Application of zerovalent iron (Fe\textsuperscript{0}) to enhance degradation of HCHs and DDX in soil from a former organochlorine pesticides manufacturing plant

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Abstract

Remediation of pesticide-polluted soil is particularly challenging when pesticides in soil are aged and a mixture of pesticides is present. Application of zerovalent iron (Fe\textsuperscript{0}) was investigated to accelerate the degradation of HCHs (\textalpha-, \textbeta-, \textgamma- and \textdelta-hexachlorocyclohexane) and DDX (DDT, DDE and DDD) in soil from a former organochlorine pesticide manufacturing plant. Ultrasonic extraction was used to extract the organochlorine pesticides from soil. The identification and quantification of organochlorine pesticides in the extracts were accomplished by gas chromatography. The addition of Fe\textsuperscript{0} facilitated the degradation of the \textbeta-HCH isomer, but had little effect on the degradation of \textalpha-HCH, \textgamma-HCH and \textdelta-HCH. Zerovalent iron significantly increased the degradation of \textp,p\textsubscript{-}DDT and \texto,p\textsubscript{-}DDT in soil, and the percentage degradation of \textp,p\textsubscript{-}DDT + \texto,p\textsubscript{-}DDT increased with increased Fe\textsuperscript{0} concentration during the first period of incubation. However, the amount of \textp,p\textsubscript{-}DDD, the main dechlorinated product of \textp,p\textsubscript{-}DDT, decreased with increased Fe\textsuperscript{0} concentration in the unamended soil. The addition of Fe\textsuperscript{0} therefore did not increase the percent degradation of \textgamma-HCH (\textp,p\textsubscript{-}DDT + \texto,p\textsubscript{-}DDT + \textp,p\textsubscript{-}DDD) markedly.

1. Introduction

Organochlorine pesticides (OCPs) such as lindane and DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) have a long history of use for control of agricultural pests (Li, 1999; Eggen and Majcherczyk, 2006). The gamma isomer of hexachlorocyclohexane (\textgamma-HCH) is commonly known as lindane, but the technical grade of lindane also contains \textalpha-, \textbeta-, and \textdelta-HCH isomers as by-products (Phillips et al., 2006). DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane) and DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) are common co-contaminants with DDT in soils, which arise from both as impurities during the manufacture of DDT and also as stable transformation products from biotic and abiotic processes (Foght et al., 2001). DDT, DDE and DDD are collectively referred to as DDX. Organochlorine pesticides (OCPs) have been recognized as a potential health risk due to their bioaccumulation, recalcitrance to degradation, and potential toxicity to humans and wildlife (Jones and de Voogt, 1998; Turusov et al., 2002; Behrooz et al., 2009; Trejo-Acvedo et al., 2009). Furthermore, DDT and DDE are known endocrine disrupting compounds (Amaral mendes, 2002). Therefore, the production and usage of HCHs and DDX were banned or restricted in many countries during the 1970s and 1990s (Voldner and Li, 1995; Li, 1999). However, concentrations of HCHs and DDX in soils in many former organochlorine pesticides manufacturing plants are still high due to spills, discharges or leaking storage tanks (Phillips et al., 2006; Yang et al., 2009).

To remediate and restore functions of soil polluted by HCHs and DDX, effective technologies are necessary. Conventional treatments for organochlorine-contaminated soils include excavation and incineration, thermal desorption (Foght et al., 2001), microwave-enhanced thermal treatment (Kawala and Atamanczuk, 1998), soil washing with surfactants (Kile and Chiou, 1989), supercritical fluid extraction (Sahle-Demessie and Richardson, 2000) and biological treatment (Yao et al., 2006). Among these treatment technologies, bioremediation was more cost-effective and less destructive (Foght et al., 2001). Many pesticides, such as \textbeta-HCH and DDX are normally considered persistent in aerobic environments, are not persistent under anaerobic conditions (Aislabie et al., 1997; Van Eekert et al., 1998). Therefore, generating a reduced environment in water, soils and sediments may facilitate the degradation of organochlorine pesticides (OCPs) (Satapanajaru et al., 2006). Zerovalent iron (Fe\textsuperscript{0}) is a good reducing agent and can generate a reduced environment in water, soils and sediments (Sayles et al., 1997; Comfort et al., 2001; Eggen and Majcherczyk, 2006; Yao et al., 2006). Some studies observed zerovalent iron can facilitate the degradation of DDT in water and soil freshly spiked with DDT (Sayles et al., 1997; Yao et al., 2006). However, there is a lack of knowledge about the...
potential of zerovalent iron to stimulate the degradation of HCHs and DDx in contaminated site soils.

