toxic and pH effects with no prolonged consequences according to our study. Hence, this nanomaterial is probably of no environmental concern not prohibiting its application for groundwater remediation. During field application synergetic effects of abiotic dechlorination and biological dechlorination as well as microbial hydrogen consumption can enhance the efficiency of NZVI groundwater remediation. The results of our investigations can be used in the international discussion on the use of standard test procedures for the testing of nanomaterials.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This work is part of the joint project NAPASAN (Nanoparticles for ground water remediation) which was funded by German Federal Ministry for Education and Research (BMBF) under the Grant Number 03X0097 within the research program NanoNature (Nanotechnologies for Environmental Protection—Value and Impact) which is part of the framework program WING (Material Innovations for Industry and Society).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.05.051.

References

Altenburger, R., Schmitt-Jansen, M., Riedl, J., 2008. Bioassays with unicellular algae: deviations from exponential growth and its implications for toxicity test results. J. Environ. Qual. 37, 16–21.
Aruoja, V., Dubourguier, H.C., Kasemets, K., Kahru, A., 2009. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. Sci. Total Environ. 407, 1461–1468.
Baalousha, M., 2009. Aggregation and disaggregation of iron oxide nanoparticles: influence of particle concentration, pH and natural organic matter. Sci. Total Environ. 407, 2093–2101.
Barnes, R.J., Riba, O., Gardner, M.N., Singer, A.C., Jackman, S.A., Thompson, I.P., 2010. Inhibition of biological TCE and sulphate reduction in the presence of iron nanoparticles. Chemosphere 80, 554–562.
Barzan, E., Mehrabian, S., Irian, S., 2014. Antimicrobial and genotoxicity effects of zero-valent iron nanoparticles. Jundishapur J. Microbiol. 7, e10054.
Behrens, S., Azizian, M.F., McMurdie, P.J., Sabalowsky, A., Dolan, M.E., Semprini, L., Spormann, A.M., 2008. Monitoring abundance and expression of "*Dehalococcoides*" species chloroethene-reductive dehalogenases in a tetrachloroethene-dechlorinating flow column. Appl. Environ. Microbiol. 74, 5695–5703.
Bhawana, P., Fulekar, M., 2012. Nanotechnology: remediation technologies to clean up the environmental pollutants. Res. J. Chem. Sci. 2231, 606X.
Bruton, T.A., Pycke, B.F.G., Halden, R.U., 2015. Effect of nanoscale zero-valent iron treatment on biological reductive dechlorination: a review of current understanding and research needs. Crit. Rev. Environ. Sci. Technol. 45, 1148–1175.
Cantrell, K.J., Kaplan, D.I., Wietsma, T.W., 1995. Zero-valent iron for the *in situ* remediation of selected metals in groundwater. J. Hazard. Mater. 42, 201–212.
Chang, M.-C., Shu, H.-Y., Hsieh, W.-P., Wang, M.-C., 2005. Using nanoscale zero-valent iron for the remediation of polycyclic aromatic hydrocarbons contaminated soil. J. Air & Waste Manag. Assoc. 55, 1200–1207.
Chen, P.J., Tan, S.W., Wu, W.L., 2012. Stabilization or oxidation of nanoscale zerovalent iron at environmentally relevant exposure changes bioavailability and toxicity in medaka fish. Environ. Sci. Technol. 46, 8431–8439.
Chen, P.J., Wu, W.L., Wu, K.C., 2013. The zerovalent iron nanoparticle causes higher developmental toxicity than its oxidation products in early life stages of medaka fish. Water Res. 47, 3899–3909.
Comba, S., Di Molfetta, A., Sethi, R., 2011. A comparison between field applications of nano-, micro-, and millimetric zero-valent iron for the remediation of contaminated aquifers. Water Air Soil Pollut. 215, 595–607.
Efecan, N., Shahwan, T., Eroğlu, A.E., Lieberwirth, I., 2009. Characterization of the uptake of aqueous Ni^{2+} ions on nanoparticles of zero-valent iron (nZVI). Desalination 249, 1048–1054.
Fu, P.P., Xia, Q., Hwang, H.M., Ray, P.C., Yu, H., 2014. Mechanisms of nanotoxicity: generation of reactive oxygen species. J. Food Drug Anal. 22, 64–75.
Gillham, R.W., O'Hannesin, S.F., 1994. Enhanced degradation of halogenated aliphatics by zero-valent iron. Ground Water 32, 958–967.
Hafner, C., Gartiser, S., Garcia-Kaufer, M., Schiwy, S., Hercher, C., Meyer, W., Achten, C., Larsson, M., Engwall, M., Keiter, S., Hollert, H., 2015. Investigations on sediment toxicity of German rivers applying a standardized bioassay battery. Environ. Sci. Pollut. Res. Int. 22, 16358–16370.
