When chicken manure compost meets iron nanoparticles: an implication for the remediation of chlorophenothane-polluted riverine sediment

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Abstract

The remediation of contaminated sediment is an intractable problem as both the remediation efficiency and the ecological environmental impact should be carefully considered. In this study, nanoscale zero-valent iron (nZVI) was applied to assist the remediation of chlorophenothane (DDT)-polluted sediment by chicken manure compost. The response of sediment bacterial community to the remediation treatments was evaluated a month later after two strategies to add the remediation materials (simultaneous and phased) were implemented. Using nZVI could enhance the degradation efficiency of chlorophenothane by the compost. Compared with simultaneous addition of chicken manure compost and nZVI at the beginning of remediation, using nZVI as a forerunner followed by adding the compost a week later further enhanced p,p'-DDT degradation but increased ∑DDT residual amount. The used chicken manure compost increased the richness, evenness, and diversity of sediment bacterial community. According to β-diversity analysis, the compost made a greater difference in the bacterial community than nZVI at the experimental using levels. Using 0.5 wt% nZVI could help to activate the bacterial metabolism in the compost-remediated sediment and increase functional abundance for DDT degradation. This study contributes to the improvement of sediment remediation technologies and the understanding of bacterial community variations in chlorophenothane-contaminated sediment remediated with chicken manure compost and nZVI.

Keywords: Nanoscale zero-valent iron; Chlorophenothane; Sediment; Remediation; Compost

1. Introduction

The riverine sediment is susceptible to pesticide pollution due to the frequent agricultural activities occurred in the river basin. During the application of pesticides, only a very small proportion (less than 5%) of the pesticides can reach the targets, and the majority of pesticides are released to the surrounding environment.[1] A considerable amount of pesticides can enter nearby river through agricultural effluent discharge, rain wash, and surface runoff, and gradually accumulate in the sediment. The long-term heavy use of pesticides has caused their occurrence in many riverine sediments, with the concentration level varying from ng/kg to mg/kg.[2, 3] The pesticides in sediment can be ingested by aquatic organisms, and enter the food chain. Chronic exposure to pesticides may contribute to the disease incidence of cancer, nervous disorder, reproductive dysfunction, and immune dysregulation.[4] On the other hand, as the sink of pesticides in the aquatic ecosystem, riverine sediment may release pesticides back into the overlying water when the sediment is disturbed, posing serious threat to the water quality and the aquatic ecosystem.[5] Considering the serious pesticide pollution and increasing requirement for environmental security, developing innovative technologies for treating pesticide-contaminated sediment is urgently needed.

Using compost for the remediation of contaminated sites has been widely studied as it can help to improve soil/sediment texture and establish a lot of microbial population quickly for degrading organic pesticides.[6] By the microbial metabolism and co-metabolism, organic pesticides can be effectively degraded and even completely

mineralized, which is considered eco-friendly. However, the hydrophobic characteristic of most organic pesticides makes them easy to combine with soil/sediment particles, limiting their bio-accessibility to microorganisms.[7] Additionally, treating contaminated sites with compost is subject to variable weather conditions and long remediation period. In order to enhance the remediation efficiency, some additives are used to assist the degradation process, such as specific degrading bacteria,[8] activated sludge,[9] and surfactant.[10] The development of nanotechnology brings a new strategy for facilitating the remediation by compost. Nanoscale zero-valent iron (nZVI) is a powerful reductant extensively studied for pollution abatement.[11-14] The nZVI can serve as an electron donor when it is in contact with pesticides, thus facilitating the degradation reactions.[15-17] Therefore, coupling the biological degradation of compost and the chemical degradation of nZVI is expected to further enhance the remediation efficiency. However, the regulating effects of nZVI on the remediation of pesticide-contaminated sediment by compost are unclear. Generally, it needs to use relatively more nZVI to treat the pesticide pollution by relying completely on nZVI, and the resulting adverse effects of nZVI on sediment organisms are not conducive to the recovery of sediment ecological functions.[18] Additionally, illuminating the interactions among pesticide, nZVI, and microorganisms (including both indigenous microorganisms and compost microorganisms) has directive significance to the engineering practice of sediment remediation.

