Water Air Soil Pollut (2015) 226: 183
DOI 10.1007/s11270-015-2436-0

# Depletion of Pentachlorophenol Contamination in an Agricultural Soil Treated with *Byssochlamys nivea*, *Scopulariopsis brumptii* and Urban Waste Compost: A Laboratory Microcosm Study

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Received: 5 November 2014 / Accepted: 27 April 2015 / Published online: 15 May 2015


**Abstract** Pentachlorophenol (PCP) has been used worldwide as a wood treatment agent and biocide. Its toxicity and extensive use have placed it among the most hazardous environmental pollutants. The response of a PCP-contaminated agricultural soil to the addition of solid urban waste compost and two exogenous Ascomycota fungal strains *Byssochlamys nivea* and *Scopulariopsis brumptii* was evaluated. The experiments were conducted in soil microcosms incubated for 28 days at 25 °C and 60 % moisture content. The depletion of PCP and the changes in biochemical soil properties (i.e. microbial biomass, soil respiration, dehydrogenase and fluorescein diacetate hydrolysis activities) were detected. The addition of PCP severely depressed some of the tested biochemical properties such as microbial biomass, dehydrogenase and fluorescein diacetate hydrolysis activities. By contrast, compost limited the negative effect of PCP on the dehydrogenase activity and soil respiration. When compost and fungal strains were contemporary present, a synergistic effect was observed with a reduction of more than 95 % of the extractable PCP after 28 days of incubation. No differences in PCP depletion resulted when fungi or compost were individually used. Our results indicate that many processes (i.e. microbial degradation and sorption to organic matter) likely occurred when PCP was added to the soil. The compost and the fungal strains, *B. nivea* and *S. brumptii*, showed good capability to tolerate and degrade PCP so that they could be successfully used in synergistic effect to treat PCP polluted soils.

**Keywords** PCP · Microbial degradation · Chlorophenols · Compost · Bioremediation

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## 1 Introduction

Pentachlorophenol (PCP) is a toxic compound widely used as a wood treatment agent and general biocide. PCP is persistent in the environment and has been classified as a priority contaminant to be reclaimed in many countries. In fact, uncontrolled PCP use and release caused contamination of soil, water and ground water (McAllister et al. 1996). Although PCP is recalcitrant to biodegradation, numerous bacterial and fungal isolates have been reported to be able to degrade it (McAllister et al. 1996; Bosso and Cristinzio 2014). The use of microorganisms for the depletion of PCP in contaminated soil and water has become an important alternative in bioremediation strategies.

Mycoremediation is a widely used process for this purpose, and numerous fungi are capable to tolerate and remove PCP. White-rot and brown-rot fungi such as *Phanerochaete chrysosporium*, *Antracophyllum discolor*, *Trametes versicolor*, *Ganoderma lucidum*, *Armillaria mellea* and *Gleophyllum striatum* have demonstrated to be able to degrade and mineralize PCP at very high initial concentrations (McAllister et al. 1996; Bosso and Cristinzio 2014; Bosso et al. 2015). At

present, only a few reports about PCP degradation and adsorption by using fungi different from white-rot and brown-rot fungi are available in literature (Seigle-Murandi et al. 1991, 1992, 1993). This justifies the importance to investigate on fungi belonging to other ecological and taxonomic fungal groups that may tolerate high PCP concentrations, grow faster and potentially increase degradation and adsorption efficiency of PCP and other pollutants.

Soil microbial degradation may be limited by several factors such as suboptimal nutrient levels, water content, temperature, pH level, organic matter and compost (McAllister et al. 1996). The use of compost to stimulate the microbial activity in PCP degradation was successfully applied to restore contaminated soils (Semple et al. 2001; Miller et al. 2003; Scelza et al. 2008). Compost, being an excellent soil ameliorant for structure and composition with diverse microbial populations and a nutrient source for indigenous degraders, can be used in contaminated soils with excellent results (McAllister et al. 1996; Bosso and Cristinzio 2014).

