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Environmental risk or benefit? Comprehensive risk assessment of groundwater treated with nano Fe0-based Carbo-Iron®

Mirco Weil[a], Katrin Mackenzie[b], Kaarina Foit[c], Dana Kühnel[d], Wibke Busch[d], Mirco Bundschuh[e, f],
Ralf Schulz[f], Karen Duis[a]

[a] ECT Oekotoxikologie GmbH, Böttgerstrasse 2-14, 65439 Flörsheim, Germany
m.weil@ect.de; k-duis@ect.de

[b] Helmholtz Centre for Environmental Research – UFZ, Department of Environmental Engineering,
Permoser Strasse 15, 04318 Leipzig, Germany
katrin.mackenzie@ufz.de

[c] Helmholtz Centre for Environmental Research – UFZ, Department of System Ecotoxicology,
Permoser Strasse 15, 04318 Leipzig, Germany
kaarina.foit@ufz.de

[d] Helmholtz Centre for Environmental Research – UFZ, Department of Bioanalytical Ecotoxicology,
Permoser Strasse 15, 04318 Leipzig, Germany
dana.kuehnel@ufz.de; wibke.busch@ufz.de

[e] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences,
Uppsala, Sweden

[f] Institute for Environmental Sciences, University of Koblenz-Landau, Forststrasse 7, 76829 Landau,
Germany
bundschuh@uni-landau.de

schulz@uni-landau.de

Corresponding author: Mirco Weil, ECT Oekotoxikologie GmbH, Böttgerstrasse 2-14, 65439
Flörsheim, Germany, m.weil@ect.de, +49 6145 956411

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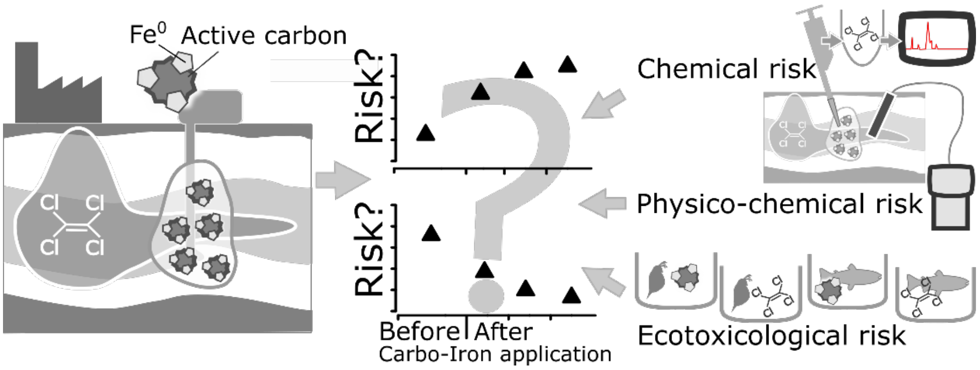
Graphical abstract



Highlights

- Environmental concentrations for the nFe^0 agent Carbo-Iron derived from field study
- Ecotoxicity data set for effects assessment was completed
- Standard risk assessment could be performed for a novel remediation agent
- Evaluation of benefit and risk of groundwater treatment for the environment
- Method applicable to identify environmental risks of other treatment techniques

Abstract

Groundwater is essential for the provision of drinking water in many areas around the world. The performance of the groundwater-bearing aquifer relies on the ecosystem services provided by groundwater-related organisms. Therefore, if remediation of contaminated groundwater is necessary, the remediation method has to be carefully selected to avoid risk-risk trade-offs that might impact these ecosystems. In the present study, the environmental risk of the *in situ* remediation agent Carbo-Iron was performed. Carbo-Iron® is a composite of zero valent nano-iron and active carbon. Existing ecotoxicity data were complemented by studies with *Daphnia magna* (crustacea)*, Scenedesmus vacuolatus* (algae)*, Chironomus riparius* (insecta) and nitrifying soil microorganisms. The predicted no effect concentration of 0.1 mg/L was derived from acute and chronic ecotoxicity studies. It was compared to measured and modelled environmental concentrations of Carbo-Iron applied in a groundwater contaminated with chlorohydrocarbons in a field study and risk ratios were derived. A comprehensive assessment approach was developed further based on existing strategies and used to identify changes of the environmental risk due to the remediation of the contaminated site with Carbo-Iron. With the data used in the present study, the total environmental risk decreased by approximately 50% in the heavily contaminated zones after the application of Carbo-Iron. Thus, based on the results of the present study, the benefit of remediation with Carbo-Iron seems to outweigh its negative effects on the environment.

1 Introduction

Groundwater constitutes more than 97% of the world’s unfrozen fresh water and is the major source for drinking water in most developed and many developing nations (Gibert et al., 1994). Thereby, the groundwater-bearing aquifers are an important habitat for highly specialized and endemic species (Danielopol, 1989; Danielopol and Griebler, 2008). which provide important ecosystem services and play a critical role in carbon and nutrient cycling (Swartjes, 2011) and improve the water quality (Danielopol, 1989; Hahn, 2009). As a consequence of the unique and central properties of groundwater, the remediation of contaminated sites (e.g. in abandoned industrial sites) needs to be environmentally compatible.

Thus, the environmental risks of a remedial agent need to be assessed prior to the application into an aquifer. Obviously, a remediation method is only suitable, if the environmental risk after application is lower than before the start of the treatment (Lemming et al., 2010). Existing risk assessment strategies are implemented in several supporting tools which consider the remediation efficiency, treatment time, costs and the potential harm of the remedial method in comparison with alternative methods (Khadam and Kaluarachchi, 2003; Li et al., 2014; Ren et al., 2017; Tartakovsky, 2013; Yang et al., 2012). However, despite the high importance of groundwater for the environment, these tools often focus on the human health risk, whereas the environmental risk is only rarely considered (Wang et al., 2016).

For the treatment of groundwater contaminated with halogenated hydrocarbons, the Fe^0-based remediation agent Carbo-Iron® was developed. It is a colloidal composite of nano Fe^0 structures embedded in active carbon (AC) particles (Bleyl et al., 2012; Mackenzie et al., 2016, 2012). In several pilot studies, Carbo-Iron was applied for treating aquifers polluted by chlorohydrocarbons and the application of particles at a site mainly contaminated by tetrachloroethene (PCE) has recently been described (Mackenzie et al., 2016). Though nano Fe^0-based groundwater remediation methods are promising, it is necessary to assess their potential harm to the environment and inform decision makers about possible risks (Grieger et al., 2010). In order to provide a comprehensive environmental risk-benefit anaylsis of Carbo-Iron, the present study comprises of three main aspects: 1) an effects assessment for Carbo-Iron, 2) an environmental risk assessment for Carbo-Iron, 3) a site-specific environmental risk assessment for the treated groundwater, considering all pollutant concentrations before and after application of Carbo-Iron.

Since previous ecotoxicity data for Carbo-Iron (Hjorth et al., 2017; Nguyen et al., 2018; Weil et al., 2016, 2015) were not sufficient for an environmental risk assessment, further ecotoxicity tests were performed. A predicted environmental concentration (PEC) of Carbo-Iron was estimated based on available data from a field study and compared to the predicted no effect concentration (PNEC) derived in this manuscript, providing a first estimation of the environmental risk of Carbo-Iron. The Triad approach for comparison of environmental risks of sediments (Chapman, 1990) was applied which was already refined for a general use in risk assessment (Dagnino et al., 2008; Jensen and Pedersen, 2006; Weeks and Comber, 2005), and a specific use for risk assessment in groundwater (Crévecoeur et al., 2011). In the present study, we further modified this approach to evaluate the risk caused by the application of the remediation agent Carbo-Iron into an aquifer during a field study.