Currently, only a few attempts have been made on this topic and the emphasis is

usually placed on variations of the pollutant content in soil system. Few studies were performed on this topic in riverine sediment system and the interactions among pesticide, nZVI, and microorganisms during the remediation process.[19-21] Chlorophenothane, also called dichlorodiphenyl trichloroethane (DDT), is a typical organochlorine pesticide, and its main pollution sources include the historical heavy use as pesticide, the recent input as raw material and major metabolite of dicofol, and the legitimate use for preventing mosquito-borne diseases such as malaria, dengue, and yellow fever.[22] The highly toxic and persistent of chlorophenothane in natural sediment make the risks lasting for several years to decades.[23] In this study, nZVI was used to assist the remediation of chlorophenothane-polluted sediment by chicken manure compost. Primary emphasis is placed on the bacterial community response in the sediment after the addition of compost and nZVI. This study may be helpful in improving sediment remediation technologies and understanding ecological environmental effects of the remediation treatment.

2. Materials and methods

2.1. Sediment, compost, and nZVI particles

The riverine sediment was sampled from the Xiangjiang River near Hunan University. After being air-dried, triturated, and sieved with an 18-mesh screen, the sediment samples were used for determining the basic physicochemical properties and preparing the chlorophenothane-contaminated sediment. The sediment has a texture of

70.9% sand, 23.3% silt, and 5.8% clay. The pH and organic matter content are 7.2 and 1.8 wt%, respectively. Technical chlorophenothane product typically consists of p,p'-dichlorodiphenyl trichloroethane (p,p'-DDT, 77.1%), o,p'-dichlorodiphenyl trichloroethane (o,p'-DDT, 14.9%), p,p'-dichlorodiphenyl dichloroethylene (p,p'-DDE, 4.0%), p,p'-dichlorodiphenyl dichloroethane (p,p'-DDD, 0.3%), and some trace impurities.[24] Thus, the chlorophenothane-polluted sediment was simulated by spiking a mixture of p,p'-DDT, o,p'-DDT, p,p'-DDD, p,p'-DDE into the sediment following a procedure proposed by the US Environmental Protection Agency.[25] In order to approach chemical equilibrium of chlorophenothane between the sediment and interstitial water, the spiked sediment was stored for one month. After one-month equilibrium period, the contaminated sediment was used for the following experiments, and the initial levels of p,p'-DDT, o,p'-DDT, p,p'-DDD, p,p'-DDE before the remediation experiment were 16.5, 15.9, 7.7, and 0.7 mg/kg, respectively. Chicken manure compost used in this study is a commercially available composting product. According to our measurement, the compost possesses a pH value of 6.3, a total humic acid content of 10.8 wt%, an organic carbon content of 2.2 wt%, and a carbon-nitrogen ratio of 58.2. The used nZVI particles have an average size of 50 nm, a purity of 99.9% (metals basis), and a surface area of 23.5 m^2/g. The morphological and structural features of nZVI were determined by electron microscope, X-ray photoelectron spectroscopy, and X-ray diffractometer, and the results are displayed in Fig. S1. The nZVI particles show typical microcosmic sphere, and contain an oxide layer which has

an estimated thickness of 4.1 nm on their surface.

2.2. Design of the remediation treatment

Two kinds of treatment strategies were implemented in the remediation process of chlorophenothane-contaminated sediment. The first (Set I) is simultaneously adding chicken manure compost and nZVI at the beginning of remediation. The using amount of chicken manure compost was 5.0 wt% and at this level the compost can help to partly degrade the pollutant but not completely according to the preliminary experiment. This using amount of the compost is suitable for the research purpose of this study. Three different dosages (0.25 wt%, 0.5 wt%, and 1.0 wt%) of nZVI were used to investigate the effects of nZVI on the remediation system. The second (Set II) is adding nZVI at the beginning of remediation and then adding 5.0 wt% of the compost a week later. This design aims to avoid the adverse effect of highly reactive nZVI on the microorganisms in chicken manure compost at the beginning of remediation.[26] The contaminated sediment without any remediation treatments was used as the control. After one month of remediation treatment, sediment samples were taken for determining the residual chlorophenothane and analyzing the bacterial community.