The aim of this study was to evaluate, in a short-term experiment under controlled conditions, the efficiency of soil fungi such as *Byssochlamys nivea* (Westling, 1909) and *Scopulariopsis brumptii* (Salvanet-Duval, 1935) (Fungi: Ascomycota) and urban waste compost in depleting PCP in agricultural soil microcosms with no history of PCP contamination. Soil biochemical properties i.e. microbial biomass, soil respiration, dehydrogenase and fluorescein diacetate hydrolysis activities were detected.

## 2 Materials and Methods

### 2.1 Chemicals

PCP (>99 % purity) was purchased from Sigma-Aldrich (USA). All solvents and chemical reagents were purchased from Carlo Erba Reagents (Italy).

### 2.2 Fungal Strains and Cultivation Conditions

*B. nivea* and *S. brumptii* were stored into slant tubes containing potato dextrose agar (PDA; 5 g $l^{-1}$ potato, 20 g $l^{-1}$ dextrose, 15 g $l^{-1}$ agar) at 20 °C at the laboratories of Forest Pathology of the Department of Agriculture (University of Naples Federico II, Italy).

### 2.3 Physical and Chemical Properties of Soil and Compost

Soil was collected (0–20-cm depth) from an orchard in southern Italy (Naples) and had no history of PCP contamination. It was sieved to 2 mm and stored at 4 °C before analyses.

Physical and chemical soil analyses were carried out in triplicates on air-dried soil samples according to the standard techniques (Sparks 1996). The texture was evaluated according to USDA Textural Soil Classification (Soil Survey Staff 1975). Electrical conductivity (EC) and pH were measured in 1:5 and 1:2.5 soil–water suspensions, respectively; cation exchange capacity (CEC) was measured after soil treatment with a barium chloride and triethanolamine solution at pH 8.2; available phosphate was measured by bicarbonate extraction. Organic C content was assayed by the chromic acid titration method, while total N was determined by the ash combustion procedure with a Fisons 1108 Elemental Analyzer. Water-holding capacity of soil was determined in according to Alef and Nannipieri (1995). The maturated compost was obtained from solid urban waste, and its composition is described in Scelza et al. (2007).

### 2.4 Soil Microcosms

The PCP disappearance experiment in soil microcosms was performed in closed 1-l jars containing 100 g of spiked fresh soil (25 mg PCP $kg^{-1}$)whose moisture was adjusted to 60 % to avoid possible stressing conditions for microorganisms with consequences on their activity and growth (Scelza et al. 2008).

The spiking procedure started by adding 100 ml of 0.250 g $l^{-1}$ PCP solution in acetone (suitable to reach the final concentration of 25 mg $kg^{-1}$) to approximately 50 g of soil in a glass jar (1 l) and mixed with a stainless steel spatula. Similar soil aliquots were gradually added into the glass jar under extensive mixing until the entire amount (1 kg) of soil was mixed. The jars containing the spiked soil were hermetically closed, darkened with aluminium foil, and left under shaking overnight for inversion. Thereafter, the acetone was left to evaporate for about 2 h under a flow hood and the soil was immediately used. A similar procedure with acetone but without PCP was used to prepare control soil samples.

Each microcosm was inoculated with 30 plugs (5 mm of diameter) of active mycelia of *B. nivea* or *S. brumptii*, cut from 7-day cultures on PDA medium. When the fungi were simultaneously present, 15 plugs from each isolate were added.

Compost was added to soil at a rate of 7.14 g $kg^{-1}$ corresponding to a field amount of 30 t $ha^{-1}$. The control was performed in the same experimental conditions without addition of inoculum, compost and PCP. All jars were incubated at 25 °C for 28 days in dark. Experimental design is summarized in Table 1.

