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# Effect of nitrate addition on reductive transformation of pentachlorophenol in paddy soil in relation to iron(III) reduction

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ABSTRACT

Reductive dechlorination is a crucial pathway for anaerobic biodegradation of highly chlorinated organic contaminants. Under an anoxic environment, reductive dechlorination of organic contaminants can be affected by many redox processes such as nitrate reduction and iron reduction. In the present study, batch incubation experiments were conducted to investigate the effect of nitrate addition on reductive dechlorination of PCP in paddy soil with consideration of iron transformation. Study results demonstrate that low concentrations (0, 0.5 and 1 mM) of nitrate addition can enhance the reductive dechlorination of PCP and Fe(III) reduction, while high concentrations (5, 10, 20 and 30 mM) of nitrate addition caused the contrary. Significant positive correlations between PCP degradation rates and the formation rates of dissolved Fe(II) (pearson correlation coefficients $r = 0.965$) and HCl-extractable Fe(II) ($r = 0.921$) suggested that Fe(III) reduction may enhance PCP dechlorination. Furthermore, consistent variation trends of PCP degradation and the abundances of the genus *Comamonas*, capable of Fe(III) reduction coupled to reductive dechlorination, and of the genus *Dehalobacter* indicated the occurrence of microbial community variation induced by nitrate addition as a response to PCP dechlorination.



## 1. Introduction

Pentachlorophenol (PCP), extensively used as a herbicide, insecticide, fungicide, wood preservative, resin, lubricant and dye intermediate, has been commonly found in ground waters, sediment and surface soils (Field and Sierra-Alvarez, 2008). It can be absorbed into the body through inhalation, diet, or skin contact (Eisler, 1989) and has caused numerous occupational illnesses and deaths (Wood et al., 1983). Its acute toxicity results from its ability to interfere with the production of high-energy phosphate compounds essential for cell respiration (Eisler, 1989; Liu et al., 2008). PCP can be degraded in the environment by chemical, microbiological and photochemical processes (Choudhury et al., 1986). In soil, microbial decomposition appears to be an important and potentially dominant removal mechanism (Choudhury et al., 1986). In addition, anaerobic reductive dechlorination achieved mainly by anaerobic microbes is a crucial pathway for the degradation of highly chlorinated organic compounds such as PCP (Adrian and Gorisch, 2002).

Iron, the most abundant element on earth and the most frequently utilized transition metal in the biosphere, plays a particularly important role in environmental biogeochemistry (Kappler and Straub, 2005). Previous studies have indicated that iron is involved in the transformation of many elements and contaminants, such as nitrogen and organic pollutants (Borch et al., 2010; Lovley et al., 2004). In particular, iron is closely linked to the transformation of nitrogen (Borch et al., 2010). For example, pyrite oxidation is able to couple denitrification in fertilizer-impact aquifers (Postma et al., 1991). In addition, nitrate-dependent Fe(II)-oxidizing bacteria mediate Fe(II) oxidation in neutrophilic, anoxic environments, where nitrate can serve as an electron acceptor (Kappler and Straub, 2005). Moreover, under anaerobic conditions, the dissimilatory reduction of Fe(III) can be coupled with ammonium oxidation (Clement et al., 2005). Furthermore, dissolved and adsorbed Fe(II) can effectively enhance the reductive dechlorination of organic pollutants (Amonette et al., 2000; Li et al., 2008). On the other hand, nitrogen, as a necessary element for microorganisms, can influence the growth and abundance of many

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microorganisms (Vitousek and Howarth, 1991). It has been reported that N addition can accelerate the degradation of organic contaminants by stimulating microbial activity (Braddock et al., 1997), while N addition can also inhibit enzymatic systems responsible for the degradation of organic pollutants, resulting in an inhibition of the degradation of organic pollutants (Abdelhafid et al., 2000). In addition, the application of nitrate, which serves as an electron acceptor, can compete for electrons with the reductive dechlorination of chlorinated organic compounds (Yoshida et al., 2007). As mentioned above, iron and nitrogen can each affect the degradation of organic pollutants separately, but the transformations of iron and nitrogen can also affect one another. Therefore, iron and nitrogen together can also affect the degradation of organic pollutants. However, few studies have considered the effects on the degradation of contaminants with respect to this interaction.

Currently, China consumes approximately 30% of global nitrogen fertilizer. Between 1961 and 1999, the global application of nitrogen fertilizer increased from $11.6 \times 10^6$ t to $85.5 \times 10^6$ t (approximately 6.4 times), whereas, during the same period, the application amount of nitrogen fertilizer in China increased 43.8 times (Peng et al., 2002). Guangdong province, located in southeast China, is one of China's most economically prosperous regions and has been well developed agriculturally. Results from a study investigating the nitrate and nitrite pollution of vegetables in typical areas of Guangdong province showed that 36.5% of samples contained nitrate at higher levels than national standards (Yang et al., 2007). Therefore, the excessive application of nitrogen fertilizer has resulted in high residual levels of nitrate in the agricultural environment of Guangdong province. In addition, PCP has also been used extensively as a wood preservative, molluscicide and clean-pond reagent in Guangdong province (Hong et al., 2005). Relatively high levels of PCP are found in pond sediment of this area, especially in Zhongshan, containing comparable levels of PCP to those found in a historic severe schistosomiasis plague area in China (Peng et al., 2002). In addition, the soil in Guangdong province is characteristic of red soil, which is rich in iron oxides (Li et al., 2006).