Hartmann, N.B., Von der Kammer, F., Hofmann, T., Baalousha, M., Ottofuelling, S., Baun, A., 2010. Algal testing of titanium dioxide nanoparticles- Testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology 269, 190–197.
He, F., Zhao, D., 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.
Heger, S., Bluhm, K., Agler, M.T., Maletz, S., Schäffer, A., Seiler, T.-B., Angenent, L.T., Hollert, H., 2012. Biotests for hazard assessment of biofuel fermentation. Energy Environ. Sci. 5, 9778.
Hund-Rinke, K., Simon, M., 2006. Ecotoxic effect of photocatalytic active nanoparticles (TiO_2) on algae and daphnids. Environ. Sci. Pollut. Res. Int. 13, 225–232.
ISO, 2012. 11350-Water Quality - Determination of the Genotoxicity of Water and Waste Water - Salmonella/microsome Fluctuation Test (Ames Fluctuation Test), ISO 11350. ISO International Organization for Standardization, p. 42.
Jang, M.H., Lim, M., Hwang, Y.S., 2014. Potential environmental implications of nanoscale zero-valent iron particles for environmental remediation. Environ. Health Toxicol. 29, e2014022.

Kanel, S.R., Manning, B., Charlet, L., Choi, H., 2005. Removal of arsenic(III) from groundwater by nanoscale zero-valent iron. Environ. Sci. Technol. 39, 1291–1298.
Karn, B., Kuiken, T., Otto, M., 2009. Nanotechnology and *in situ* remediation: a review of the benefits and potential risks. Environ. Health Perspect. 117, 1813–1831.
Keenan, C.R., Goth-Goldstein, R., Lucas, D., Sedlak, D.L., 2009. Oxidative stress induced by zero-valent iron nanoparticles and Fe(II) in human bronchial epithelial cells. Environ. Sci. Technol. 43, 4555–4560.
Keller, A.A., Garner, K., Miller, R.J., Lenihan, H.S., 2012. Toxicity of nano-zero valent iron to freshwater and marine organisms. PLoS One 7, e43983.
Kirschling, T.L., Gregory, K.B., Minkley Jr., E.G., Lowry, G.V., Tilton, R.D., 2010. Impact of nanoscale zero valent iron on geochemistry and microbial populations in trichloroethylene contaminated aquifer materials. Environ. Sci. Technol. 44, 3474–3480.
Köber, R., Hollert, H., Hornbruch, G., Jekel, M., Kamptner, A., Klaas, N., Maes, H., Mangold, K.M., Martac, E., Matheis, A., Paar, H., Schäffer, A., Schell, H., Schiwy, A., Schmidt, K.R., Strutz, T.J., Thümmler, S., Tiehm, A., Braun, J., 2014. Nanoscale zero-valent iron flakes for groundwater treatment. Environ. Earth Sci. 72, 3339–3352.
Kocur, C.M., Lomheim, L., Boparai, H.K., Chowdhury, A.I., Weber, K.P., Austrins, L.M., Edwards, E.A., Sleep, B.E., O'Carroll, D.M., 2015. Contributions of abiotic and biotic dechlorination following carboxymethyl cellulose stabilized nanoscale zero valent iron injection. Environ. Sci. Technol. 49, 8648–8656.
Kranzioch, I., Stoll, C., Holbach, A., Chen, H., Wang, L., Zheng, B., Norra, S., Bi, Y., Schramm, K.W., Tiehm, A., 2013. Dechlorination and organohalide-respiring bacteria dynamics in sediment samples of the Yangtze Three Gorges Reservoir. Environ. Sci. Pollut. Res. Int. 20, 7046–7056.
Kumar, N., Auffan, M., Gattacceca, J., Rose, J., Olivi, L., Borschneck, D., Kvapil, P., Jublot, M., Kaifas, D., Malleret, L., Doumenq, P., Bottero, J.Y., 2014a. Molecular insights of oxidation process of iron nanoparticles: spectroscopic, magnetic, and microscopic evidence. Environ. Sci. Technol. 48, 13888–13894.
Kumar, N., Omoregie, E.O., Rose, J., Masion, A., Lloyd, J.R., Diels, L., Bastiaens, L., 2014b. Inhibition of sulfate reducing bacteria in aquifer sediment by iron nanoparticles. Water Res. 51, 64–72.
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. Ecotoxicol. Environ. Saf. 72, 684–692.
