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Response of compost maturity and microbial community composition to pentachlorophenol (PCP)-contaminated soil during composting

Guangming Zeng [a,b,*], Zhen Yu [a,b], Yaoning Chen [a,b,*], Jiachao Zhang [a,b], Hui Li [a,b], Man Yu [c], Mingjie Zhao [a,b]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China
[b] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, PR China
[c] Environmental Resources and Soil Fertilizer Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, PR China

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ABSTRACT

Two composting piles were prepared by adding to a mixture of rice straw, vegetables and bran: (i) raw soil free from pentachlorophenol (PCP) contamination (pile A) and (ii) PCP-contaminated soil (pile B). It was shown by the results that compost maturity characterized by water soluble carbon (WSC), TOC/TN ratio, germination index (GI) and dehydrogenase activity (DA) was significantly affected by PCP exposure, which resulted in an inferior degree of maturity for pile B. DGGE analysis revealed an inhibited effect of PCP on compost microbial abundance. The bacteria community shifts were mainly consistent with composting factors such as temperature, pH, moisture content and substrates. By contrast, the fungal communities were more sensitive to PCP contamination due to the significant correlation between fungal community shifts and PCP removal. Therefore, the different microbial community compositions for properly evaluating the degree of maturity and PCP contamination were suggested.

1. Introduction

Pentachlorophenol (PCP) is a highly chlorinated organic compound which has been extensively used as wood preservative, pesticide and general biocide in agriculture and industry. Although most of PCP in the environment is utilized and destroyed, a high percentage is released into the water and soil, representing a potential environmental hazard (Alexander, 1995; Scelza et al., 2008). As with many organic compounds, PCP can be irreversibly bound to soil. As a result, a legacy of contaminated soils requiring attention exists, and a variety of remediation techniques, including the physical and chemical remediation strategies and biological techniques, have been developed for the clean-up of PCP in soils (Semple et al., 2001).

As a promising bioremediation technology, composting has been successfully applied to the bioremediation of PCP-contaminated substances with the evidence that mineralization of the xenobiotic (Laine and Jorgensen, 1997) or the maturity and stability of composts (Jiang et al., 2006) is/are achieved. A composting bioremediation strategy relies on mixing the contaminated substances with other necessary materials, wherein the pollutants are degraded by the active microflora as the compost matures (Semple et al., 2001). Currently, compost maturity has been widely recognized as one of the most important factors concerning the compost process and the application of these by-products (Chikae et al., 2006). It could be a criterion for the success of bioremediation of PCP-contaminated soil and mineralization of the xenobiotic compound PCP by composting.

Microorganisms are the essential factors for the successful operation of composting, in which biodegradable organic wastes are stabilized and converted by the activities of all kinds of microorganisms under controlled conditions (Khalil et al., 2001; Tang et al., 2004). Usually, microorganisms that tend to dominate within the contaminated ecosystems are those capable of utilizing and/or surviving toxic contamination. The structure and diversity of the dominant microbial communities changed substantially (MacNaughton et al., 1999). Hence, in addition to studies of the bioavailability of hazardous chemicals, investigations of the stress of these chemicals, PCP included, are needed to properly understand the shifts in microbial communities.

Up to date, several studies have been carried out to explore the effect of PCP on the microbial community composition in soils. Relative abundance changes of microbial communities have been detected in the PCP-amended soils (Mahmood et al., 2005). While, microbial community succession in the composting system might be different from that in the soil. Microorganisms present in compost are metabolically active and able to degrade contaminants under optimized composting conditions (Antizar-Ladislao

* Corresponding authors at: College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China. Tel.: +86 731 88822754, fax: +86 731 88823701.
E-mail addresses: zgming@hnu.cn (G. Zeng), imscyn@263.net (Y. Chen).

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et al., 2007; Miller et al., 2004; Tang et al., 2009). Thus, the implementation of composting technology as a remediation strategy requires an understanding of the succession of compost microbial communities. Advanced molecular biological techniques such as polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) have proved useful in detecting this succession (Cahyani et al., 2003; Nakasaki et al., 2009). The effects of different treatment regimes on the microbial community composition can be evaluated using such technique at molecular level (Vivas et al., 2009).

For complete elucidation of the biota responsible for the bioremediation of PCP-contaminated soil, the present study aimed at revealing the advances in composting process. This was accomplished by evaluating the compost maturity with such parameters as the ordinary physico-chemical properties, dehydrogenase activity (DA), germination test and PCP removal, and the microbial community composition using PCR–DGGE analysis.