2.3. Determination of chlorophenothane in sediment

The chlorophenothane in sediment was quantitatively measured by gas chromatography-mass spectrometry based on the Chinese standard HJ 835-2017.

Concretely, the chlorophenothane was extracted from 20 g of the sediment sample by 100 mL of a mixed solvent of n-hexane and acetone in a Soxhlet extractor. The extracting solution was condensed to 1.0 mL by a nitrogen sample concentrator and then purified with a magnesium silicate column. The resulting solution was concentrated again and made to a constant volume of 1.0 mL. The concentration of chlorophenothane was quantitatively determined with an internal standard method. The detection limits of this method for p,p'-DDT, o,p'-DDT, p,p'-DDD, p,p'-DDE were 0.09, 0.08, 0.08, and 0.04 mg/kg, respectively.

2.4. Analysis of bacterial community in sediment

Sediment bacterial community information was obtained by 16S rRNA gene sequencing. The bacterial DNA was obtained from sediment by magnetic bead method with a commercial DNA extracting kit (E.Z.N.A., Omega Bio-tek, USA). The DNA integrality and concentration were measured by agarose gel electrophoresis and Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA), respectively. Polymerase chain reactions (PCR) were performed with primer 341F and 805R for amplifying the V3-V4 regions of the bacterial 16S rRNA gene. After being purified, the obtained PCR product was accurately quantified, and the amplicons were then sequenced on an Illumina MiSeq platform.

2.5. Sequencing data analysis

The original image data gotten from the Illumina MiSeq platform were converted into sequenced reads by base calling. The sequenced reads of primers and adaptors were first removed, and then the paired-end reads were merged into one according to the overlap. The data for each sample were segmented from the merged reads based on the differentiated barcode. All the sample reads were converted to various operational taxonomic units (OTUs) based on their similarity. The corresponding bacterial species of each OTU was identified by the ribosomal database project (RDP) classifier. The α-diversity of the bacterial community was analyzed with Chao1, Shannoneven, and Simpson indexes, which were calculated with the software Mothur (v. 1.43.0). Fisher LSD test was used to identify the significant difference between the α-diversity indexes of different groups at a significant level of 0.05. The β-diversity between different treatment groups was quantified and presented as Bray-Curtis distance, which is calculated by a weighted method considering both the presence and the abundance of bacterial species. Significantly different species between different treatments were analyzed with Welch's t-test by the software STAMP (v. 2.1.3).[27] The function of bacterial community was predicted with the software PICRUSt (v. 1.1.4) and annotated based on the KEGG database.[28]

3. Results and discussion

3.1. Degradation of chlorophenothane by compost in the presence of nZVI

The addition of nZVI enhanced the degradation efficiency of chlorophenothane in

191 both Set I and Set II (Fig. 1). When the contaminated sediment was remediated with
192 only compost, the degradation efficiency of p,p'-DDT, o,p'-DDT, p,p'-DDD, p,p'-DDE,
193 and ∑DDT (total DDT including the first four) was 27.9%, 17.0%, 3.4%, −28.8%, and
194 18.5%, respectively. The negative value indicates that considerable amount of p,p'-DDT
195 was degraded and transformed into p,p'-DDE by the chicken manure compost.
196 Simultaneous addition of chicken manure compost and nZVI at the beginning of
197 remediation (Set I) enhanced the degradation of all DDT and the derivatives. With the
198 assistance of 1.0 wt% nZVI, 61.6% of ∑DDT was removed by chicken manure compost.
199 When nZVI was used as a forerunner and chicken manure compost was added a week
200 later (Set II), the degrading transformation of p,p'-DDT was promoted but the residual
201 p,p'-DDD increased compared with that of Set I. This result suggests that considerable
202 amount of p,p'-DDT was degraded to p,p'-DDD by nZVI in the compost remediation
203 system. As a result, the removal efficiency of ∑DDT in treatments of Set II was less
204 than that of Set I, but still higher than that with only compost.