### 2.5 PCP Extraction and Quantification

PCP was extracted and analyzed according to protocol of Khodadoust et al. (1999) after 1, 7, 14, and 28 days. Briefly, 1 g of soil was extracted with 20 ml of water–ethanol (50:50, *v*/*v*) on a horizontal shaker (190 rev $min^{-1}$) for 1 h. After that, the supernatant was separated from the residual soil by centrifugation at 3000 *g* for 15 min and then concentrated by evaporation under vacuum (Laborota 4000, Heidolph). Each concentrate was re-suspended in 1 ml of methanol and the PCP concentration was measured by HPLC (R1100, Agilent Technologies) with diode-array detector, equipped with Phenomenex 250×4.6 mm C18 column. PCP was detected with a gradient elution by using at the beginning 80 % of buffered water (1 % acetic acid) and 20 % methanol as mobile phase; methanol reached 100 % within 10 min and remained constant for 5 min. The system came back to the initial ratio in further 5 min. Detection was carried out at 220 nm.

**Table 1** Soil microcosms and their composition

| Microcosms | Composition |
|---|---|
| S | Soil |
| N | Soil + *B. nivea* |
| B | Soil + *S. brumptii* |
| NB | Soil + *B. nivea* + *S. brumptii* |
| C | Soil + compost[a] |
| NC | Soil + *B. nivea* + compost |
| BC | Soil + *S. brumptii* + compost |
| NBC | Soil + *B. nivea* + *S. brumptii* + compost |
| P | Soil + PCP[b] |
| NP | Soil + *B. nivea* + PCP |
| BP | Soil + *S. brumptii* + PCP |
| NBP | Soil + *B. nivea* + *S. brumptii* + PCP |
| CP | Soil + compost + PCP |
| NCP | Soil + *B. nivea* + compost + PCP |
| BCP | Soil + *S. brumptii* + compost + PCP |
| NBCP | Soil + *B. nivea* + *S. brumptii* + compost + PCP |

[a] Compost rate=7.14 g $kg^{-1}$

[b] PCP=25 mg $kg^{-1}$

### 2.6 Soil Biochemical Analyses

Soil respiration (SR) was measured after 1, 3, 7, 13, 18, and 28 days of incubation according to Piotrowska et al. (2006).

Microbial biomass-C (MB-C) was measured with a fumigation–extraction method that converts the extractable C to microbial C using standard factors (Vance et al. 1987).

Among soil enzyme activities, dehydrogenase activity (DH) was measured according to Trevors (1984) using tetrazolium salts (TTC) as substrate and fluorescein diacetate hydrolase activity (FDA) was determined according the method of Green et al. (2006) based on soil treatment with fluorescein diacetate solutions. MB-C, DH and FDA were analyzed after 1 and 28 days.

### 2.7 Statistical Analysis

Statistical analysis was carried out using XLSTAT version 2013.1. Analyses of variance followed by Tukey's multiple comparison test at the 0.05 level were performed to determine significant difference means. All experiments were performed in triplicates.

## 3 Results

### 3.1 Physical and Chemical Properties of Soil

Soil was classified as a loamy sand soil according to USDA Textural Soil Classification (sand 69 %, silt 20 % and clay 11 %). Soil pH was 7.53, moisture content was approximatively 20 %, and the organic matter was 34.25 g $kg^{-1}$ of soil. Other soil physical and chemical properties are shown in Table 2.

**Table 2** Main physical and chemical properties of the agricultural soil

| Soil property | Unit | Mean | SD |
|---|---|---|---|
| Electrical conductivity | dS $cm^{-1}$ | 0.88 | 0.45 |
| pH | | 7.53 | 0.25 |
| $NO_3^-N$ | mg $kg^{-1}$ | 11.17 | 1.04 |
| $NH_3^-N$ | mg $kg^{-1}$ | 3.43 | 0.93 |
| TOC | g $kg^{-1}$ | 19.87 | 1.53 |
| Organic matter | g $kg^{-1}$ | 34.25 | 2.64 |
| TN | g $kg^{-1}$ | 1.9 | 0.02 |
| $P_2O_5$ | mg $kg^{-1}$ | 46.66 | 4.34 |
| $K_2O$ | mg $kg^{-1}$ | 200.05 | 9.16 |