2 Material and Methods

2.1 Effect assessment for Carbo-Iron

Active Carbo-Iron rapidly alters chemically by the target reaction with halogenated hydrocarbons and by oxidation reactions with water. This ageing process of the material in groundwater is unavoidable and starts immediately after injection. Therefore, all toxicity tests were performed with aged Carbo-Iron, which means that the originally zero-valent iron was treated in slightly acidic deoxygenated water leading to formation of Fe^{2+} and, by further reaction with dissolved oxygen, to Fe^{3+}. This way, the ageing process of Carbo-Iron in the aquifer was simulated. Henceforth, the term Carbo-Iron is used for aged Carbo-Iron, consisting mainly of FeOOH, Fe_3O_4 and less Fe_2O_3 embedded in the AC with an iron content of approx. 22% (w/w)

The characteristics of Carbo-Iron particles in aqueous media were extensively examined in previous studies with *Danio rerio* (Weil et al., 2015) and *Hyalella azteca* (Weil et al., 2016). In these studies, particle diameters measured in the stock suspensions varied only marginally. In the present study, the same methods for preparation of stock and test suspensions were used (see SI section 1.1.1). Briefly, stock suspensions were prepared by homogenizing Carbo-Iron with an ultrasonic probe (Hielscher UP200S, Teltow, Germany) in deionized water. Test suspensions were prepared by dilution of the stock suspension with the culture medium for the respective test organisms. As stabilizing additive, carboxymethyl cellulose (CMC; 70,000 g/mol and a range in substitution degree from 0.65 to 0.9, which correspond to a concentration of carboxylic groups of 2.8 to 3.9 mval/g, respectively; Antisol FL 30, Wolff Cellulosics) was used in the stock and test suspensions with 20% w/w relative to the highest used test concentration to prevent rapid sedimentation of Carbo-Iron. CMC was also used as particle stabilizer for Carbo-Iron suspensions during injection into the groundwater. In each toxicity test, a control group in culture medium was included. Additionally, a CMC dispersant control group with culture medium and CMC at the same concentration as in the test suspensions was used in all tests except the test with *Scenedesmus vacuolatus*. Supplemental to the tests described here, a nitrogen-transformation test was performed as a sensitive indicator for effects on soil-inhabiting microorganisms (see SI section 1.1.5 for method description).

2.1.1 Toxicity tests with Daphnia magna

The 48-h acute toxicity test with *D. magna* (Crustacea, Branchiopoda, Cladocera) was based on OECD (2004a), extended by a 5-d post-exposure period. Daphnids were exposed to Carbo-Iron concentrations between 0.562 and 100 mg/L. For each treatment and control, 4 replicates with 5 daphnids each were used. During the 48-h exposure, no food was provided. Immobility was evaluated after 48 h of exposure. Then, daphnids were transferred to M7 medium without Carbo-Iron and CMC and fed daily with 4.0×10^5 cells/mL (1.67 mg C/L) of a suspension of batch-cultured green algae (*Desmodesmus subspicatus*). During a post-exposure period of 5 d, immobility was assessed daily.

The *D. magna* chronic reproduction test was based on OECD (2008). For each treatment and control, 10 replicates with 1 daphnid each were used; the investigated Carbo-Iron concentrations were between 0.1 and 10 mg/L. During the 21 d exposure, daphnids were fed three times per week with green algae (*D. subspicatus*, 9.6×10^5 cells/mL corresponding to 4.0 mg C/L). Three times weekly, the survival of adult daphnids and number of living offspring per animal per day were assessed, and test suspensions were renewed. This interval of the test media renewal coincides with the finding of a previous study with *Hyalella azteca*, where Carbo-Iron suspensions with concentrations up to 100 mg/L were stable for approx. 3 d after start of the exposure (Weil et al., 2016). To verify this assumption, Carbo-Iron concentrations in the test suspensions with 3.16 and 31.6 mg/L were measured after 24, 48 and 72 h with the dynamic light scattering-based method described in Weil et al (2015). Further details on exposure conditions are described in SI section 1.1.2.

2.1.2 Algal growth inhibition test

The algal growth inhibition test with *Scenedesmus vacuolatus* (Chlorophyta, Chlorophyceae, Sphaeropleales) was performed with Carbo-Iron and with active carbon (AC; Norit® SA Super, Cabot, USA; milled to d_{50} = 0.8 µm). This pure AC powder is used as a basis for the preparation of Carbo-Iron and was applied in the algae test to investigate whether effects were a result of the reduced light intensity due to shading by the particles. Carbo-Iron test concentrations were between 16 - 62.5 mg/L and the test concentrations for AC (15 – 43.5 mg/L) correspond to the content of AC of the tested Carbo-Iron concentrations. The test suspensions were prepared by adding 0.8 mL of algae suspension (cell density 7-9.5 in 10-fold concentrated culture medium) to 7.2 mL of a respective Carbo-Iron or AC suspension. Aeration of the test suspensions kept the algae cells as well as the Carbo-Iron particles in suspension and no precipitates were observed during the exposure. Light intensity was measured at the beginning of exposure in each culture tube of the lowest and highest investigated test concentration with Carbo-Iron and AC, respectively.

The effect of Carbo-Iron and AC on algal growth based on cell numbers could not be measured in a cell counter or under the microscope, because Carbo-Iron and AC particles had a similar size as the algal cells after cell division. Further, effects on photosynthesis could not be quantified, because the particles interfered with the measurement of fluorescence. For these reasons, the parameter cell volume was used as alternative to assess effects on algal growth (Faust et al., 1992). Cell volume was measured after an exposure duration of 16 h, when cells had increased in volume, but not yet divided. The cell diameter at this time point was 6-15 µm and a clear distinction from the particles (< 5 µm) was possible. Further information on exposure conditions is provided in SI section 1.1.3. Additionally, the toxicity of PCE to *S. vacuolatus* was investigated, details are provided in SI sections 1.1.3 and 2.3.3.

2.1.3 Sediment-water test with Chironomus riparius

The sediment-water toxicity test was performed according to OECD (2004) with *C. riparius* (Arthropoda, Insecta, Diptera). This test organism was chosen for its relevance for particle suspensions with limited stability, since Carbo-Iron precipitated to the sediment surface would remain available for the sediment-dwelling larvae. Test vessels with artificial sediment and medium M4 were prepared. Immediately after addition of Carbo-Iron test suspensions to the water phase of the water-sediment systems, first instar larvae were introduced to the test vessels (10 organisms per vessel). For each treatment and control, 8 replicates were used. Test vessels were incubated at 20 ± 2°C with slight aeration and a photoperiod of 16 h light and 8 h dark. During the 28 d exposure, the emergence and development rate were evaluated (for further details, see SI section 1.1.4).

2.1.4 Statistical analysis

Statistical analyses were carried out using R Version 3.3.0 (R Development Core Team, 2011). Statistical analysis by null hypothesis significance testing was based on replicate means; proportional data were arcsine-transformed before analysis. For comparison of differences between control and dispersant control, the two-sided Welch two-sample t-test was used. If the requirements for parametric testing (homogeneity of variances and normal distribution) were fulfilled, ANOVA and Dunnett's two-sided post-hoc test were performed, while the Kruskal-Wallis test with subsequent Wilcoxon ranks sum test were used as non-parametric alternative. For all tests, a significance level of $p \leq 0.05$ was used (OECD, 2006). Treatments were compared to the dispersant (CMC) control. Where appropriate, the log-normal model with 3 parameters was fitted to the data to calculate effective concentrations (Ritz and Streibig, 2005).