2. Methods

2.1. Composting materials

PCP was purchased from American ADL Co. with a purity >98%. The raw soil was obtained from Yuelu Mountain (Changsha, China). The soil was air-dried and ground to pass through a 2 mm sieve, and then stored at 4 °C in amber-colored jars. The common physico-chemical characteristics were as follows: water holding capacity 26.1%, organic matter (OM) 9.5%, total organic C (TOC) 5.5%, total N (TN) 0.24%, and pH 4.73.

A stock solution of PCP (10 g L^{-1}) was prepared in acetone and stored under refrigeration at 4 °C. Approximately 100 g of the sieved soil was spiked with 40 ml of acetone and 9.8 ml of the PCP-stock solution. Soil was gradually added to a 2 L specimen container in 100 g aliquots and extensively mixed with the spiked soil. This procedure was repeated until the entire amount of soil (1 kg) was added and mixed. The specimen containers were hermetically closed and left to shake for inversion. Then the acetone was left to evaporate overnight under a flow hood. The final concentration of PCP-spiked soil was 133 mg kg^{-1} (dry weight).

The other composting materials were collected from suburb of Changsha, China. Rice straw, which was air-dried and cut into 10–20 mm lengths, was used as recalcitrant organic composting materials. Several kinds of vegetables chopped into 10–20 mm pieces were used as easy metabolizing materials. Bran was used to adjust the initial TOC/TN ratio of composting.

2.2. Composting set-up and sampling

Two experimental composting piles were set up indoors in this study. Soil, rice straw, vegetables, and bran were homogenized at a ratio of 38:43:12:7 (moist weight). Soil contained in the control (pile A) was free from PCP contamination. In the treatment (pile B), the raw soil was replaced by PCP-contaminated soil. The initial concentration of PCP in compost mixture was 50 mg kg^{-1} (dry weight). These compost materials (40 kg moist weight) were packed in 120 dm^3 polyethylene bins (65 cm long, 45 cm wide, 42 cm height). The dimension of the bins ensured adequate homogenization of the initial mixture of composting materials and self heating. The initial OM content and TOC/TN ratio of this mixture were 58% and 30:1, respectively. The initial water content was adjusted to approximately 60% and composts were monitored every 3 days throughout the process, with sterile deionized water being added when needed to maintain appropriate moisture. To provide some aeration, the piles were turned twice a week during the first 2 weeks and then once a week afterwards.

The experiment was conducted for 60 days. Samples were collected on day 1, 3, 6, 9, 15, 21, 36 and 60, respectively. Combination samples of 5–10 different subsamples in each pile were mixed by shaking and sieved through an 8 mm sieve. Samples for total DNA extraction were stored immediately at −20 °C until use.

2.3. Composting parameters and PCP analyses

Average temperature was monitored daily by inserting a thermometer into five different locations of the piles. The pH was determined after mechanically shaking the fresh sample in water suspension at a ratio of 1:10 (w/v) at 200 rpm for 40 min. Moisture content was measured after drying the samples overnight at 105 °C. The dried sample was analyzed for TOC by dry combustion at 550 °C, and TN content by the Kjeldahl method (K435, Buchi, Switzerland).

Water soluble carbon (WSC) and germination index (GI) were determined according to the method described by Cayuela et al. (2009) and Jiang et al. (2006), respectively. DA was measured with the substrate of 3% of 2,3,5-triphenyltetrazolium chloride following the modified Thalmann method (Benito et al., 2003).

PCP was extracted with acetone and *n*-hexane as described by Scelza et al. (2008). The PCP was analyzed and quantitated by HPLC (Agilent Technologies, USA) using an UVD detector and an Eclipse XDB-C18 (4.6 × 150 mm) column with 5 μm particle size. Analysis was conducted using 68% of acetonitrile and 32% of buffered water (1% acetic acid) as mobile phase with a column temperature at 25 °C and 1.0 ml min^{-1} flow rate. Detection was carried out at 220 nm.

2.4. DNA extraction and PCR–DGGE

Total genomic DNA was extracted according to the method described by Yang et al. (2007). Fragments of 16S rDNA and 18S rDNA genes were amplified with bacterial universal primers 338F/518R (Muyzer et al., 1998) and fungal universal primers NS1/Fung (Hoshino and Morimoto, 2008), respectively. GC clamp was attached to the forward primers to prevent complete separation of the strands during DGGE.