205 A critical step of chlorophenothane degradation is dechlorination, which can be
206 realized via microbial metabolism and co-metabolism.[29] The addition of chicken
207 manure compost may intensify the microbial degradation of chlorophenothane by
208 introducing exogenous degrading bacteria and rising the abundance and activity of
209 indigenous bacteria.[30] The degradation of chlorophenothane by nZVI is mainly by
210 reductive dechlorination, and p,p'-DDD is the main degradation product of p,p'-DDT.[11,
211 15] Additionally, p,p'-DDT may undergo oxidative degradation via non-DDD pathway.[31]

212 The increase of residual p,p'-DDD in the treatment of Set II shows that reductive
213 dechlorination was the major degrading mechanism of chlorophenothane in this case.
214 When the contaminated sediment was remediated with only nZVI, the degradation
215 efficiency of chlorophenothane was higher than that with both compost and nZVI in
216 some cases (Fig. S2). The best performance for ∑DDT degradation was observed with
217 0.5 wt% nZVI alone, showing a degradation efficiency of 63.0%. These results indicate
218 the negative effect of chicken manure compost on the nZVI reactivity, which is closely
219 related to the changes of nZVI properties during the remediation process.[32] Xu *et al.*[33]
220 investigated the effects of particle properties of nZVI on its reactivity to chlorinated
221 organic compounds, and found that the Fe^0 content and the reactivity of nZVI decreased
222 with time as a result of oxidation. This result supports the high degradation capacity of
223 nZVI to p,p'-DDT in Set II. Adding high Fe^0 content nZVI at the beginning of
224 remediation showed excellent reactivity to p,p'-DDT degradation. However, using only
225 nZVI for the remediation had a significantly negative impact on the sediment bacterial
226 community and the incorporation of compost could mitigate it. This point will be
227 described and discussed below. Considering the pros and cons of both sides, proper
228 combination of nZVI and compost is necessary to ensure the benefits outweigh the
229 ecological risks when using them for the remediation of chlorophenothane-polluted
230 riverine sediment.
231
232 *3.2. Response of bacterial community structure*

According to the taxonomy of 1652 OTUs, the sediment bacterial communities involve at least 24 bacterial phyla, and the relative abundance of them in various treatments is displayed in Fig. 2. *Proteobacteria* is the most abundant bacterial phylum and their relative abundance ranges from 41.4% to 52.8%, followed by 14.1%–27.2% of *Bacteroidetes*, 6.5%–11.5% of *Actinobacteria*, and 3.8%–15.3% of *Gemmatimonadetes*. All remediation treatments increased the proportion of other bacteria except the first four phylum. The relative abundance of other bacteria in the contaminated sediment without any treatment showed the lowest proportion of 9%, while that in the sediment remediated with only compost showed the highest proportion of 19%. The presence of relatively high proportion of *Planctomycetes*, *Firmicutes*, and others in the used chicken manure compost (Fig. S3) contributed to a more diversified structure of the bacterial community after remediation treatment. Additionally, high using amount (1.0 wt%) of nZVI resulted in a relatively higher abundance of *Proteobacteria*, but lower abundance of *Bacteroidetes*. This effect was more obvious in the contaminated sediment remediated with only nZVI (Fig. S4). Similar results were found in some other studies,[34, 35] but the underlying causes need further investigation.