2.2 Assessment of the environmental risk of aged Carbo-Iron

2.2.1 Exposure assessment for Carbo-Iron

The relevant release of Carbo-Iron into the environment is not, as for many industrial chemicals, via diffuse routes over the whole product life cycle. Instead, Carbo-Iron release is intended and immediate, i.e. a major portion of the produced amounts is pumped into contaminated groundwater. Thus, the present study focuses on the exposure to Carbo-Iron in the area close to the treated site. Carbo-Iron was used in a pilot study for remediation of groundwater contaminated with chlorohydrocarbons (Mackenzie et al., 2016). The contaminated site (Figure 1) was a former military area with a chemical cleaning facility that caused groundwater pollution by chlorohydrocarbons, mainly tetrachloroethene (PCE). Several sampling ports were used to monitor concentrations of the pollutants before and during treatment with Carbo-Iron (see section 2.3.2). Based on the measured PCE concentrations, the site was subdivided in four contamination zones (I – IV, Figure 1A). The groundwater flow velocities were between 30 cm/day in the southern part of the site and of 6 cm/day in the northern part. For remediation of the site, Carbo-Iron suspensions (10 g/L) were prepared by dispersion of dry Carbo-Iron into deoxygenized tap water using a high-speed homogenizer. CMC (the same as described in section 2.1) at a concentration of 2 g/L was used as colloid stabilizer. The first injection campaign with 20 kg of Carbo-Iron was applied in two injection ports (IP1 and IP3; Figure 1A) and was designed to build a fence-like reactive barrier in the aquifer. In a second injection campaign Carbo-Iron was applied in a two-dimensional injection pattern (Mackenzie et al., 2016). For the present study, only data from the first campaign are used to exploit the rare opportunity of a localized remediation of groundwater with a very uniform distribution of the remediation agent Carbo-Iron. Groundwater conditions are often highly complex and the added complexity of the two-dimensional injection pattern using 15 injection ports would have impeded the attribution of the observed effects to the remediation.

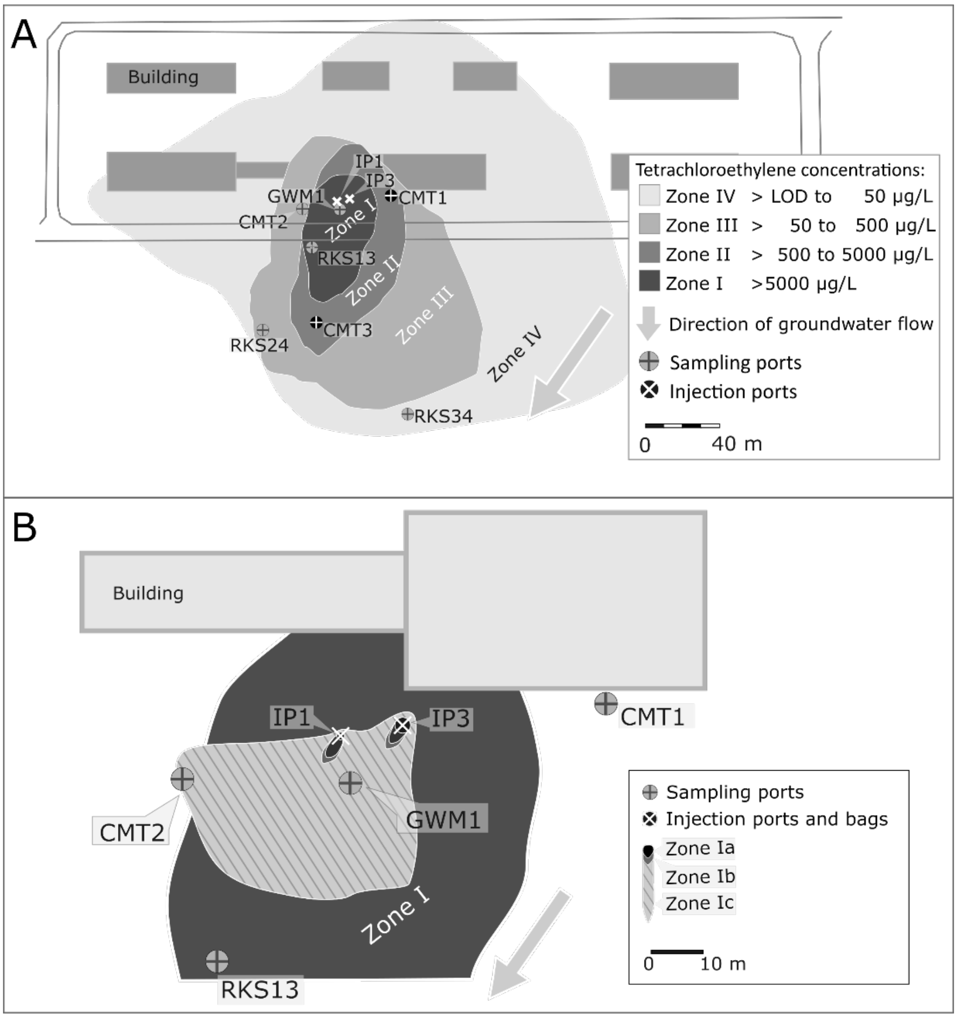


Figure 1: Site treated with Carbo-Iron in the pilot study of Mackenzie et al. (2016). Groundwater monitoring well (GWM1) with a sampling depth of 8 m below ground level; continuous monitoring well with multichannel-tubing (CMT1, CMT2, CMT3) with seven ports in depths between 6 and 25 m below ground level; window sampling tube (RKS 13, RKS 24, RKS 34) with two sampling ports at approx. 6.5 and 8 m below ground level. **A**: Overview of the area and allocation of zones based on measured concentrations of PCE above analytical limit of detection (LOD=0.5 µg/L). **B**: Distribution of Carbo-Iron after injection in zone I. Subdivisions Ia, Ib, and Ic indicate the calculated Carbo-Iron concentrations (Table 1) based on data from the column studies (SI section 2.1).

Before termination of the monitoring period, sediment samples were taken by direct push from zone I and analysed for its Carbo-Iron content (see SI section 2.2.2). Concentrations of Carbo-Iron in the wells were not measured with common analytical methods, since the Carbo-Iron constituents would be unnoticeable at the high natural background values of iron and carbon. Therefore, concentrations (Table 1) and distribution of Carbo-Iron in the groundwater (Figure 1B) were estimated based on information on the time-dependent distribution pattern of Carbo-Iron from soil column studies with various standard and natural porous materials (such as Dorsilit and sediment from the site; see SI section 3.1) using columns with different lengths (Batka and Hofmann, 2016).

Table 1: Estimated concentrations of Carbo-Iron in the distribution zones (Figure 1). Carbo-Iron concentrations were estimated based on data from soil column studies (SI section 2.1).

Contamination zone	Sampling well	Distance from Carbo-Iron injection (m)	Estimated mean Carbo-Iron concentration (mg/L)
Ia	-	0 – 1.3	650
Ib	-	1.3 – 1.9	475
Ic	GWM1	1.9 – 12.9	1.3
I	RKS13	0 to approx. 20	0.5
II	CMT2	approx. 20	1.3*
	CMT3	approx. 40	0
III	RKS24	approx. 80	0
IV	RKS34	approx. 100	0
II	CMT1	5 to approx. 20 (Opposite to direction of groundwater flow)	0

*: Sampling well CMT2 is in zone II but bordering to zone Ic. For a conservative risk assessment approach, the Carbo-Iron concentration was based on concentrations estimated for zone Ic.

2.2.2 Derivation of a PNEC, environmental risk assessment for Carbo-Iron

A predicted no effect concentration (PNEC) for Carbo-Iron was derived based on the effect concentrations generated in the present study, and by Weil et al. (2016, 2015) and Hjorth et al. (2017). The PNEC value was then calculated as the quotient of the most sensitive endpoint and the related assessment factor. The latter was selected depending on the availability of results from (a) acute and chronic tests, and (b) trophic/functional groups algae, crustaceans, fish as recommended for the risk characterisation of chemicals (ECHA, 2017, 2008). The risk quotient (i.e. PEC/PNEC ratio) indicates the degree of risk expected to be caused by Carbo-Iron in the treated aquifer. A risk quotient below 1 is generally considered acceptable (ECHA, 2016).