There are many differences between soil freshly spiked with organochlorine pesticides and contaminated sites soil which have been polluted by organochlorine pesticides for several years. Firstly, organochlorine pesticides such as DDT and HCHs in contaminated sites soil may have become less bioavailable after being naturally aged for many years (Singh and Agarwal, 1992; Quintero et al., 2005). Furthermore, soils from former organochlorine pesticides manufacturing plant may be impacted by more than one contaminant. Co-contaminants may complicate biodegradation (Foght et al., 2001). They provide more readily utilized substrates for the micro flora, which can divert enzymatic activity from the contaminant of concern. Furthermore, they may have specific or nonspecific toxic effects on the soil micro flora and may affect the solubility or adsorption of the contaminant of concern (Foght et al., 2001).

The objective of the present study was to investigate the effect of zerovalent iron (Fe0) on the degradation of hexachlorocyclohexane isomers (α-, β-, γ-, δ-HCH) and 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and its metabolites (DDE and DDx) in a highly contaminated soil from a former organochlorine pesticides manufacturing plant.

2. Materials and methods
2.1. Chemicals and reagents

Standard solutions of organochlorine pesticides including α-, β-, γ- and δ-HCH [hexachlorocyclohexane], p,p'-DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane], p,p'-DDE [1,1,1-trichloro-2-p-chlorophenyl]-2-(o-chloro-phenyl)ethane], p,p'-DDD [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] and pentachloronitrobenzene (PCNB) were purchased from the National Research Center for Certified Reference Materials of China. Hexane and dichloromethane (HPLC-graded) were offered by Tedia Company, USA. Anhydrous sodium sulfate (Na2SO4, AR, Beijing Chemical Reagent Plant, PR China) was oven-dried at 450 °C for 6 h to act as desiccant. Silica gel (80 mesh, Dalian Chemo-physical Institute, PR China) and alumina (Beijing Chemical Factory, 100 mesh) were activated in an oven at 180 °C and 250 °C respectively, then deactivated by adding 3% deionized water. Zerovalent iron (Fe0) (size < 100 mesh, 98% of pure iron), was purchased from Beijing Chemical Factory, China.

2.2. Soil samples

To remediate a former organochlorine pesticides contaminated site in Beijing, China, about 10 m³ of heavily organochlorine pesticides contaminated soil was removed from the contaminated site in 2008 and stored in storehouse in Beijing, China. In this study, about 10 kg soil was sieved (2 mm) and homogenized. The soil was characterized as: sand soil (53.37% sand, 46.38% silt and 0.25% clay), pH 8.34, total organic carbon 13.7 g kg⁻¹, total nitrogen 0.82 g kg⁻¹, and moisture 2.1%. The soil was sampled from each treatment (three replicates) on day 0 of the incubation to measure initial concentrations of HCHs and DDx in soil (Table 1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>mg kg⁻¹ (d.w.)</th>
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</thead>
<tbody>
<tr>
<td>α-HCH</td>
<td>3.73 ± 0.46</td>
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<tr>
<td>β-HCH</td>
<td>26.52 ± 1.70</td>
</tr>
<tr>
<td>γ-HCH</td>
<td>2.35 ± 0.32</td>
</tr>
<tr>
<td>δ-HCH</td>
<td>4.69 ± 0.37</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>31.40 ± 1.34</td>
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<tr>
<td>o,p'-DDT</td>
<td>7.37 ± 0.69</td>
</tr>
<tr>
<td>p,p'-DDD</td>
<td>7.70 ± 0.38</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>4.79 ± 0.29</td>
</tr>
</tbody>
</table>