Li, X.-q., Elliott, D.W., Zhang, W.-x., 2006. Zero-valent iron nanoparticles for abatement of environmental pollutants: materials and engineering aspects. Crit. Rev. Solid State 31, 111–122.
Liu, A., Liu, J., Zhang, W.X., 2015. Transformation and composition evolution of nanoscale zero valent iron (nZVI) synthesized by borohydride reduction in static water. Chemosphere 119, 1068–1074.
Lohner, S.T., Becker, D., Mangold, K.M., Tiehm, A., 2011. Sequential reductive and oxidative biodegradation of chloroethenes stimulated in a coupled bioelectroprocess. Environ. Sci. Technol. 45, 6491–6497.
Lohner, S.T., Tiehm, A., 2009. Application of electrolysis to stimulate microbial reductive PCE dechlorination and oxidative VC biodegradation. Environ. Sci. Technol. 43, 7098–7104.
Lowry, G.V., Johnson, K.M., 2004. Congener-specific dechlorination of dissolved PCBs by microscale and nanoscale zerovalent iron in a water/methanol solution. Environ. Sci. Technol. 38, 5208–5216.
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.
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. Int. 19, 550–558.
Mueller, N.C., Nowack, B., 2010. Nanoparticles for remediation: solving big problems with little particles. Elements 6, 395–400.
O'Carroll, D., Sleep, B., Krol, M., Boparai, H., Kocur, C., 2013. Nanoscale zero valent iron and bimetallic particles for contaminated site remediation. Adv. Water Resour. 51, 104–122.
OECD, 2004. Test No. 202: *Daphnia* sp. Acute Immobilisation Test.
OECD, 2011. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test.
OECD, 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test.
Patil, S.S, Shedbalkar, U.U., Truskewycz, A., Chopade, B.A., Ball, A.S., 2016. Nanoparticles for environmental clean-up: a review of potential risks and emerging solutions. Environ. Technol. Innov. 5, 10–21.
Peddinghaus, S., Brinkmann, M., Bluhm, K., Sagner, A., Hinger, G., Braunbeck, T., Eisentrager, A., Tiehm, A., Hollert, H., Keiter, S.H., 2012. Quantitative assessment of the embryotoxic potential of NSO-heterocyclic compounds using zebrafish (*Danio rerio*). Reprod. Toxicol. 33, 224–232.
Phenrat, T., Long, T.C., Lowry, G.V., Veronesi, B., 2009. Partial oxidation ("aging") and surface modification decrease the toxicity of nanosized zerovalent iron. Environ. Sci. Technol. 43, 195–200.
Ponder, S.M., Darab, J.G., Mallouk, T.E., 2000. Remediation of Cr(VI) and Pb(II) aqueous solutions using supported, nanoscale zero-valent iron. Environ. Sci. Technol. 34, 2564–2569.
Reifferscheid, G., Maes, H.M., Allner, B., Badurova, J., Belkin, S., Bluhm, K., Brauer, F., Bressling, J., Domeneghetti, S., Elad, T., Fluckiger-Isler, S., Grummt, H.J., Gurtler, R., Hecht, A., Heringa, M.B., Hollert, H., Huber, S., Kramer, M., Magdeburg, A., Ratte, H.T., Sauerborn-Klobucar, R., Sokolowski, A., Soldan, P., Smital, T., Stalter, D., Venier, P., Ziemann, C., Zipperle, J., Buchinger, S., 2012. International round-robin study on the Ames fluctuation test. Environ. Mol. Mutagen 53, 185–197.
Rushton, E.K., Jiang, J., Leonard, S.S., Eberly, S., Castranova, V., Biswas, P., Elder, A., Han, X., Gelein, R., Finkelstein, J., Oberdörster, G., 2010. Concept of assessing nanoparticle hazards considering nanoparticle dosemetric and chemical/biological response metrics. J. Toxicol. Environ. Health, Part A 73, 445–461.
Schiwy, S., Braunig, J., Alert, H., Hollert, H., Keiter, S.H., 2015. A novel contact assay for testing aryl hydrocarbon receptor (AhR)-mediated toxicity of chemicals and whole sediments in zebrafish (*Danio rerio*) embryos. Environ. Sci. Pollut. Res. Int. 1–14.
Schmidt, K.R., Stoll, C., Tiehm, A., 2006. Evaluation of 16S-PCR detection of *Dehalococcoides* at two chloroethene-contaminated sites. Water Sci. Technol. 6, 129.
Schrand, A.M., Rahman, M.F., Hussain, S.M., Schlager, J.J., Smith, D.A., Syed, A.F., 2010. Metal-based nanoparticles and their toxicity assessment. Wiley Interdiscip. Rev. NanomedNanomed. Nanobiotechnol. 2, 544–568.