2.3 Site-specific risk-benefit analysis

2.3.1 Theoretical impact of Carbo-Iron on the relevant pollutants

The desired remedial effect of Carbo-Iron is promoted by two mechanisms: (a) after Carbo-Iron is introduced into the groundwater, the sorption capacity of the AC component in Carbo-Iron increases the retention time of the pollutants, i.e. the migration of PCE through the groundwater slows down by a calculated factor of approx. 30 compared to migration before Carbo-Iron treatment (Georgi et al., 2015). This leads to a rapid decrease of the PCE concentrations in zone I. (b) The chemical degradation of the pollution induced by the Fe^0-component of Carbo-Iron leads to an additional decrease of the pollutant concentration.

2.3.2 Exposure assessment for the relevant pollutants

In the field study (Mackenzie et al., 2016), Carbo-Iron was applied to an aquifer polluted by chlorohydrocarbons (see section 2.2.1), mainly by PCE at concentrations up to 120 mg/L. In groundwater samples from wells distributed over the contaminated area (Figure 1 and Table 1), chemical analysis was performed for trichloroethene and PCE. Additionally, transformation products of the reduction by Carbo-Iron, 1,1-dichloroethene, 1,2-dichloroethene (cis and trans), ethane and ethene (as a sum parameter) and vinyl chloride, were analysed. Furthermore, the redox potential and total organic carbon (Table S16), pH and iron concentration (Table S 17) and oxygen concentration (Table S18) were measured. In the present study, data for day 0 (i.e. before Carbo-Iron injection) and days 9, 31, 58, 93 and 190 after application of Carbo-Iron into the groundwater were evaluated. For the contamination zones I to IV, the wells GWM1, RKS13, CMT2, CMT3, RKS24 and RKS34 were selected based on the availability of continuous data for chemical analysis. To evaluate groundwater dynamics that are independent from the application of Carbo-Iron, the sampling well CMT1 (located in a distance of approx. 10 m from the injection points in opposite direction of the groundwater flow) was included. For all calculations, the median value per well and sampling day was used.

2.3.3 Derivation of PNECs for the relevant pollutants / chlorinated hydrocarbons

For the determination of PNECs for the analysed pollutants, effect data for at least three trophic/functional groups (algae, crustaceans and fish) were retrieved from ECHA registrations dossiers (echa.europa.eu), the ECOTOX database (http://cfpub.epa.gov/ecotox/) and the QSAR Toolbox platform (https://www.qsartoolbox.org/, Dimitrov et al., 2016) that retrieves data from the Aquatic OASIS and Aquatic Japan databases. This database search was extended by a literature search using the name of each substance in combination with the trophic/functional group or presentative species in these groups. In the case of multiple data for one trophic/functional group, the lowest relevant and reliable (criteria were e.g. guideline test, accompanying chemical analysis) value was chosen. PNEC values were then calculated as described for Carbo-Iron in section 2.2.2.

2.3.4 Triad-based risk-benefit analysis

2.3.4.1 General aspects

The Triad approach was developed for the quality assessment of sediments (Chapman, 1990; Critto et al., 2007; Long and Chapman, 1985) and integrates data on (1) the concentrations of the contaminants compared to target values (chemical component), (2) alterations in biodiversity of the local community compared to a reference site (ecological component) and (3) potential adverse effects of the contaminants on environmental organisms (ecotoxicological component). Dagnino et al. (2008) further developed the approach for the assessment of contaminated sites and calculated risk indices for each component. To assess the environmental risk before and after the application of Carbo-Iron, the approach of Dagnino et al. (2008) was used with the following modifications:

(1) The chemical component of the assessment integrates concentrations of the relevant pollutants (section 2.3.2) before and after treatment with Carbo-Iron. Carbo-Iron concentrations were not considered, because the calculation of risk indices requires target values lacking for Carbo-Iron.

(2) The ecotoxicological component covers data for the relevant pollutants as well as for Carbo-Iron. Instead of ecotoxicity tests with groundwater samples, data from single-substance standard ecotoxicity tests were used. This was done because groundwater samples, usually with low oxygen concentrations, do not provide suitable conditions for the commonly used test organisms. An adjustment of groundwater samples to the requirements of these test organisms would most likely have an impact on the pollutant concentration (e.g. stripping of volatile compounds by aeration of the samples). Moreover, a culture of aquifer-inhabiting organisms in the laboratory is often problematic and standard test guidelines as recommended for risk assessment are not available for groundwater organisms.

(3) The ecological component was excluded from analysis. The determination of the ecological status of a groundwater is extremely difficult, as observations on biodiversity cannot be performed directly in the aquifer. Sampling of the aquifer fauna is not only time-consuming but in most cases incomplete due to the patchy distribution (Hahn, 2006; Steube et al., 2009), and standardized sampling protocols are lacking (Stein et al., 2010). Additionally, the ecological component requires an appropriate reference site with minimal anthropogenic pollution but similar fauna and physico-chemical parameters (Dagnino et al., 2008; Jensen and Pedersen, 2006). For groundwater, selection of a reference site is complicated because of the aforementioned patchy distribution of fauna in groundwater and a high number of endemic species (Stein et al., 2010). Furthermore, in case of highly contaminated groundwater, it is very likely that the abundance of organisms is poor, or organisms are completely absent which makes it impossible to calculate a risk index for the ecological component.

(4) A physico-chemical component was introduced, as suggested by Crévecoeur et al. (2011). Changes in physico-chemical parameters due to the injection of Carbo-Iron may increase the environmental risk for the groundwater fauna. The physico-chemical component includes parameters that are very likely to change after application of Carbo-Iron into the groundwater including redox potential, conductivity and pH.

2.3.4.2 Chemical component

Risk indices were calculated for each sampling well and day in three steps: (1) risk quotients (RQ) were calculated as quotients of the mean measured concentrations of the relevant pollutants per day and well and the respective target values, (2) chemical toxic pressure (cTP) for each sampling well per day was then calculated as sum of the risk quotients for all 6 pollutants, (3) chemical risk indices (cRI) were calculated (Eq. 1 to Eq. 3) depending on the cTP value in comparison to two defined threshold values (Th’ and Th’’) representing two levels of risk.

The target values for vinyl chloride, 1,1-dichloroethene, 1,2-dichloroethene (cis and trans), trichloroethene and tetrachloroethene of 5, 5.8, 20, 500 and 40 µg/L were generally taken from Swartjes (1999), who derived intervention values for the assessment of groundwater. These values are based on potential risks to humans and ecosystems and should, if exceeded, trigger a remediation of the site (Swartjes, 1999). For ethane and ethene, no quality standards for groundwater were identified, and a maximum permissible concentration of 8500 µg/L in surface water was only available for ethene (Crommentuijn et al., 2000). This value was used as target value for the sum parameter ethane/ethene.