The PCR reaction mixture was prepared with 1 μl of template DNA, 5 μl of 10×PCR buffer, 1 μl of dNTPs (10 mM each), 10 pmol of each primer, 2 μg of bovine serum albumin, 2 U *Taq* polymerase, and adjusted to a final volume of 50 μl with sterile deionized water. The PCR thermal cycling scheme of 16S rDNA consisted of 5 min at 94 °C, then 45 s at 94 °C, 40 s at 56 °C, 40 s at 72 °C (35 cycles) followed by a final chase at 72 °C (10 min), and end at 4 °C. The 18S rDNA PCR program consisted of 5 min at 94 °C, then 45 s at 94 °C, 50 s at 55 °C, 1 min at 72 °C (35 cycles) followed by a final chase at 72 °C (7 min), and end at 4 °C.

DGGE was carried out using a Dcode Universal Mutation Detection System (Bio-Rad, USA). Approximately equal amounts of PCR samples (30 μl) were loaded onto the 0.75 mm thick 8% (w/v) polyacrylamide gels in 1×TAE buffer using a denaturing gradient ranging from 30% to 65% and 15% to 50% for bacterial and fungal PCR samples, respectively. Electrophoresis was performed at 60 °C and 120 V for 12 h. After stained with SYBR Green I nucleic acid gel stain for 30 min, the gels were scanned and analyzed for understanding the DGGE profiles.

2.5. Data analysis

All data were expressed on dry basis except for moisture, which was expressed on a moist basis.

Three replicates were used for each analysis. Data were presented as the mean values of triplicates. Student–Newman–Keuls test (S–N–K test) was performed to compare the mean values of

different sampling time. Correlations between parameters were calculated by Pearson's correlation coefficient (SPSS, version 16.0). DGGE banding profiles were digitized and band numbers were counted using Quantityone software (version 4.5, Bio-Rad laboratories, USA). Discriminant and principal component analyses (PCA) were performed using the SPSS 16.0 and Canoco 4.5 statistical packages, respectively.

3. Results

3.1. Physico-chemical and biochemical analyses

Similar pile temperature dynamics were obtained in the two treatments. Both piles achieved rapidly thermophilic temperatures (>45 °C) according to the criterion reported by Barrena et al. (2008). The thermophilic stage maintained for more than 10 days (from day 3 to day 12), which ensured the organic matter stabilization and the pathogenic microorganism suppression. The pile temperature decreased gradually to ambient temperature level afterwards. The main difference between the two piles occurred at the mesophilic and the beginning of the thermophilic phases (from day 1 to day 6). The temperature in pile A rose to 35 °C after one day of composting and reached its peak of 61 °C at day 6, while in pile B, it rose slowly and the peak temperature was 57 °C (Table 1).

The pH increased rapidly in the first 9 days in pile A (from 7.2 to 9.0), and then decreased to 7.9 at day 36, slightly increasing afterwards, while in pile B, it increased for 15 days (from 7.0 to 8.6), before decreasing to 7.9 at day 21. The final pH turned to be 8.6 and 8.1 for piles A and B, respectively (Table 1). The OM percentage decreased continuously in both piles, and reached 35% and 38% at the end of the process in piles A and B, respectively (Table 1).

The TOC decreased from 34% to 25% in both piles (Table 1). Considering the absolute amount of TOC in the compost, mineralization losses at the end of the process accounted for about 52% and 49% of the C initially present in the starting mixtures for piles A and B, respectively (data not shown). Although the TN loss was higher in pile B than in pile A (data not shown), the TN contents were similar in the two piles during the composting process (Table 1).

The TOC/TN ratio showed a clearly decreasing trend from initial values of around 30 to final values of 15.7 and 17.2 for piles A and B, respectively. The greatest decrease occurred during the first 21 days in pile A (from 30 to 18.5); while in pile B, it decreased slowly throughout the whole process (Table 1). The WSC showed similar change trends in both piles. It was significantly increased in the first 6 days for piles A and B, respectively. After then, it was decreased to around 40% of the initial values at the end of the process. No significant changes were found in WSC after 36 days for the two piles (Table 1).