2.3. Experimental procedure

To evaluate the effect of zerovalent iron (Fe0) on HCHs and DDx biodegradation, experiments were carried out in 250 mL flasks with 100 g of fresh soil (wet weight) and a series of zerovalent iron after intermixing thoroughly. The soil was mixed with a certain amount of zerovalent iron in the 500 mL beaker. Mixed the soil and iron powder by glass rod thoroughly, then transferred the mixture into the flask. Forty-seven milliliters deionized water was transferred to each flask by measuring cylinder to keep the moisture of the mixture to 50% (dry weight) at 0 d of incubation, then the flasks were tightly closed. The soil had a water content greater than 140% of its water holding capacity (WHC) after the deionized water was added to each flask and the soil in flask was saturated. Four treatments were then initiated: (1) control (no Fe0), (2) 0.5% (w/w) Fe0, (3) 2% (w/w) Fe0, (4) 5% (w/w) Fe0. Each treatment included 6 replicates. The soil samples in the flasks were incubated at 30 °C in the dark. The soil was sampled from three flasks of each treatment after 0, 10, 20, 30, 40 d of incubation, respectively, to measure concentrations of HCHs and DDx in the soil. About 4 g of soil (wet weight) were sampled from each flask and wrapped in pre-cleaned aluminum foil and immediately stored at −20 °C until analysis.

Oxidation-reduction potential in the soil was measured with Pt-electrode and calomel electrode reference connected to a portable pH meter (PHS-3, China) after 0, 1, 3, 5, 10, 20, 30, 40 d of incubation, respectively, in the other three flasks of each treatment (see above). The reading at 30 min without mixing was used as the measurement. The Eh values were converted to a standard hydrogen electrode (SHE) reference by adding (251-X) mV to the mV reading obtained from the calomel electrode reference used experimentally. The electrodes adjusted by redox potential standard solutions (KeTu electrode Company, Nanjing, China) were applied for measurement.

2.4. Extraction and analytical procedures

Ultrasonic extraction was applied to extract the organochlorine pesticide from the soil (USEPA, 2000). Briefly, 1 g of homogenized and freeze-dried soil was placed in 50 mL centrifuge tubes and 30 mL of dichloromethane/hexane (1:1, v/v) was added. The sample was extracted three times by ultrasonication for 60 min, 30 min, and 30 min, respectively, in an ultrasonic cleaning bath (400 W average power output and mean operating frequency of 40 kHz). The extract was separated by centrifugation at 1500 rpm for 15 min in order to obtain clear organic supernatants. The combined extracts were concentrated to about 3 mL in eggplant-type bottle with a vacuum rotary evaporator at a temperature below 39 °C. An additional 20 mL of hexane was added to the eggplant-type bottle and the combined extracts were again concentrated to about 3 mL in eggplant-type bottle, and then the hexane extracts were transferred to a 1.2 alumina/silica gel glass column to be purified and fractionated. The column was eluted with 15 mL of hexane and the eluate was discarded. The second organochlorine pesticides containing fraction was eluted with 70 mL of dichloromethane/hexane (3:7, v/v). The eluate containing organochlorine pesticides was diluted with hexane to fit the GC calibration range.

The identification and quantification of organochlorine pesticides in the extracts were accomplished by a gas chromatograph...
equipped with an electron capture detector (ECD) (HP 5890 series II, Agilent). The capillary column was HP-5, 30 m long × 0.32 mm width × 0.25 μm film thickness. The carrier gas was nitrogen (purity = 99.99%). The injector and detector temperature were 250 °C and 300 °C, respectively. The oven temperature program consisted of an initial temperature of 60 °C for 1 min, which was increased at a rate of 20 °C min⁻¹ to 140 °C, held for 5 min, and then raised at a rate of 10 °C min⁻¹ up to 230 °C, and held for 2 min. The injection volume was 1 μL in the splitless mode. The quantification was determined by internal calibration. The quantification of organochlorine pesticide was accomplished by a 8-point internal calibration curve using peak area and the internal standard for determining the OCPs was pentachloronitrobenzene (PCNB).

