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Oxidized Carbo-Iron causes reduced reproduction and lower tolerance of juveniles in the amphipod *Hyalella azteca*

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ABSTRACT

For in situ remediation of groundwater contaminated by halogenated hydrocarbons Carbo-Iron®, a composite of microscale activated carbon and nano Fe^0, was developed. Against the background of intended release of Carbo-Iron into the environment in concentrations in the g/L-range, potential ecotoxicological consequences were evaluated in the present study. The nano Fei^0 in Carbo-Iron acts as reducing agent and is oxidized in aqueous systems by chlorinated solvents, groundwater constituents (e.g. dissolved oxygen) and anaerobic corrosion. As Carbo-Iron is generally oxidized rapidly after application into the environment, the oxidized state is environmentally most relevant, and Carbo-Iron was used in its oxidized form in the ecotoxicological tests. The amphipod *Hyalella azteca* was selected as a surrogate test species for functionally important groundwater crustaceans. Effects of Carbo-Iron on *H. azteca* were determined in a 10-d acute test, a 7-d feeding activity test and a 42-d chronic test. Additionally, a 56-d life cycle test was performed with a modified design to further evaluate effects of Carbo-Iron on adult *H. azteca* and their offspring. The size of Carbo-Iron particles in stock and test suspensions was determined via dynamic light scattering. Potential uptake of particles into test organisms was investigated using transmission and scanning electron microscopy. At the termination of the feeding and acute toxicity test (i.e. after 7 and 10 d of exposure, respectively), Carbo-Iron had a significant effect on the weight, length and feeding rate of *H. azteca* at the highest test concentration of 100 mg/L. While an uptake of Carbo-Iron into the gut was observed, no passage into the surrounding tissue was detected. In both chronic tests, the number of offspring was the most sensitive endpoint and significant effects were recorded at concentrations $\geq$50 mg/L (42-d experiment) and $\geq$12.5 mg/L (56-d experiment). Parental exposure to oxidized Carbo-Iron significantly exacerbated the acute effects of the nanocomposite on the subsequent generation of *H. azteca* by a factor >10. The present study indicates risks for groundwater species at concentrations in the mg/L range. Carbo-Iron may exceed these effect concentrations in treated aquifers, but the presence of the pollutant has most likely impaired the quality of this habitat already. The benefit of remediation has to be regarded against the risk of ecological consequences with special consideration of the observed increasing sensitivity of juvenile *H. azteca*.

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1. Introduction

Groundwater pollution is among the most severe environmental challenges (Dimitriou et al., 2008; Fatta et al., 2002). Contamination may arise from a multitude of anthropogenic activities, which include landfills, industrial effluents or accidental spillage (Rail, 1989). Groundwater aquifers can provide a habitat for a high diversity of micro- and macroorganisms (among them various crustaceans) including many endemic species (Danielopol and Griebler, 2008; Hahn and Fuchs, 2009). Thus, the protection and remediation of groundwater bodies is of high importance, but also represents a

Abbreviations: CMC, Carboxymethyl cellulose; EC_x, Concentration leading to an effect in x% of organisms (e.g. EC_{50}); EDX, Energy dispersive X-ray microanalysis; LC_x, Concentration leading to mortality of x% of organisms (e.g. EC_{50}); nFe^0, Nanoscaled zerovalent iron; PBS, Phosphate buffered saline sd:Standard deviation of the group of data values used to calculate e.g. a mean; SEM, Scanning electron microscopy.

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scientific challenge considering the variety of contaminants. In this context, the use of nanoscaled zerovalent iron (nFe^0) for remediation of contaminated groundwater is a promising technique (Xu et al., 2012). For efficient groundwater treatment with nFe^0, particles should have a certain mobility to build a broad reactive barrier in the aquifer. Yet, mobility of nFe^0 in the receiving medium is impeded by quick agglomeration and sedimentation (Johnson et al., 2013; Yin et al., 2012). To improve mobility and maintain reactivity, Carbo-Iron, a nanocomposite of zerovalent iron nanoparticles and activated carbon was developed (Bleyl et al., 2012; Mackenzie et al., 2012), which is a promising alternative to nFe^0. For in situ groundwater remediation, several thousand L of a suspension with a Carbo-Iron concentration in the g/L range have to be applied into the aquifer (Mackenzie et al., 2016).

As so far very few ecotoxicity data are available for Carbo-Iron (Weil et al., 2015), potential risks for aquatic ecosystems deserve further investigation. In ecotoxicity testing, semi-static or flow-through test systems are often employed to overcome sedimentation behavior of particles in suspension and increase the probability of the relevant species to interact with the particles, recently especially focused on experiments with nanoparticles (Bundschuh et al., 2012; Seitz et al., 2013). These experimental designs simulate the continuous release of particles via point sources such as wastewater treatment plant effluents. However, sediments represent a sink for particles in suspension (Baun et al., 2008; Poynton et al., 2013). Therefore, ecotoxicity tests with sediment-dwelling organisms are of high relevance for an estimation of environmental risk of Carbo-Iron. Additionally, to minimize the risk that a remediation of an aquifer with Carbo-Iron deteriorates the conditions for groundwater organisms, information on potential effects on such organisms is necessary. For these reasons, the benthic amphipod *Hyalella azteca* was chosen as test organism in the present study as a surrogate species for amphipods inhabiting groundwater. In a study on risk assessment of chemical stressors in groundwater, Schäfers et al. (2001) recommended to place special emphasis on tests with higher crustaceans (e.g. amphipods). Surface water-inhabiting higher crustaceans show comparable sensitivity as related groundwater species (Schäfers et al., 2001). *H. azteca* are living on the surface of and in the upper few mm of the sediment (Doig and Liber, 2010).