Scown, T.M., van Aerle, R., Tyler, C.R., 2010. Review: do engineered nanoparticles pose a significant threat to the aquatic environment? Crit. Rev. Toxicol. 40, 653–670.
Simon, A., Maletz, S.X., Hollert, H., Schaffer, A., Maes, H.M., 2014. Effects of multi-walled carbon nanotubes and triclocarban on several eukaryotic cell lines: elucidating cytotoxicity, endocrine disruption, and reactive oxygen species generation. Nanoscale Res. Lett. 9, 396.
Stumm, W., Morgan, J.J., 2012. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. John Wiley & Sons.
Su, C., Puls, R.W., Krug, T.A., Watling, M.T., O'Hara, S.K., Quinn, J.W., Ruiz, N.E., 2012. A two and half-year-performance evaluation of a field test on treatment of source zone tetrachloroethene and its chlorinated daughter products using emulsified zero valent iron nanoparticles. Water Res. 46, 5071–5084.
Tiehm, A., Schmidt, K.R., 2011. Sequential anaerobic/aerobic biodegradation of chloroethenes–aspects of field application. Curr. Opin. Biotechnol. 22, 415–421.
Tilston, E.L., Collins, C.D., Mitchell, G.R., Princivalle, J., Shaw, L.J., 2013. Nanoscale zerovalent iron alters soil bacterial community structure and inhibits chloroaromatic biodegradation potential in Aroclor 1242-contaminated soil. Environ. Pollut. 173, 38–46.
Tratnyek, P.G., Johnson, R.L., 2006. Nanotechnologies for environmental cleanup. Nano Today 1, 44–48.
Velimirovic, M., Simons, Q., Bastiaens, L., 2015. Use of CAH-degrading bacteria as test-organisms for evaluating the impact of fine zerovalent iron particles on the anaerobic subsurface environment. Chemosphere 134, 338–345.
Vuori, K.-M., 1995. Direct and indirect effects of iron on river ecosystems. Ann. Zool. Fenn. 32, 317–329.
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.
Wei, Y.T., Wu, S.C., Yang, S.W., Che, C.H., Lien, H.L., Huang, D.H., 2012. Biodegradable surfactant stabilized nanoscale zero-valent iron for *in situ* treatment of vinyl chloride and 1,2-dichloroethane. J. Hazard. Mater. 211–212, 373–380.
Weil, M., Meissner, T., Busch, W., Springer, A., Kuhnel, D., Schulz, R., Duis, K., 2015. The oxidized state of the nanocomposite Carbo-Iron(R) causes no adverse effects on growth, survival and differential gene expression in zebrafish. Sci. Total Environ. 530–531, 198–208.
Wyrwoll, A.J., Lautenschlager, P., Bach, A., Hellack, B., Dybowska, A., Kuhlbusch, T.A., Hollert, H., Schaffer, A., Maes, H.M., 2016. Size matters - the phototoxicity of TiO_2 nanomaterials. Environ. Pollut. 208, 859–867.
Xiu, Z.M., Gregory, K.B., Lowry, G.V., Alvarez, P.J., 2010a. Effect of bare and coated nanoscale zerovalent iron on *tce*A and *vcr*A gene expression in *Dehalococcoides* spp. Environ. Sci. Technol. 44, 7647–7651.
Xiu, Z.M., Jin, Z.H., Li, T.L., Mahendra, S., Lowry, G.V., Alvarez, P.J., 2010b. Effects of nano-scale zero-valent iron particles on a mixed culture dechlorinating trichloroethylene. Bioresour. Technol. 101, 1141–1146.
Yan, L., Gu, Z., Zhao, Y., 2013. Chemical mechanisms of the toxicological properties of nanomaterials: generation of intracellular reactive oxygen species. Chem Asian J.
Yin, J.-J., Fu, P.P., Lutterodt, H., Zhou, Y.-T., Antholine, W.E., Wamer, W., 2012. Dual role of selected antioxidants found in dietary supplements: crossover between anti- and pro-oxidant activities in the presence of copper. J. Agric. Food Chem. 60, 2554–2561.
Zabetakis, K.M., Nino de Guzman, G.T., Torrents, A., Yarwood, S., 2015. Toxicity of zero-valent iron nanoparticles to a trichloroethylene-degrading groundwater microbial community. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 50, 794–805.
Zhang, W.-x., 2003. Nanoscale iron particles for environmental remediation: an overview. J. Nanopart. Res. 5, 323–332.
Zhu, X., Tian, S., Cai, Z., 2012. Toxicity assessment of iron oxide nanoparticles in zebrafish (*Danio rerio*) early life stages. PLoS One 7, e46286.