3.3. Variation of bacterial community diversity

The remediation treatments significantly changed the α-diversity of sediment bacterial community (Table 1). The α-diversity characterizes the species diversity within a community or habitat, and herein describes the richness, evenness, and

254 diversity of bacterial species in each treatment. These characteristics are presented as
255 Chao1, Shannoneven, and Simpson indexes, respectively. The Chao1 index quantifies
256 the bacterial richness by estimating the number of OTU in each treatment. Compared
257 with the sediment without any treatment, all the sediments containing chicken manure
258 compost showed higher bacterial richness. This can be attributed to the positive effect
259 of compost on sediment indigenous bacteria and the presence of abundant bacterial
260 species in the compost. However, no significant difference was observed after the
261 contaminated sediment was remediated with only nZVI. Within the group of sediments
262 remediated with only nZVI, high using amount (1.0 wt%) of nZVI decreased the
263 bacterial richness. The reason for this phenomenon might be that high concentration of
264 nZVI caused obvious toxicity to sediment bacteria. The Shannoneven index describes
265 the evenness of relative abundance distribution of bacterial species. Using chicken
266 manure compost increased the evenness of bacterial community, which is consistent
267 with the relative abundance increase of the other bacterial species except
268 *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Gemmatimonadetes*. Additionally,
269 when the sediment was remediated with only nZVI, the evenness of bacterial
270 community increased with the using amount of nZVI. The Simpson index is widely
271 used to reflect the species diversity based on the probability that two randomly selected
272 individuals in a community belong to the same species. A higher value of Simpson
273 index denotes a lower community diversity. Applying chicken manure compost boosted
274 the diversity of bacterial community both in the presence and absence of nZVI. The

addition of only nZVI at relatively high concentration (0.5 wt% and 1.0 wt%) decreased the Simpson index, which might result from greater environmental heterogeneity caused by nZVI [36].

The β-diversity of sediment bacterial communities between different treatment groups is illustrated in Fig. 3 based on the Bray-Curtis distance. All the remediation treatments increased the β-diversity of sediment bacterial communities, resulting in a Bray-Curtis distance of 0.32–0.58 (the first column or row in Fig. 3). The bacterial community difference between the sediment remediated with only compost and the control without any treatment reached a distance of 0.54. Within the treatments of Set I or Set II, the community difference was relatively small. The maximum distance within Set I and Set II was 0.25 and 0.24, respectively. Such a result indicates that the used compost made a greater difference in the bacterial community than the nZVI in the experiments. Additionally, the treatments of Set I had a more significant impact on the bacterial community than the treatments of Set II, which suggests the greater impact of adding chicken manure compost and nZVI simultaneously at the beginning of remediation. Staggering the addition of compost and nZVI could to some extent mitigate the bacterial diversity change.

3.4. Species difference analysis

Significantly different bacterial species between different treatments were identified and displayed in Fig. 4. The addition of chicken manure compost mainly

296 increased the abundance of *Actinomadura* (3.3%), *Luteimonas* (2.8%), and
297 *Parasegetibacter* (2.5%), but decreased the abundance of *Microvirga* (2.0%). The
298 increased three bacterial genera were members of the phylum *Actinobacteria*,
299 *Proteobacteria*, and *Bacteroidetes*, respectively (Table S1). The increased abundance
300 of *Actinomadura* and *Luteimonas* primarily resulted from their high abundance in the
301 chicken manure compost, where the two accounted for 4.8% and 9.6% of the total
302 abundance of all bacterial genera. *Parasegetibacter* is a widely studied bacterial genus
303 that is related to the microbial dechlorination, and it is often dominant in the site
304 contaminated with organochlorine compounds.[37, 38] *Microvirga* is a symbiotic nitrogen-
305 fixing bacterium, and its abundance usually increased only in the presence of plants and
306 decreased after the addition of compost.[39] The use of nZVI mainly increased abundance
307 of *Lysobacter* (5.3%) and *Ramlibacter* (2.7%), but decreased the abundance of
308 *Microvirga* (1.2%). *Lysobacter* and *Ramlibacter* both belong to the bacterial phylum
309 *Proteobacteria* (Table S2), and the abundance increase of these two bacterial genera
310 might be related to the nZVI treatment and iron oxidation.[40, 41] The *Ohtaekwangia*,
311 *Luteimonas*, *Actinomadura*, *Mycobacterium*, *Gp3*, *Mesorhizobium*, *Lysobacter*, and
312 *Sphaerobacter* were the main bacterial genera significantly different in both Set I and
313 Set II after the remediation (Fig. S5). The abundance increase of *Ohtaekwangia* and
314 *Luteimonas*, respectively belonging to the bacterial phylum *Bacteroidetes* and
315 *Proteobacteria* (Table S3 and S4), is considered to be related to the degradation of
316 aromatic ring.[42, 43] When comparing the sediment bacterial communities of Set I and