2.5. Quality control

To reduce or eliminate contamination problems, glassware was prepared by successive treatments in the following order: washing with acetic acid and water, soaking in 5% H₂Cr₂O₇ sulfuric acid solution overnight, washing with water and distilled water in turn, then rinsing with acetic acid and hexane just before use. For accuracy and precision of analysis, the method blank and the spiked sample with organochlorine pesticide standards were measured in duplicate with each batch of 20 samples. Method blanks were run firstly using the same solvents as for soil samples. No contaminants of organochlorine pesticides were found in the method blanks. The recovery of the pesticides were calculated by spiking with 230 μg L⁻¹ of organochlorine pesticide standards (HCHs and DDX) in soil. These spike soils were allowed to equilibrate for at least 24 h before extraction. The spiked recoveries of OCPs were in the range of 89.5–112.9% (except δ-HCH, where it was 65.1 ± 5.9%). The quantification was determined by internal calibration using peak area. The calibrations were made daily and the correlation coefficients (r) of calibration curves of HCHs and DDX were all higher than 0.998. DDT thermal breakdown was checked periodically by injecting p,p'-DDT standards, according to 8081B guidelines (USEPA, 2007). The method detection limits (MDL) were in the range from 0.02 mg kg⁻¹ to 0.04 mg kg⁻¹ for the OCPs following the method of Qiao et al. (2004).

3. Results and discussion

3.1. The effects of Fe₀ on oxidation–reduction potential of soil

The oxidation–reduction potential of the soil in the treatment without Fe₀ remained about 400 mV for the duration of the experiment and the soil was in an aerobic state. Zerovalent iron (Fe₀) had little effect on the oxidation–reduction potential of soil. The strong effect on the oxidation–reduction potential of soil in the treatments with addition of Fe₀ decreased rapidly from a level of +400 mV to less than 200 mV and became anaerobic. The more Fe₀ added, the more negative the oxidation–reduction potential became during the first period of the incubation (Fig. 1).

3.2. The effect of Fe₀ on the degradation of HCHs

The apparent degradation of α-HCH, γ-HCH and δ-HCH had been observed in the treatment without Fe₀ after 40-d-incubation (Fig. 2). The percentage degradation of α-HCH, γ-HCH and δ-HCH was 77%, 80% and 62%, respectively, after 40-d-incubation. However, little effect of Fe₀ on the degradation of α-HCH, γ-HCH and δ-HCH was observed (Fig. 2). Compared with α-, γ- and δ-HCH, the story of β-HCH was more interesting. The concentration of β-HCH in the treatment without Fe₀ had no apparent change in the total 40 d (Fig. 2). The percentage degradation of β-HCH is less than 44%, respectively, after 40-d-incubation. It is evident that zerovalent iron (Fe₀) had noticeable effect on the degradation of α-HCH, γ-HCH and δ-HCH, but had noticeable effect on the degradation of β-HCH. This could be attributed to the characteristics that α-HCH, γ-HCH and δ-HCH might biodegrade under both aerobic condition and anaerobic condition, and the biodegradation of β-HCH need anaerobic condition. Our results agreed with the literature (Doelman et al., 1990; Johri et al., 1998; Van Eekert et al., 1998).

HCH biodegradation was initially thought to be an aerobic process (Setunathan et al., 1983; MacRae et al., 1984) and reductive dechlorination of the four commonly found HCH isomers (α-, β-, γ-, δ-HCH) had been observed under anoxic conditions in soil microcosms (Jagnow et al., 1977), soil slurry mixtures (MacRae et al., 1984) and pure cultures (Jagnow et al., 1977). The proposed pathway for the aerobic transformation of HCH showed that HCH was reductively dechlorinated to tetrachlorocyclohexene (TCH) and 5,6-dichloro-1,3-cyclohexadiene (DCDN), the latter of which was reductively dechlorinated to monochlorobenzene (MCB) and benzene (Phillips et al., 2005). However, α-HCH and γ-HCH were also biodegraded anaerobically in the soil and soil slurry mixtures (MacRae et al., 1984; Wada et al., 1989), field plots of soil (Doelman et al., 1990) and pure cultures (Johri et al., 1998), but a strongly negative redox potential was needed for β-HCH removal (Van Eekert et al., 1998). The proposed pathway for the aerobic transformation of γ-HCH showed that γ-HCH was oxidized to pentachlorocyclohexene (PCHC), 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) and 1,2,4-trichlorobenzene (1,2,4-TCB). The latter of which was again oxidized or proceeded with aerobic aromatic ring cleavage (Nagata et al., 1999; Phillips et al., 2005).