The DA levels in pile A significantly increased and peaked on day 9 (2.67 mg TPF g^{-1}), decreasing afterwards to a final value of 0.49 mg TPF g^{-1}. Similarly, in pile B, DA levels increased from 0.58 mg TPF g^{-1} at day 1 to 2.36 mg TPF g^{-1} at day 15 and significantly decreased afterwards to a final value of 0.56 mg TPF g^{-1} (Table 1). In pile A, the GI showed an increasing trend throughout the whole process with final values of 119%. While in pile B, the values of the GI increased up to 15 days (from 21% to 68%), and then decreased to 54% at day 21, increasing afterwards to the final value of 102% (Table 1).

PCP removal presented a clearly increasing trend in the investigated pile. It was less than 5% after 3 days of composting and significantly increased up to around 72% by the end of the process. The highest PCP removal rate occurred at the thermophilic phase of composting (Table 1).

Physico-chemical and biochemical parameters were subjected to discriminant analysis in order to elucidate the parameters most responsible for differentiation of the two piles for separating the compost samples of different maturity. Discriminant analysis showed a clear differentiation of compost samples of different ages. The first two function axes explained 76.9% and 16.2% of the total variance, respectively. Compost samples of 6, 9, 15, 21, 36 and 60 days were separated according to their age along the first function axis. In addition, samples from the different piles of the same age of 1, 3, 6 and 9 days were more distant in the plot space than those of 15, 21, 36 and 60 days. The variable with the highest degree of correlation with Function 1 was temperature. The parameters most responsible for this classification (parameters with the higher correlation coefficient with discriminant Function 2) were WSC, GI, and TOC/TN ratio (Fig. 1).

3.2. Microbial community composition

The bacterial and fungal community compositions were obtained in the resulting DNA band profiles (Fig. 2 and Fig. 3). Fifty-

Table 1
Selected physico-chemical and biochemical parameters in compost sample.

Pile	Days	Temp[a] (°C)	Moisture (%)	pH	OM (%)	TOC (%)	TN (%)	TOC/TN	WSC[b] (%)	DA[c] (mgTPF/g)	GI[d] (%)	PCP removal (%)
A	1	35.0 cd	56.1 a	7.20 d	56.4 a	34.4 a	1.17 d	29.4 a	2.21 bc	1.03 e	34.5 e	n.d. [e]
	3	55.0 ab	52.5 b	7.89 bc	52.2 b	35.2 a	1.30 c	27.1 ab	2.54 ab	1.95 c	37.7 e	n.d.
	6	61.2 a	50.9 c	8.57 ab	48.5 bc	33.6 a	1.33 c	25.3 b	2.68 a	2.48 ab	42.1 de	n.d.
	9	51.8 b	52.7 b	9.04 a	49.7 bc	29.7 b	1.26 cd	23.6 bc	2.27 bc	2.67 a	50.4 d	n.d.
	15	39.5 c	51.8 bc	8.87 a	46.6 c	28.4 b	1.32 c	21.5 c	1.72 cd	2.32 b	62.8 cd	n.d.
	21	34.3 cd	53.2 b	8.25 b	42.1 d	26.6 c	1.44 bc	18.5 cd	1.33 de	1.43 d	73.5 c	n.d.
	36	29.2 d	46.4 d	7.92 bc	39.6 de	26.3 c	1.49 b	17.6 cd	1.01 f	0.65 f	98.4 b	n.d.
	60	24.8 e	44.5 e	8.58 ab	35.3 e	25.1 c	1.60 a	15.7 d	0.89 f	0.49 f	118.5 a	n.d.
B	1	25.3 e	58.4 a	7.01 d	57.6 a	33.9 a	1.14 d	29.9 a	2.16 bc	0.58 e	20.8 e	1.1 f
	3	52.7 ab	54.0 b	7.33 cd	53.8 ab	35.0 a	1.25 bc	28.0 ab	2.44 ab	1.16 cd	22.5 e	4.5 f
	6	57.0 a	49.2 c	7.96 b	51.0 b	34.2 a	1.26 bc	27.1 ab	2.62 a	2.02 b	27.4 de	13.6 ef
	9	51.7 ab	53.1 b	8.42 a	50.4 b	32.3 a	1.20 c	27.0 ab	2.11 bc	2.17 ab	39.2 d	25.9 de
	15	42.2 bc	50.3 bc	8.59 a	47.9 bc	30.1 b	1.25 bc	24.1 c	1.56 cd	2.36 a	67.8 c	40.6 cd
	21	36.5 cd	53.5 b	7.90 b	44.6 c	27.6 c	1.32 b	21.0 cd	1.02 de	1.32 c	53.5 cd	53.2 bc
	36	27.5 de	48.3 c	7.78 b	41.7 cd	26.7 c	1.40 ab	19.0 d	0.83 e	0.73 de	85.7 b	65.8 ab
	60	25.3 e	46.2 d	8.11 b	38.2 d	25.5 c	1.49 a	17.2 d	0.77 e	0.56 e	102.4 a	72.3 a