The objective of the present study was to examine the effect of nitrate addition on reductive dechlorination of PCP in paddy soil with consideration of the involvement of the process of iron transformation. It is expected that the results derived from the interactions among nitrate, iron and PCP in the microcosm will offer theoretical guides for further research on the interaction mechanisms among the nitrogen cycle, iron cycle and reductive dechlorination of organic pollutants under natural conditions.

## 2. Materials and methods

### 2.1. Soil sampling

Paddy soil sample used in the present study was collected from the town of Shahu in the city of Enping (22°23.11′N, 112°26.85′E), located in southwest Guangdong province in south China during September and December 2008. Upon return to the laboratory, the soil was sealed in polytetrafluoroethylene (PTFE) bag and stored in glass bottles at 4 °C prior to use.

### 2.2. Chemicals

PCP (≥98% purity) and 1,4-piperazinediethanesulfonic acid (PIPES) (≥98% purity) were purchased from Sigma–Aldrich (St. Louis, MO, USA). $NaNO_3$ was purchased from Sinopharm Chemical Reagent Co., Ltd, China. All other analytical-grade chemicals were obtained from Guangzhou Chemical Co., China. Deaerated deionized water was prepared by deoxygenating ultrapure water (18 MΩ-cm, Easy Pure II RF/UV, USA).

### 2.3. PCP transformation experiments in soil

Experiments were conducted in triplicate at a constant pH of 7.0 ± 0.2 with 30 mM PIPES as a buffer solution. The batch experiment procedures were as follows: the soil samples (0.5 g dry weight) were transferred into 20 mL serum bottles with silicone-lined septa and aluminum sealing caps, and 10 mL PIPES buffer solution was then added. Seven batch experiments, including control, were conducted in this study: (1) control (0 mM $NaNO_3$); (2) 0.5 mM $NaNO_3$; (3) 1 mM $NaNO_3$; (4) 5 mM $NaNO_3$; (5) 10 mM $NaNO_3$; (6) 20 mM $NaNO_3$; and (7) 30 mM $NaNO_3$. Subsequently, PCP and lactic acid at final concentrations of 0.0188 mM and 10 mM, respectively, were added to each vial. The mixture was then purged with $O_2$-free $N_2$ for 30 min and sealed with teflon-coated butyl rubber stoppers and crimp seals. The closed bottles were mixed on a rotary shaker and incubated at 30 ± 1 °C in an anaerobic chamber. Finally, at predetermined sampling intervals, the bottles were removed for analysis.

### 2.4. Chemical analysis methods

The PCP in the soil suspension was extracted using water/ethanol mixtures (50:50 by volume) on a horizontal shaker (180 rpm) for 1 h. The suspension was then filtered through a 0.45 μm syringe filter and collected for high performance liquid chromatography analysis. The detailed analysis procedures for PCP have been described in a previous study (Lan et al., 2008). HCl-extractable Fe(II) and dissolved Fe(II) were measured using the 1,10-phenanthroline colorimetric method at 510 nm on a UV–Vis spectrophotometer (TU-1810PC, Beijing Purkinje General Instruments, China) (Viollier et al., 2000). Concentrations of $NO_3^-$/$NO_2^-$ were determined by ion chromatography (Dionex ICS-90) with an ion column (IonPac AS14A4 × 250 mm) and detection limits for both $NO_3^-$ and $NO_2^-$ were 0.003 mM. The mixed solution of $Na_2CO_3$ (8.0 mM) and $NaHCO_3$ (1.0 mM) was used as mobile phase at a flow rate of 1.0 mL $min^{-1}$.

### 2.5. DNA extraction and terminal restriction fragment length polymorphism (T-RFLP) analysis

Total soil DNA was extracted using the PowerSoil™ DNA isolation kit (Mo Bio Laboratories, USA) according to the manufacturer's instructions. The bacterial 16S rRNA gene was amplified using PCR primers 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′; 5′ end labeled with 6-FAM) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′). The PCR mixture contained 1.2 μL of each primer (20 μM), 15 μL of the 2× PCR mix containing Taq polymerase, dNTP and buffer solution, 1 μL of template DNA and 11.6 μL of ultrapure water. PCR was performed using an initial denaturation step of 94 °C for 4 min followed by a program of 30 cycles consisting of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1.5 min, with a final extension step at 72 °C for 10 min. Triplicate PCRs per sample were performed, and the labeled PCR products were purified with a commercial PCR purification kit (OMEGA biotek, USA). Aliquots of these products were digested with restriction enzyme AluI (TaKaRa Biotechnology, China) at 37 °C for 6 h. The digested PCR products were resolved by electrophoresis using an ABI 3730xl sequencer (Applied Biosystems, USA), with GS-500 Rox as an internal size standard in each lane. The fragment sizes and peak fluorescence intensities were analyzed using GENESCAN software. The relative abundance of individual terminal restriction fragment (T-RF) was calculated as the percentage of its peak area