3.3. The effects of Fe₀ on the degradation of DDX

The concentration of p,p'-DDT and o,p'-DDT in treatment without Fe₀ had no apparent change during the total 40 d (Fig. 3). However, the addition of Fe₀ could facilitate the degradation of p,p'-DDT and o,p'-DDT markedly. Apparent degradation of p,p'-DDT and o,p'-DDT had been observed in treatment with the addition of Fe₀ during the first 20 d. Furthermore, the percentage degradation of p,p'-DDT and o,p'-DDT increased with increased Fe₀ during the first 20 d (Fig. 3). After 20 d, a small quantity of p,p'-DDT and o,p'-DDT in the treatment with the addition of 5% Fe₀ degraded, while a great quantity of p,p'-DDT and o,p'-DDT in the treatment with the addition of 0.5% Fe₀ and 2% Fe₀ degraded. It is evident that zerovalent iron (Fe₀) had noticeable effect on the degradation of
Fig. 2. Effect of zerovalent iron on HCHs degradation by the initial amounts of Fe\(0\): 0.0% ( ), 0.5% ( ), 2.0% ( ), 5.0% ( ) (w/w, n = 3).

Fig. 3. Effect of zerovalent iron on degradation of DDX by the initial amounts of Fe\(0\): 0.0% ( ), 0.5% ( ), 2.0% ( ), 5.0% ( ) (w/w, n = 3).
p,p'-DDT and o,p'-DDT, and the more Fe$^0$ added, the greater percentage of p,p'-DDT and o,p'-DDT degraded during the first period of incubation. This could be due to two main facts. Firstly, a negative redox potential was needed for biodegradation of DDT (Aislabie et al., 1997; Van Eekert et al., 1998). Furthermore, zerovalent iron (Fe$^0$) lowered the oxidation–reduction potential of soil, and the more Fe$^0$ added, the more negative oxidation–reduction potential of the soil reached during the first period of incubation (Fig. 1).

The quantity of p,p'-DDT in the treatment without Fe$^0$ had no apparent change during the total 40 d (Fig. 3). However, the addition of Fe$^0$ can increase the degradation of p,p'-DDE markedly, and the more Fe$^0$ added, the higher percentage of p,p'-DDE degraded. Compared with p,p'-DDE, the mechanism behind p,p'-DDT degradation was more complicated. Except for control tests, the amount of p,p'-DDT in the treatments with the addition of Fe$^0$ showed a pronounced increase in the first period of the incubation and the more Fe$^0$ added, the more amount of p,p'-DDT increased. With time, the amount of p,p'-DDT in the treatments with the addition of Fe$^0$ had increased slightly, and the difference of the amount of p,p'-DDT in the treatments with the addition of Fe$^0$ was less obvious.

DDT degradation in the soil appears to proceed by one of two main routes depending on the prevailing environmental conditions (Foght et al., 2001). In anoxic soils, transformation of DDT to DDD by reductive dechlorination is considered to be the dominant reaction (Boul, 1996). However, minor level of DDE also has been detected in this process (Boul, 1996; Foght et al., 2001). Considering DDD and DDE are also toxic and recalcitrant to degradation (Foght et al., 2001), Alternating anaerobic and aerobic incubation conditions can enhance DDX biodegradation by promoting reductive dechlorination of DDT–DPB with subsequent aerobic aromatic ring cleavage (Foght et al., 2001).

**4. Conclusion**

The effects of zerovalent iron on the degradation of HCHs and DDX were investigated in the soil from a former organochlorine pesticide manufacturing plant. The results showed that zerovalent iron could facilitate the degradation of β-HCH, p,p'-DDT and o,p'-DDT, but had little effect on the degradation of α-HCH, γ-HCH and δ-HCH. However, the amount of p,p'-DDD, the main dechlorinated product of p,p'-DDT, basically kept increased except in the unamended soil. The addition of Fe$^0$ therefore not increased the percent degradation of p,p'-DDT in p,p'-DDT + p,p'-DDE. The factors that influence DDX degradation have to be clarified in further studies to enhance the degradation of ΣDDT.

**Acknowledgements**

This work was supported by National S & T Infrastructure Program of China (No. 2007BAC28B04) and the Knowledge Innovation Project of Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences (No. 200904002).

**References**


Eggen, T., Majcherzyck, A., 2006. Effects of zerovalent iron (Fe$^0$) and temperature on the transformation of DDT and its metabolites in lake sediment. Chemosphere 62, 1116–1125.