317 Set II, the abundance of *Sphingomonas* and *Lysobacter* in Set II was 4.9% and 4.0%
318 more than that in Set I, while the abundance of *Parasegetibacter*, *Cupriavidus*, and
319 *Luteimonas* in Set II was 3.5%, 2.8%, and 1.6% less than that in Set I, respectively.
320 These bacterial abundance changes can be typical responses of the sediment bacterial
321 community to the remediation treatments, but the specific functions and behaviors need
322 further investigation.
323
324 *3.5. Functional difference analysis*
325 The functional differences of sediment bacterial communities between different
326 treatment groups are illustrated in Fig. 5. It can be found that on the whole the sediment
327 bacterial communities possessed relatively high functional abundance on the amino
328 acid metabolism, carbohydrate metabolism, membrane transport, and replication and
329 repair. Amino acid and carbohydrate are the main substrates of basic metabolic
330 activities for the survival, growth, reproduction, and adaptation of bacterial cells. The
331 high functional abundance of the two indicates their significant position in the
332 metabolic function of sediment bacterial community. Membrane transport ensures the
333 normal substance exchange in cellular activities such as metabolism, and it is also the
334 functional basis of environmental information recognition and transfer. Genetic
335 replication and repair regulate the bacterial proliferation, which is a highlighted
336 function of the bacterial community in genetic information processing. Similar results
337 of the high functional abundance in these aspects were documented by.[44] Additionally,

synergistic effect between compost and 0.5 wt% nZVI enhanced the overall functional abundance of sediment bacterial community in the treatments of both Set I and Set II. The abundance differences in the function concerning DDT degradation between different treatment groups were further studied (Fig. S6). Using 0.5 wt% nZVI could help to activate the metabolic function of the bacterial community in the compost-remediated sediment and increase the functional abundance of DDT degradation. However, relatively high concentration of nZVI (1.0 wt%) inhibited the bacterial function for DDT biodegradation even in the presence of compost, and the obvious decrease of residual DDT in this case should be mainly attributed to the chemical degradation by nZVI. The predicting outcomes of PICRUSt might not accurately show the real situation of DDT biodegradation in the remediated sediments, as the expression of functional genes is often affected by the environmental factors.[45]

4. Conclusions

In this work, the remediation of chlorophenothane-contaminated sediment by chicken manure compost in the presence of nZVI was investigated. Overall, the presence of nZVI could enhance the degradation efficiency of chlorophenothane by the compost. Compared with simultaneous addition of chicken manure compost and nZVI at the beginning of remediation, using nZVI as a forerunner followed by adding the compost a week later further enhanced p,p'-DDT degradation but increased ∑DDT residual amount. The used chicken manure compost contributed to more diversified

359 structure of the bacterial community and increased the bacterial α-diversity after
360 remediation treatment. Analyzing the β-diversity of sediment bacterial communities
361 between different treatment groups suggested that the compost caused a greater
362 difference in the bacterial community than nZVI at the experimental using levels.
363 Various feature bacteria in response to the addition of chicken manure compost and
364 nZVI were identified. Using 0.5 wt% nZVI could help to activate the metabolic function
365 of bacterial community in the compost-remediated sediment and increase the functional
366 abundance for DDT degradation. This study investigated the community level
367 variations of chlorophenothane-contaminated sediment before and after remediation
368 based on the bacterial community structure. Further research is needed to reveal the
369 actual response of metabolic function and correlate it with the degradation efficiency
370 of chlorophenothane.
371

Author contributions

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Conflicts of interest

There are no conflicts of interest to declare.

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