For each pile, values in a column followed by different letters are statistically different according to S–N–K test ($p < 0.05$).
[a] Temp, temperature.
[b] WSC, water soluble carbon.
[c] DA, dehydrogenase activity.
[d] GI, germination index.
[e] n.d., not detected.

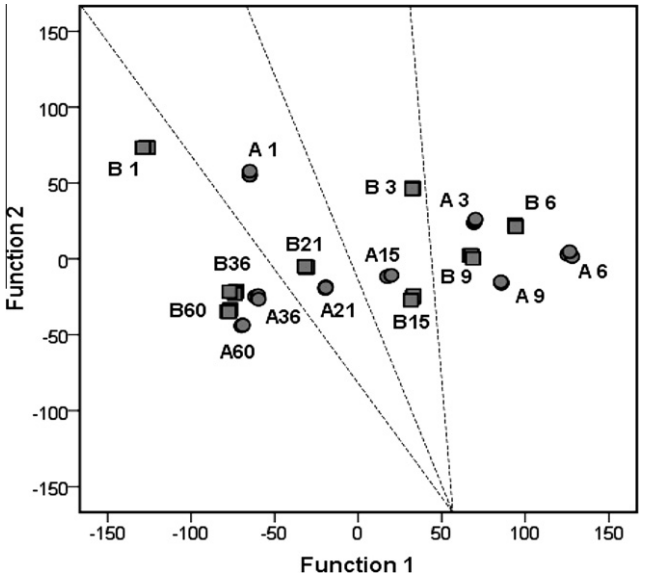

Fig. 1. Discriminant analysis loading plot of physico-chemical and biochemical data. The letter denotes the pile type, the number refers to the age of the sample.

two different 16S rDNA bands and 41 different 18S rDNA bands were detected in these profiles. Microbial communities were very dynamic, as strong shifts in the DGGE profiles were determined between the samples of different ages. Differences in DGGE profiles between the samples from the different piles of the same age suggested the effect of PCP stress on microbial community composition.

The band number in bacterial DGGE profiles changed clearly for both piles in the whole process. It peaked on day 1 (42) for pile A, and then significantly decreased during the thermophilic phase, trending afterwards towards stabilization. Similar shifts occurred for pile B except an obvious fluctuation during the thermophilic phase. The final number was 33 and 32 for pile A and pile B, respectively. On the other hand, the band number in fungal DGGE profiles was rather low for both piles. It did not significantly change until the thermophilic temperature was reached for pile A. However, this number decreased to its minimum (13) after one day for pile B, and then slightly increased for 3 days, ranging afterwards between 17 (day 15) and 24 (day 60) (Table 2).

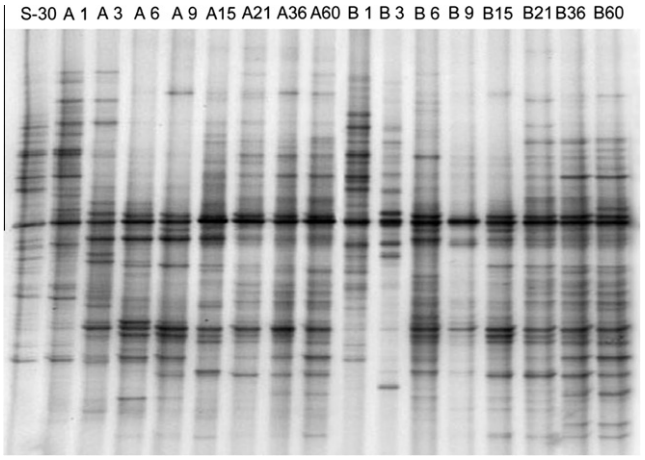

Fig. 2. Denaturing gradient gel electrophoresis (DGGE) profiles of amplified 16S rDNA fragments for raw soil, piles A and B. The letter denotes the type of the raw soil and different piles, the number refers to the age of the sample.