Since ingestion of Carbo-Iron was supposed to be the major route of uptake, the presence of Carbo-Iron particles on and in *H. azteca* was evaluated in a 10-d acute toxicity test in addition to the standard test endpoints survival and growth (US EPA, 2000). The impact of Carbo-Iron on ingestion rates of leaves was investigated during a 7-d exposure. Potential chronic toxicity of Carbo-Iron was studied in a 42-d exposure including reproduction of *H. azteca* as an endpoint (US EPA, 2000). Assuming a higher sensitivity of juveniles released from adults exposed to higher Carbo-Iron concentrations, as has been shown for nano TiO_2 (Bundschuh et al., 2012), the potential impact of Carbo-Iron on amphipod offspring was investigated in an additional chronic experiment.

2. Material and methods

2.1. Culture of Hyalella azteca

H. azteca were obtained in 2002 from Dresden University of Technology. They were kept at 20–25 °C and 16:8 h light:dark in glass tanks with quartz sand and approximately 5 L culture medium according to Borgmann (1996) with $CaCl_2$ (110.98 mg/L), $MgSO_4$ (30.09 mg/L), $NaHCO_3$ (84.01 mg/L), KCl (3.72 mg/L) and NaBr (1.03 mg/L). The amphipods were fed twice per week with TetraMin® flakes *ad libitum*, the culture medium was exchanged every other week.

Prior to each experiment, a synchronized culture was initiated. Organisms from the culture were siphoned through sieves. *H. azteca* passing through sieves with 500 μm but being retained by 355 μm were considered to be approximately 6 d old (US EPA, 2000). They were transferred to a new culture vessel and kept under the same conditions as the original culture until test start. Before each experiment, a group of 20 animals was removed from the synchronized culture, and length and weight were measured to allow for the quantification of growth during the tests.

2.2. Preparation of test suspensions and artificial sediment

The toxicity tests with *H. azteca* were performed with aged Carbo-Iron, which means that the originally zero-valent iron oxidized to Fe^{2+} and Fe^{3+}. Aged Carbo-Iron was provided by the producers Mackenzie and colleagues (see Bleyl et al., 2012; Mackenzie et al., 2012, 2016 for more details on composition and other particle characteristics); the iron content was approx. 22% (w/w). Stock suspensions were prepared as described by Weil et al. (2015). As stabilizing additive, 2 g carboxymethyl cellulose (CMC; Antisol® FL 30, Sigma-Aldrich, Germany) were added to 1 L of deionized water supplemented with 0.1 mL NaOH (1 M, Sigma-Aldrich, Germany). CMC is also used for stabilization of Carbo-Iron in suspensions used for application into the groundwater. The CMC solution was stirred overnight at room temperature, subsequently filtered through a 0.4 μm filter (MN GF-5, Macherey-Nagel, Germany) and stored for a maximum of 7 d at 4 °C. Before use, the CMC solution was diluted with deionized water to 200 mg CMC/L. Carbo-Iron stock suspensions (1 g/L) with 200 mg/L CMC (i.e. 20% w/w relative to Carbo-Iron) were prepared by adding 100 mg Carbo-Iron to 100 mL CMC solution. The suspensions were placed on ice and treated with an ultrasonic probe (Hielscher UP200S, Germany, 14 mm probe diameter) at approx. 80 W for 7 min. If volumes >100 mL were needed, this procedure was repeated and the obtained suspensions were pooled until sufficient volume was prepared. The Carbo-Iron stock suspensions were used within 2 h after preparation.

Test suspensions were prepared immediately before starting the ecotoxicity tests by diluting the stock suspension with test medium. Final CMC concentration was 20 mg/L in all test suspensions irrespective of the Carbo-Iron concentration. All tests included controls (culture medium) and dispersant controls (20 mg/L CMC in culture medium).

For the water-sediment test systems, artificial sediment according to OECD (2004) with 75% quartz sand (Quarzwerke Frechen, Germany), 20% kaolin (Chinafill 100, Ziegler, Germany) and 5% peat (Thomaflor, Germany) was prepared approx. one week before use, and preconditioned at 23 ± 2 °C with aeration. Two days prior to the start of the exposure, glass beakers with screw caps and a total volume of 500 mL were filled with 80 ± 1 g (fresh weight) sediment. Subsequently, 180 mL culture medium were added to each vessel. Test vessels were then incubated at 23 ± 2 °C with slight aeration until test start. Immediately before introduction of *H. azteca*, Carbo-Iron stock suspension (1 g/L Carbo-Iron and 200 mg/L CMC) and CMC solution (200 mg/L) were mixed in the necessary ratios to achieve 10-fold nominal test concentrations in a final volume of 20 mL. This mixture was then added to the respective test vessels.

2.3. Ecotoxicity tests

To choose suitable developmental stages of *H. azteca* for the respective tests, development of the laboratory culture was observed over a period of 80 d and results are shown in Fig. 1 (details are available in Section 1 in the SI). In the water-sediment test systems, the test vessels were incubated at 23 ± 2 °C with a 16:8 h light:dark photoperiod. The test vessels were aerated with approx. 1–2 bubbles per second starting at d 2 after the onset of

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