If any measured value for a parameter was above the respective target value, the TP was > 1 and a risk for the environment was expected. A TP below Th’ will lead to calculated RI between 0 and a predefined level of risk α; a TP between Th’ and Th’’ will lead to a risk index between α and 1. Above Th’’, the risk index acquires the maximum value of 1. It should be pointed out that the value for Th’’ in the present study is higher than the value selected by Dagnino et al. (2008) who compared negative effects of the deposition of contaminated soils on xenobiotic compounds and applied Th’=1 and

Th’’=10. With these thresholds Dagnino et al. (2008) derived cRI ≤0.75 and a differentiation of the investigated soils was possible. In the present study, Th’=1 was chosen, too. However, due the high pollutant concentrations in groundwater samples from zone I, several RQ were in the range of 1000, leading to similarly high TP values. Thus, a Th’’=10 would have led to cRI and eRI above the maximum value of 1 in several cases and identification of a positive or negative influence of Carbo-Iron on these components would have been impossible. Thus, to detect changes in the risk under the influence of Carbo-Iron, Th’’=1000 was chosen for the calculation of cRI and eRI.

$$\text{If } TP_{well,\,day} \le Th' \qquad RI = \frac{TP}{Th'} \times \alpha \qquad \text{(Eq. 1)}$$

$$\text{If } Th' < TP_{well,\,day} \le Th'' \qquad RI = \alpha + \frac{TP - Th'}{Th'' - Th'} \times (1 - \alpha) \qquad \text{(Eq. 2)}$$

$$\text{If } TP_{well,\,day} > Th'' \qquad RI = 1 \qquad \text{(Eq. 3)}$$

2.3.4.3 Ecotoxicity component

Ecotoxicity risk quotients were calculated as quotients of the concentrations of the relevant pollutants (see section 2.3.2) measured in groundwater samples and the PNEC for the respective compound as target values. Toxic pressure (eTP) for each sampling well per day was then calculated as sum of the risk quotients. This differs from the method described by Dagnino et al. (2008), who used the mean of all risk quotients (instead of the sum) when evaluating the toxicity of contaminated sediments based on ecotoxicity tests with sediment samples. In the present study, additive effects of the pollutants are covered in the risk assessment by using the sum of the eRQs. Risk indices (eRI) were calculated as described in Eq. 4 to Eq. 6 with the threshold values Th’=1 and Th’’=1000. For the ecotoxicity component, a TP below Th’ will lead to an RI of 0 and TP values between Th’ and Th’’ will lead to a risk index between 0 and 1. Above Th’’, the risk index acquires the maximum value of 1 for all three components.

$$\text{If } TP_{well,\,day} \leq Th' \qquad RI = 0 \qquad \text{(Eq. 4)}$$

$$\text{If } Th' < TP_{well,\,day} \leq Th'' \qquad RI = \frac{TP - Th'}{Th'' - Th'} \qquad \text{(Eq. 5)}$$

$$\text{If } TP_{well,\,day} > Th'' \qquad RI = 1 \qquad \text{(Eq. 6)}$$

2.3.4.4 *Physico-chemical component*

The passage of groundwater through the reactive barrier of Carbo-Iron can potentially change physico-chemical characteristics of the groundwater. While a potential ecotoxicological risk due to exposure of organisms to Carbo-Iron is locally limited by the mobility and distribution of Carbo-Iron in the aquifer, extreme physico-chemical parameters in the groundwater could lead to an increased risk beyond this area. During the field study (Mackenzie et al.,2016), physico-chemical parameters were measured in the groundwater samples. In the present study, only conductivity, pH and redox potential were considered. Data for total organic carbon (Table S16), iron concentration (Table S17) and oxygen concentrations (Table S18) could not be attributed to Carbo-Iron application and were thus not included. For each of the relevant physico-chemical parameters per day and sampling well, a risk index was calculated as described in the following and the mean value of these risk indices was used as the physico-chemical risk index per well and day (pRI).

The target values of 2500 µS/cm for the parameter conductivity was taken from the EU directive on the quality of water for human consumption (European Communities, 1998).Since only a maximum but no minimum target value was available, the risk index was calculated in two steps (1) the quotient of measured data and target value for each day and well was calculated and (2) these values were used as TP to calculate a RI for conductivity as described in Eq. 1 to Eq. 3. For pH and redox potential, however, optimum ranges were defined and used to directly derive a risk index for the two parameters. For pH, the calculation of the risk index followed the description of Crévecoeur et al. (2011; see Eq. 7 to Eq. 9) in the target range of pH 6.5 to 9.0 (US EPA, 1999). For the redox potential, no target values could be identified in literature. However, due to the high potential of Carbo-Iron to affect this parameter, its observation was considered necessary. In low-oxygen groundwater, the redox potential ranges usually between -300 and 0 mV (Grenthe et al., 1992). However, due to the high permeability of sandy sediments and the low distance to the ground level, the aquifer of the field study was also markedly influenced by precipitation events and groundwater samples frequently showed relatively high oxygen concentrations carried in by rainwater (Table S18). Therefore, redox potentials representative for surface waters ranging between 0 and 500 mV were additionally considered (Pepper and Gentry, 2015; Søndergaard, 2009; Williams and Fraústo da Silva, 2006). Combining both, the target range for the parameter redox potential was set to -300 to 500 mV. This range is in accordance with live-promoting ranges mentioned in the comprehensive review on redox potentials by Husson (2013). Measured values beyond the range of -300 to 500 mV result in a risk index of 1, values from -300 to -225 and from 375 to 500 mV are attributed to a risk index of 0.5, values between -225 and 375 mV are attributed to a risk index of 0.

$$\text{If } pH_{well,\,day} < 6.5 \qquad pRI_{pH,\,well,\,day} = 1 - (0.0085 \times e^{0.7032 \times pH_{well,\,day}}) \qquad \text{(Eq. 7)}$$

$$\text{If } 6.5 \leq pH_{well,\,day} \leq 9 \qquad pRI_{pH,\,well,\,day} = 1 - (-0.163 + 0.1381 \times pH_{well,\,day}) \qquad \text{(Eq. 8)}$$

$$\text{If } pH_{well,\,day} > 9 \qquad RI_{pH,\,well,\,day} = 1 - (2.163 - 0.1481 \times pH_{well,\,day}) \qquad \text{(Eq. 9)}$$

2.3.4.5 Environmental risk

To integrate the risk indices for the chemical, ecotoxicological and physico-chemical components, the environmental risk was calculated as described by Dagnino et al. (2008) as the mean value of the three risk indices (Eq. 10).

$$EnvRI = \frac{cRI + eRI + pRI}{3} \qquad \text{(Eq. 10)}$$

3 Results & Discussion

3.1 Effect assessment for Carbo-Iron

In the additionally performed nitrogen-transformation test, no effects were observed in the investigated test concentrations up to 2828 mg Carbo-Iron/kg soil dw (see SI section 2.3.5 and Table S9 for results). In the algae test with *S. vacuolatus* and PCE, a LC_{50}= 25.6 mg/L was determined (see SI section 2.3.3 and Table S7 for the results).

3.1.1 Toxicity tests with Daphnia magna

The stability of the test suspensions decreased over time and was independent from the nominal Carbo-Iron concentrations. After exposure for 24, 48 and 72 h to 3.16 and 31.6 mg/L, measured concentrations decreased to 93, 87 and 81% of the nominal concentrations, respectively. Despite these deviations from the desired test concentrations, all exposure concentrations are given as nominal values.

Between 50 and 70% of the *D. magna* were immobile after 48-h exposure to Carbo-Iron at concentrations between 10 and 56.2 mg/L (Figure 2A and Table S1). However, in the highest test concentration only 30% of the *D. magna* were immobile. A 48-h LC_{50} of 33.5 mg/L was determined (Table S2). After the 5-d post-exposure period in the culture medium without Carbo-Iron, mean immobility was at least 90% in all test concentrations ≥ 10 mg/L (Figure 2B), and for the postexposure a LC_{50} of 3.4 mg/L was derived (Table S2).

In the 21-d chronic test, no mortality was recorded at concentrations ≤ 3.2 mg/L while exposure of *D. magna* to 10 mg/L Carbo-Iron reduced the survival to 70% (Table S3). The reproduction rate was significantly lower compared to the CMC control at the two highest test concentrations of 3.16 and 10 mg/L (Figure 2C and Table S3) and an EC_{10} of 2.0 mg/L (Table S4) was calculated.