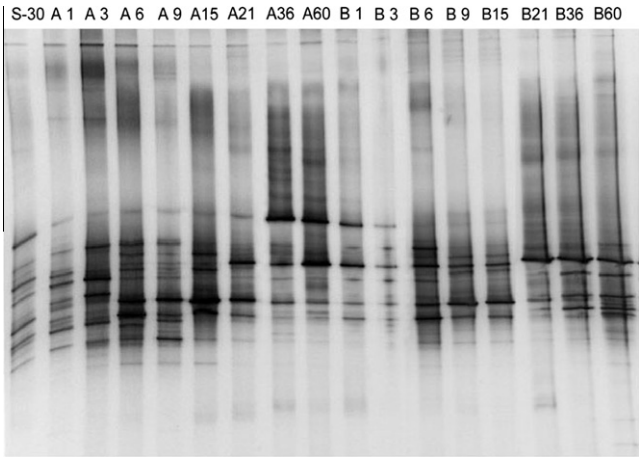

Fig. 3. DGGE profiles of amplified 18S rDNA fragments for raw soil, piles A and B. The letter denotes the type of the raw soil and different piles, the numbers refers to the age of the sample.

Table 2
The band number of DGGE profiles for each compost sample.

Pile	Days of composting								
	−30	1	3	6	9	15	21	36	60
Bacterial community profiles									
A	33.0	42.0	33.0	31.0	24.0	30.0	29.0	32.0	33.0
B	33.0	36.0	24.0	33.0	19.0	26.0	31.0	32.0	32.0
Fungal community profiles									
A	17.0	18.0	20.0	21.0	23.0	22.0	20.0	22.0	21.0
B	17.0	13.0	14.0	22.0	20.0	17.0	19.0	22.0	24.0

Statistical analysis of the DGGE profiles for bacterial and fungal communities in the raw soil and compost samples was performed by PCA. The PCA loading plot of the bacterial community profiles was shown in Fig. 4. Raw soil (S-30), early stage compost samples (A1, B1, A3, A6, A9, B3, B6 and B9) from the two piles were separated from the later stage and mature ones along axis 1, explaining 36.0% of the variance. Samples from the two piles grouped closer together with time, showing an increasing similarity in bacterial community composition. In addition, samples from the different piles separated along axis 2, indicating significant difference between the two piles. This axis explained an additional 18.0% of the variance. In total, the two axes of the PCA plot explained 54.0% of the total variance of the data.

Fig. 5 shows a PCA loading plot of the fungal community profiles, whereby the two first axes explain 42.2% of the variance. Samples from pile A of the different ages separated along axis 1, explaining 25.2% of the variance, while the positions of samples from pile B were located separately on the PCA loading plot, indicating a clear succession of fungal community. Compost samples from the different piles were separated from each other, which indicated the existence of difference between the two piles in fungal community composition throughout the whole process.

4. Discussion

4.1. Assessment of compost maturity

It is critical to the successful use of composts for agricultural purposes (Bernal et al., 2009). Classical parameters for the evaluation of compost quality such as TOC/TN ratio, WSC, DA and GI showed a proper functioning of the process and a good degree of

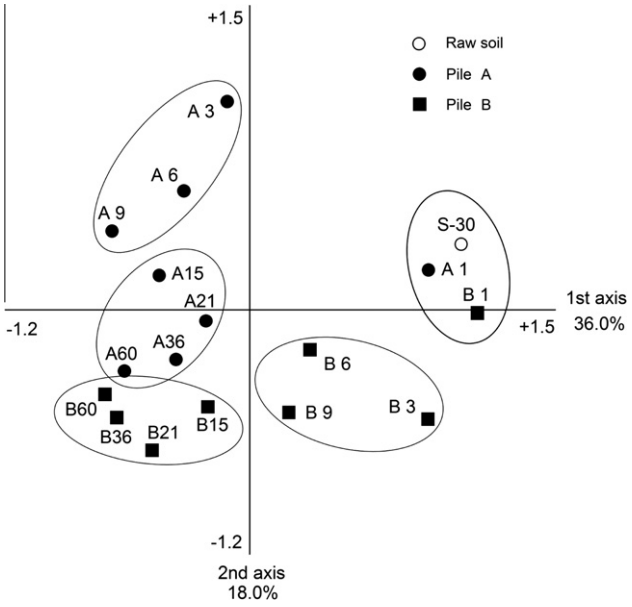

Fig. 4. Principal component analysis (PCA) of bacterial community profiles.

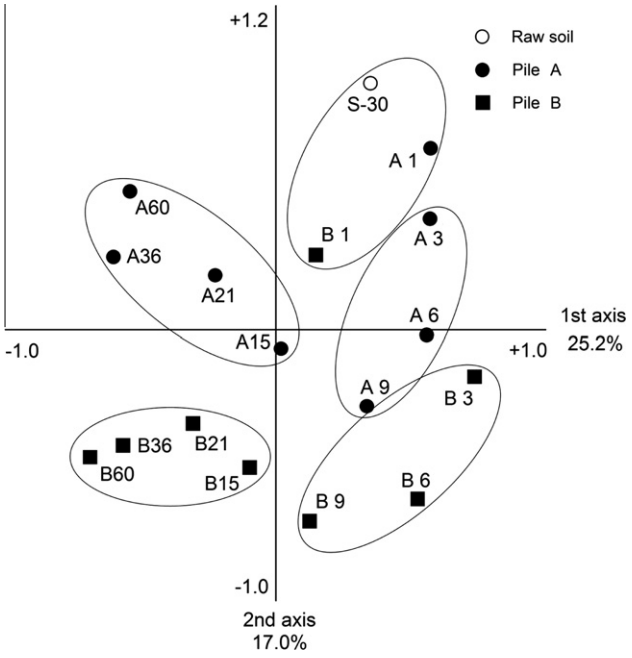

Fig. 5. PCA of fungal community profiles.

maturity of the end products for both composts. Initial values of TOC/TN ratio were consistent with values determined by Goyal et al. (2005) for a wide range of organic wastes, while final values for the two piles were lower than 20, which is thought to be the threshold limits for compost maturity (Garcia et al., 1992). The impact of existing PCP in pile B resulted in an inferior degree of maturity, as indicated by the higher levels of TOC/TN ratio and OM percentage for the final compost.

The WSC represents the most easily biodegradable C fraction during the composting process. In agreement, Goyal et al. (2005) commented that the WSC content decreased to 2.1–4.1% when the composts were mature. These values were significantly different from others suggested by Bernal et al. (1998) (WSC < 1.7%), Hue and Liu (1995) (WSC < 1.0%) and Benito et al. (2003) (WSC < 0.5%). There cannot be a single limit for WSC to judge compost maturity, and a stable low value of WSC is acceptable. In this study, the low values of WSC were stabilized around 0.8% after 36 days of composting for piles A, while a longer composting period was needed to reach these values for pile B. This observation revealed that the compost without PCP stress was mature earlier than that contaminated with PCP.

DA as a very reliable indicator of maturity was suggested by Tiquia (2005), and a value of below 35 μg TPF g^{-1} for highly mature composts also was proposed. The decrease of DA to low levels towards the end of composting for the two piles indicated that less active decomposition was going on and the compost was getting mature. However, highly mature composts were not obtained until the composting process was accomplished, suggesting that a longer period was recommended for the bioremediation of PCP-contaminated soil.

Previous reports (Jiang et al., 2006; Campittelli and Ceppi, 2007; Saidi et al., 2009) have demonstrated that GI is a sensitive parameter to evaluate compost phytotoxicity and maturity, and the values greater than 80% are generally thought to be phytotoxin-free and completely mature. The GI showed different trends of decreasing phytotoxicity in the presence of PCP in pile B. It was related to the content of PCP, as indicated by the significant correlation ($r = 0.95$, $p < 0.01$). The depletion of PCP became slower but the GI increased up to higher than 80% after 36 days of composting. This observation implied that lower concentration of PCP (<17.1 mg kg^{-1}) had almost no toxic effect on seeds and the compost was almost mature at the same time.

For accurately evaluating compost maturity, correlation tests were conducted among the four criterions previously reported. The GI and TOC/TN ratio shared the highest correlation coefficients ($r > 0.93$) at the significant level of 0.01. WSC was best correlated to the other indexes tested with the high correlations found for GI ($r = 0.90$, $p < 0.01$ for both piles) and TOC/TN ratio ($r = 0.89$, $p < 0.01$ and $r = 0.90$, $p < 0.01$ for piles A and B, respectively). These results proved that compost maturity characterized jointly by WSC, TOC/TN ratio and GI is acceptable. In contrast, DA may be an inappropriate criterion for uniformly evaluating the maturity of compost with polluted and unpolluted substances, as the pollutant strongly hinders the activity of dehydrogenase (Saidi et al., 2009).