While approx. 90% of the daphnids were immobile at 10 mg/L after the post-exposure period in the acute toxicity test, only 30% of the introduced daphnids were immobile in the chronic toxicity test at the same test concentration. In both tests, the same method was used for the preparation of the Carbo-Iron suspensions. However, as required by OECD (2012) daphnids were not fed during the 48-h exposure in the acute toxicity test, while food was provided every second day starting on d 0 in the chronic test. Potentially, daphnids in the acute test ingested more Carbo-Iron than the algae-fed daphnids in the chronic test. During the post-exposure phase after the acute toxicity test, this reduced food uptake could not be compensated for. In a previous study with Carbo-Iron and the crustacean *Hyalella azteca* (Weil et al., 2016), effects on survival of adults and offspring were related to presence of Carbo-Iron in the gut, a reduced feeding rate, and an assumed decreased gut clearing time that most likely led to nutrient depletion. The general gut physiology of the pelagic cladoceran *D. magna* and the epi-benthic malacostracan *H. azteca* is similar: It is composed of oesophagus, midgut and hindgut with setae that allow the separation between food particles and liquid compounds (Ceccaldi, 1989). Likely, similar increases of gut transit times and food uptake rates as probably caused by Carbo-Iron in *H. azteca* are the reason for the delayed mortality of *D. magna* observed in the post exposure of the acute toxicity test.

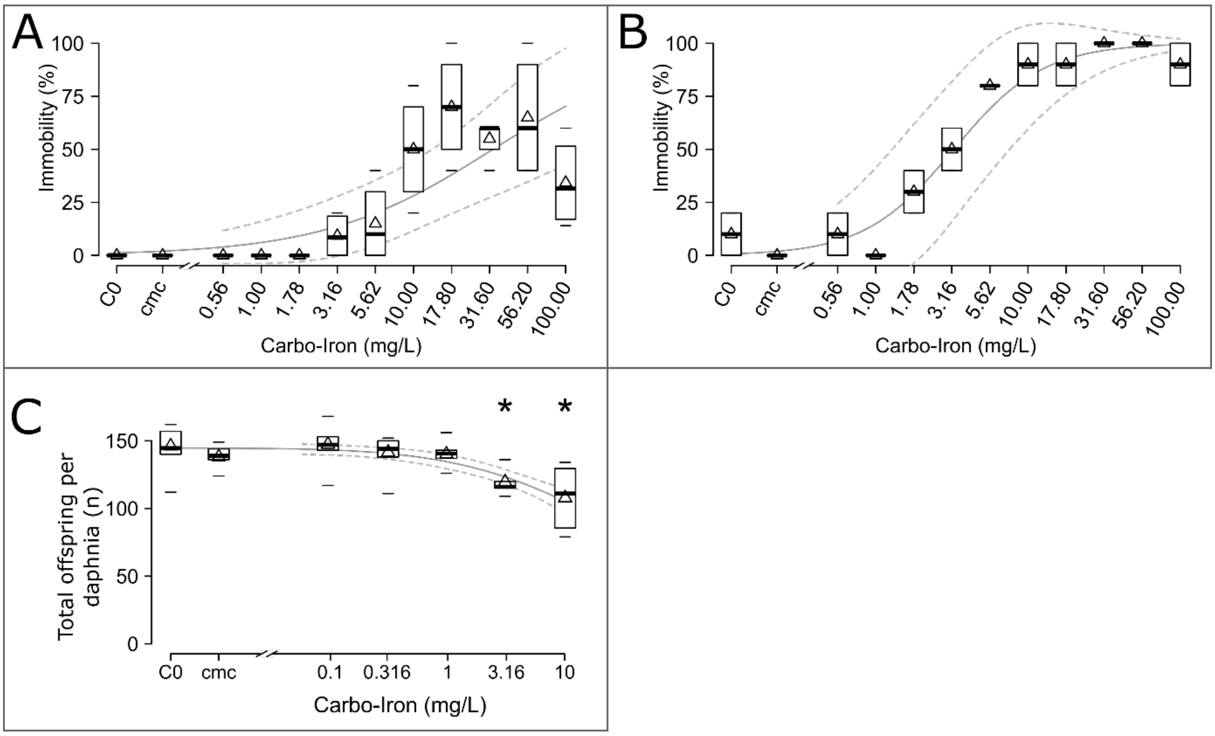


Figure 2: Results of the toxicity tests with *D. magna* and Carbo-Iron. In the acute test, immobility (n=4) was assessed after 48 h exposure (**A**). Subsequently, *Daphnia* were transferred to culture medium without Carbo-Iron and mortality was assessed after 5 d post-exposure (**B**). In the chronic test (**C**) total number of living offspring per *Daphnia* (7≤n≤10) was determined during 21 d of exposure. In all tests, negative controls with culture medium (C0) and CMC controls with 20 mg/L CMC (cmc) were investigated. *: Significant difference from the CMC control (Wilcoxon ranks sum test, p≤0.05). Triangles are mean values; boxes represent the upper and lower quartile of the data, horizontal dashes represent extreme values outside this range.

3.1.2 Algal growth inhibition test

In the algae growth inhibition test with *S. vacuolatus* the endpoint cell volume inhibition was measured after 16 h exposure to Carbo-Iron and AC, respectively. In all Carbo-Iron treatments, significant inhibition of cell volumes was found in comparison to the control (Figure 3). The observed effects on cell volume in the Carbo-Iron treatments were comparable to those observed in the AC treatments, considering the mass difference of approx. 1.2 between Carbo-Iron and active carbon with EC_{10}=7.2 and 6.4 mg/L, respectively (Table S6).

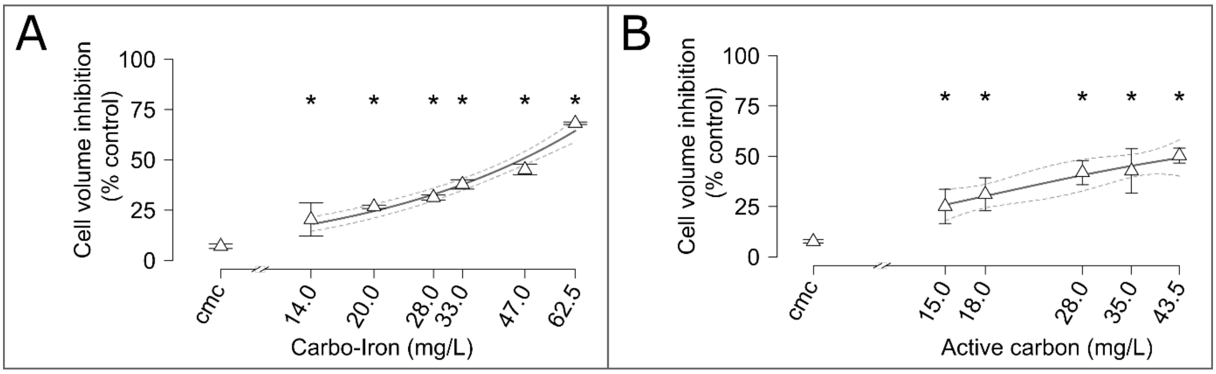


Figure 3: Cell volume inhibition (mean ± sd; n=3) in *S. vacuolatus* after 16 h exposure to Carbo-Iron (**A**) and AC (**B**). In all tests, negative controls with culture medium (C0) and CMC controls with 20 mg/L CMC (cmc) were investigated. Cell volume inhibition was normalized to the control values. *: Significant difference to the CMC control (ANOVA followed by two-sided Dunnett's test, p≤0.05).

In order to evaluate the effect of shading by the particles, the light intensity at the beginning of the exposure was measured in selected test vessels (SI section 2.3.2). Light intensities decreased with increasing Carbo-Iron and AC content (Table S5). Light intensities in Carbo-Iron treatments were significantly higher than in the corresponding AC treatment (Fig. S3). The shading effects observed in the tests with Carbo-Iron and AC are potentially the main reason for the observed effects on algae. Similar effects were reported for CNT-exposed green algae (Schwab et al., 2011). However, the binding of nutrients from the test media by AC (Bundschuh et al., 2011) could additionally have caused nutrient depletion in the culture medium and thus contributed to the observed toxicity.

3.1.3 Sediment-water test with Chironomus riparius

Carbo-Iron suspensions were initially homogenously grey in concentrations < 56.2 mg/L and nearly opaque at test concentrations ≥ 56.2 mg/L. The test suspensions became visibly clearer approx. 10 d after start of exposure and black precipitate was visible on the sediments, indicating nearly complete sedimentation of the particles in all treatments. Emergence of *C. riparius* was not affected by Carbo-

Iron and reached a similarly high value of 89% in the highest test concentration as in the CMC control with 90% (Table S8). For development rate, a significant increase was observed in the CMC control compared to the control with 0.057 and 0.061 d^{-1}, respectively; Figure 4, Table S8). Positive effects of α-cellulose as organic material (i.e. nutrient) on the growth of *Chironomus tentans* were observed by Lacey et al. (2009) in a 10-d exposure. Due to the similar chemical structure of α-cellulose and CMC it is likely that CMC had a positive effect on the development of the midge larvae. However, while all Carbo-Iron treatments had the same CMC content, exposure to Carbo-Iron minimized the positive effect of CMC and a significantly lower development rate of 0.056 d^{-1} was observed in the highest test concentration of 100 mg/L Carbo-Iron in comparison to 0.061 d^{-1} in the CMC control.

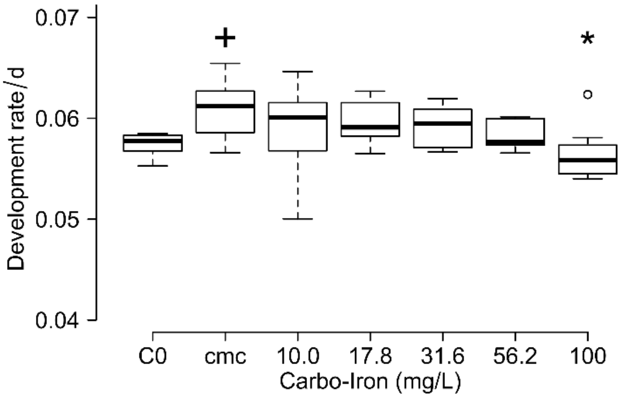


Figure 4: Development rate of *C. riparius* (n=8) during the 28-d exposure to Carbo-Iron in the water-sediment test. Triangles are mean values, boxes represent the upper and lower quartile of the data, whiskers extend to minimum and maximum values within a 1.5 quartile distance, circles represent extreme values outside this range. +: significant difference to the control (C0, two-sided t-test, $p \leq 0.05$); *: significant difference from the CMC control (cmc, two-sided Dunnett's test, $p \leq 0.05$).

3.2 Assessment of the environmental risk of aged Carbo-Iron

The results from the ecotoxicity studies with aged Carbo-Iron are summarized in Table S10. The most sensitive effect concentration observed in the chronic ecotoxicity tests was the NOEC of 1.0 mg/L derived in the 21-d study with *D. magna*. Since results of chronic studies with all three trophic levels are available, an assessment factor of 10 was used and a PNEC of 0.1 mg/L was derived for Carbo-Iron. This PNEC is relatively high when compared to PNEC values obtained for nano-Ag (0.02 µg/L), nano-ZnO (1 µg/L), fullerenes (4 µg/L), nano-TiO_2 (16 µg/L) and carbo-nano tubes (CNT; 56 µg/L) (Coll et al., 2016).

At the pilot study site, the estimated environmental concentration of Carbo-Iron in the area close to the injection points (zone Ia: 650 mg/L, see Table 1), exceeds the PNEC by a factor of 6500. The risk quotients decrease with increasing distance to the injection point to 4750 (zone Ib), 13 (zone Ic, sampling well GWM1) and 5 (zone I, sampling well RKS13, approx. 10 m from injection points). The estimated migration of Carbo-Iron through the aquifer is supported by the detection of Carbo-Iron particles in samples from GWM1 on days 30, 57, 92 and 139 after injection, and in samples from CMT2 on day 139 after injection (Fig. S1). The detection method used for this analysis is described in SI section 2.2 and was developed recently. However, the quantification of Carbo-Iron with this method is currently not possible. A further indication of the transport of Carbo-Iron is provided by the observed change in redox potential (Table S16), and Carbo-Iron can be assumed to considerably influence the aquifer for approx. 200 d after the first injection. Based on the estimated transport data for Carbo-Iron from the column studies (SI section 2.1), a distribution of Carbo-Iron beyond zone I is unlikely. Hence, the risk quotients in zones II, III and IV can be assumed to be below 1 indicating no risk due to Carbo-Iron application.

3.3 Site-specific risk-benefit analysis

The pilot study site was highly polluted with chlorohydrocarbons (Table S11) and the potential benefit or a possible risk/risk trade-off of the Carbo-Iron treatment was assessed. For this assessment, available ecotoxicity data for the most relevant hydrocarbons polluting the pilot study site were evaluated to derive PNEC values (Table S13) and used in a Triad-based environmental risk assessment. Due to the lack of target values, Carbo-Iron was not included in the calculation of the cRI. However, it was included as a pollutant, equal to the chlorohydrocarbons, for the calculations of the eRI. The calculations of the risk indices were performed with exposure data collected during the 1st application of Carbo-Iron (Mackenzie et al., 2016, see sections 2.2 and 2.3).

In Figure 5, the results of the site-specific environmental risk assessment are shown for the contamination zones I to IV as defined in Figure 1 and Table 1. The physico-chemical risk (Tables S19 and S20) was relatively stable in all contamination zones during the 190 d after Carbo-Iron injection. Increased risk indices due to a reduced redox potential are visible in zone II (CMT2) on d 190 and zone III (RKS24) on d 31 and d 58. The slightly increased physico-chemical risk in zone II (well CMT1) on d 93 was caused by an increased conductivity and a lower pH than on the sampling days before (Table S19). These observations of increased physico-chemical risk indices are very likely related to the application of Carbo-Iron into the aquifer. The effect on redox potential can be assumed to be temporary, caused by the reaction of Carbo-Iron with the pollutants and the oxygen in the groundwater, i.e. ceasing with loss of reactivity of Carbo-Iron. Yet, with the available data it is not possible to verify these assumptions. The desired remedial effect of Carbo-Iron is discernible in the decreasing chemical and ecotoxicological risk indices in all groundwater samples investigated (Figure 5 and Tables S9 and S12) during the first 58 d after Carbo-Iron injection. This effect is most pronounced in samples from the contamination zones Ic and I (wells GWM1 and RKS13). Obviously, the amount of Carbo-Iron applied into the aquifer during the first injection campaign was not sufficient to completely remediate the contamination with chlorohydrocarbons, which was not the intention of the study by Mackenzie et al. (2016). The chemical (Table S11 and S12) and ecotoxicological (Tables S14 and S15) risks increase again after 58 d (Figure 5), possibly due to inflow of chlorohydrocarbon-contaminated groundwater from the non-treated area upstream of the injection wells and depletion of reactive Carbo-Iron. However, on d 190 in zones Ic (GWM1), I (RKS13) and III (RKS24), a strong decline of the chemical and ecotoxicological risk occurred compared to d 93. This was mainly caused by a drop of measured concentrations of PCE and trichloroethene (Table S11), maybe as a result of precipitation event. In most zones investigated, the eRI and cRI reached similar but lower values on d 190 than before application of Carbo-Iron. The calculated environmental risk (Table 2) integrates all three risk indices and its highest value is usually observed on d 0 during the 190 d of monitoring. Yet, in zone II (CMT2), the eRI increased on d 93 and d 190 above any previous values and the environmental risk is higher than before the application of Carbo-Iron. On these days, concentrations of ethane, ethene and 1,1-dichloroethene and 1,2-dichloroethene reached their maximum value, after steadily increasing from 0 mg/L on d 0 (Table S11). Similar increasing concentrations are observable for all zones investigated. The measured concentrations exceed the relatively low PNEC values of 1,1-dichloroethene and 1,2-dichloroethene and this represents the main reason for the increased eRI in zone II. The presence of these transformation products and simultaneous absence of vinyl chloride corresponds to the spectrum of PCE degradation by Carbo-Iron and thus indicates proper functioning of the remediation (Mackenzie et al., 2012). Yet, the increased risk is not desirable. In the present study, effects of Carbo-Iron on the pollutants beyond d 190 were not considered and the fate of the transformation products was not examined. However, Vogel et al. (2018) observed in a field study that 600 days after treatment with Carbo-Iron microorganisms in the groundwater grow on the AC component of Carbo-Iron and biogenic dehalogenation of halogenated alkenes occurs. Thus, in the long term, further decreasing values for the eRI and cRI are expected when an exhaustive remediation with Carbo-Iron is envisaged.