Discriminant analysis based on all parameters data indicated that composting for the two piles was proceeding differently. It clearly revealed the differentiation of compost samples from the different piles in the whole process, especially at the mesophilic and thermophilic phases. The reason was due to the presence of PCP-contaminated soil in pile B, which resulted in an inferior degree of maturity of the end composts than those for pile A.

4.2. Response of microbial community composition to PCP-contaminated soil during the composting process

PCR–DGGE was conducted to assess the effect of PCP-contaminated soil on the microbial community composition during the composting process. The resulting DNA band profiles provided a fingerprint of the microbial community structure, in which each band represented a group of bacterial or fungal species (Ovreas et al., 1997). The bacterial and fungal communities were affected after PCP addition, as difference between the treatment and the control in DGGE profiles was interpreted.

DGGE band numbers were compared between the two piles in this study (Table 2). The lower number for pile B implied that PCP stress had an inhibitory effect on the microbial abundance. As the composting progressed, the band number reached the levels close to pile A, due to a drop in PCP concentration. In fact, according to the results obtained by Liu et al. (2008), microbial

abundance shifted obviously with PCP concentration changed. The lowest numbers of bacteria were consistently found in treatment piles with the highest chlorophenol concentrations (Laine and Jorgensen, 1997).

In addition, we only found the correlation between the band number in fungal DGGE profiles and PCP removal ($r = 0.72$, $p < 0.05$). The lack of significant correlation between the band number in bacterial DGGE profiles and PCP removal may be due to its predominance of bacterial communities in the composting system (Khalil et al., 2001). Usually, microbial community succession is significantly affected by kinds of composting factors, especially by temperature (Antizar-Ladislao et al., 2007). As a consequence of PCP contamination, microbial abundance of composts was reduced but not consistent with PCP concentration.

Succession of bacterial communities during the composting process was reflected in the PCA loading plot (Fig. 4), and the compost samples were divided into 5 groups based on their ages and types. The bacterial communities between the two piles were similar with those in the raw soil at the beginning of composting. However, the greatest difference occurred at the thermophilic phase was mainly attributed to the differentiation of the composting temperature. This conclusion is supported by Goyal et al. (2005), who reported that the microflora succession from mesophilic to thermophilic depending on the pile temperature. Another reason may be that the higher temperature at the thermophilic stage is favorable for the increase of water-soluble fraction of the toxic compound (Leita and DeNobili, 1991), thus resulting in an inhibited effect on bacterial communities.

Another important observation is that towards the end of the process the bacterial community composition in the two piles became similar. It suggested an adaptation of the compost bacteria to the PCP contaminant (Cea et al., 2010). Changes in bacterial communities were consistent with the previously reported composting factors such as temperature, pH, moisture content and substrates. This behavior reflected the significant effects of those factors on bacterial communities. The similar bacterial community compositions towards the end of the process in both piles could be considered as an indicator of mature composts, as appearance of some microorganisms reflects the quality of maturing compost (Ishii et al., 2000).

In contrast to bacteria, the fungal communities from the same age samples of those two piles were different throughout the whole process according to the PCA loading plot (Fig. 5). The fungal community composition in pile A at the beginning of the composting was closer to raw soil than pile B, and what is more, the fungal communities in PCP contaminated pile changed substantially compared to an uncontaminated pile. In consideration of the previous results about the significant correlation between fungal abundance and PCP removal, the shifts in fungal communities were related to the PCP contamination. The fungal community composition could be more appropriate for a response to the degree of PCP contamination than bacterial community composition.

5. Conclusions

The present experiments showed a proper functioning of the composting mixed with PCP-contaminated soil. However, the addition of PCP led to an inferior degree of maturity, and accordingly a longer composting period was recommended for obtaining highly mature composts. Of particular interest were the different roles of compost microbial community composition in the evaluation of compost maturity and PCP contamination. The bacterial communities could be considered as a potential indicator of mature composts, while the fungal communities were more useful for evaluating the PCP contamination of compost due to their higher susceptibility to PCP.

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