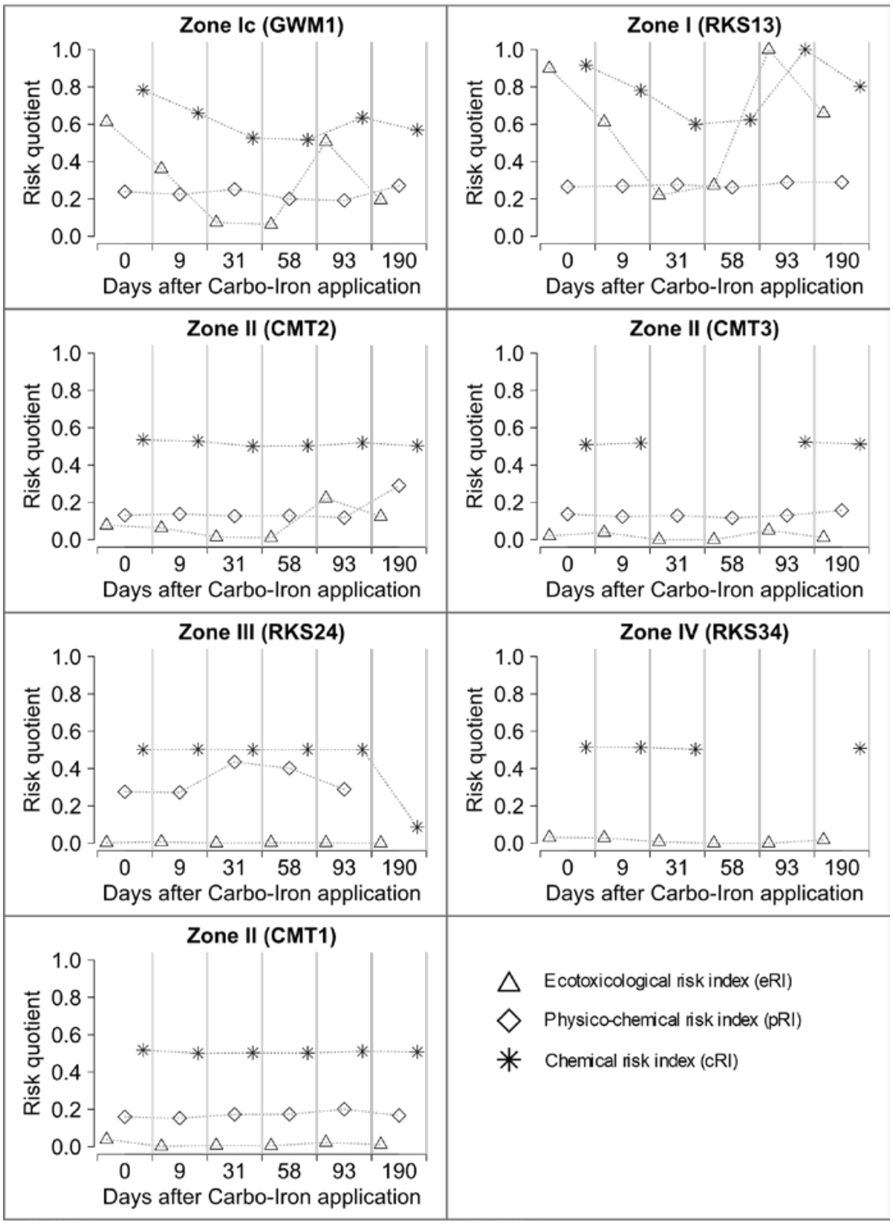


Figure 5: Comparison of the calculated risk indices for groundwater contaminated with chlorohydrocarbons during the first 190 d after treatment with Carbo-Iron using a TRIAD-based approach. No data points available for days with no measurement data.

Table 1: Environmental risk for groundwater contaminated with chlorohydrocarbons integrating the risk indices for the chemical, ecotoxicological and physico-chemical component.

Contamination zone (Sampling well)	Days after Carbo-Iron injection 0	9	31	58	93	190
Zone Ic (GWM1)	0.545	0.4152	0.2846	0.260	0.445	0.344
Zone I (RKS13)	0.694	0.554	0.365	0.386	0.763	0.583

Zone II (CMT2)	0.248	0.243	0.214	0.214	0.287	0.306
Zone II (CMT3)	0.222	0.223	- [A]	- [A]	0.234	0.227
Zone III (RKS24)	0.2606	0.260	0.312	0.302	0.264	NA
Zone IV (RKS34)	- [B]	- [B]	- [B]	- [B]	- [B]	- [B]
Zone II (CMT1)	0.239	0.219	0.228	0.227	0.245	0.229

[A]: No data for the determination of the cRI available, the environmental risk could not be calculated;

[B]: No data for the determination of the pRI available, the environmental risk could not be calculated.

4 Conclusion

With the crustacea *D. magna* a NOEC of 1 mg/L was determined, a similar range as the NOEC of 6.3 mg/L determined in a study with the crustacean *Hyalella azteca,* likely associated to reduced nutrient uptake and negative effects on the energy budget. The effect of Carbo-Iron on the algae *S. vacuolatus* however is probably caused by shading of the algae by the particles, reducing light intensity required for photosynthesis.

Estimated concentrations of Carbo-Iron were exceeding the PNEC in the areas close to the injection, but decreasing rapidly with increasing distance to injection wells. Carbo-Iron successfully reduced the concentrations of the investigated pollutants while the presence of Carbo-Iron and the increasing concentrations of the transformation products were usually not leading to environmental risks exceeding the levels before the remediation was initiated. However, in the single occurrence of increased risk, increasing concentrations of the transformation product dichloroethene was identified as the responsible compound. Pre-existing natural attenuation is not negatively affected by the presence of Carbo-Iron in the groundwater and very likely reduces the concentrations of the transformation products in the time period exceeding the monitored time in the present study. With the data analysed in the present study, the benefit of applying Carbo-Iron into the groundwater outweighs the potential negative effects on the environment and a risk/risk-trade-off is considered as very unlikely.

In case of remediation of contaminated groundwater, the best available option has to be chosen. The procedure described for environmental risk assessment in the present manuscript is not limited to

Carbo-Iron; it is suitable to support decisions on other remediation methods as well. With sufficient data, the Triad-based approach presented in the current study can be used to compare the potential environmental risks of other remedial agents, whether they are based on nano zero-valent iron or on other *in situ* treatment methods.

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Appendix A. Supplementary data.

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