### 3.2 PCP Depletion in Soil Microcosms

The PCP depletion, evaluated in soil microcosms after 1, 7, 14 and 28 days of incubation, showed different trends related to soil treatment and incubation time (Fig. 1). After 1 day of incubation, a reduction of extractable PCP was already measured in all treatments. Also in the microcosm P, having exclusively spiked soil, only 72 % of the PCP initially added (25 mg $kg^{-1}$) was extractable. The percentage further decreased (56 %) when the microcosm included inoculum of both fungi *B. nivea* or *S. brumptii* (NBP) and still more (46 %) when also compost was added (NBCP). No significant difference was found in the PCP removal between soil incubated with only *B. nivea* (NP) or *S. brumptii* (BP) also in the presence of compost (NCP and BCP) (Fig. 1)

A continuous decrease of extractable PCP was observed during the time for all the microcosms until values were close to zero. After 7 and 14 days, the extractable PCP was less than 30 and 25 %, respectively, of the initially added PCP (25 mg $kg^{-1}$). Even in these cases, the smaller PCP amount was recovered from soils with compost and both fungi (NCP and BCP) (Fig. 1). In these latter microcosms, a significant reduction by almost 95 % of the extractable PCP was detected after 28 days of incubation (Fig. 1).

### 3.3 Biochemical Properties

Biochemical properties of soils in microcosms were affected by different treatments, but firstly by PCP contamination. PCP contamination determined a strong reduction of microbial biomass (Fig. 2). After 1 day of incubation, the presence of PCP significantly decreased the MB-C values in all soil microcosms except for the microcosm NBP in which the contemporaneous presence of both fungi counteracted this negative effect (Fig. 2b). Among not contaminated soil microcosms (Fig. 2a) a large enhancement of MB-C was produced when soil was inoculated with fungi (alone or together) simultaneously with compost amendment (NC, BC, and NBC).

After 28 days, MB-C further decreased in all soil microcosms with no significant differences due neither to compost nor to fungi (Fig. 2a, b). SR significantly increased in all soil microcosms inoculated with fungi and/or amended with compost with respect to the control (S), and a common increase of SR was observed until 7 days (Fig. 3a); after that, it slightly decreased until 20–30 mg of $CO_2$ for 100 $g^{-1}$ of soil. In spiked soil (P), the produced $CO_2$ doubled with respect to the control (S) within 7 days of incubation, and all the treatments produced a depletion of SR in PCP-contaminated soil. In fact, the SR level was down in all microcosms with respect to the microcosm P (Fig. 3b) but in any case higher than the control (S) (Fig. 3a). A significant difference was observed in the presence of compost with or without fungi until the end of the experiment thus indicating a stabilizing effect of compost on SR (Fig. 3).

Some enzymatic activities of soil microcosms, involved in the cycle of the main biological nutrients, were evaluated. DH was not affected by different treatments independently on the presence of PCP, though the contamination led to smaller DH activity levels (Fig. 4). An increase of DH was observed after 28 days of incubation, and higher values were achieved in soil microcosms enriched with compost, reaching values >9 μg TFA $g^{-1}$ $h^{-1}$in not contaminated (Fig. 4a) and >6 μg TFA $g^{-1}$ $h^{-1}$contaminated (Fig. 4b) soil microcosms.

FDA activity showed a similar trend of DH except for the no positive response of contaminated soil microcosms to compost addition (Fig. 5b). Conversely, higher FDA activity was registered in not contaminated soil microcosms that received compost (Fig. 5a) in particular microcosm C.

## 4 Discussion

Organic matter in soil is the most important sorbent for the phenolic compounds. In fact, the soil organic matter presence may enhance PCP sorption (Cea et al. 2007;

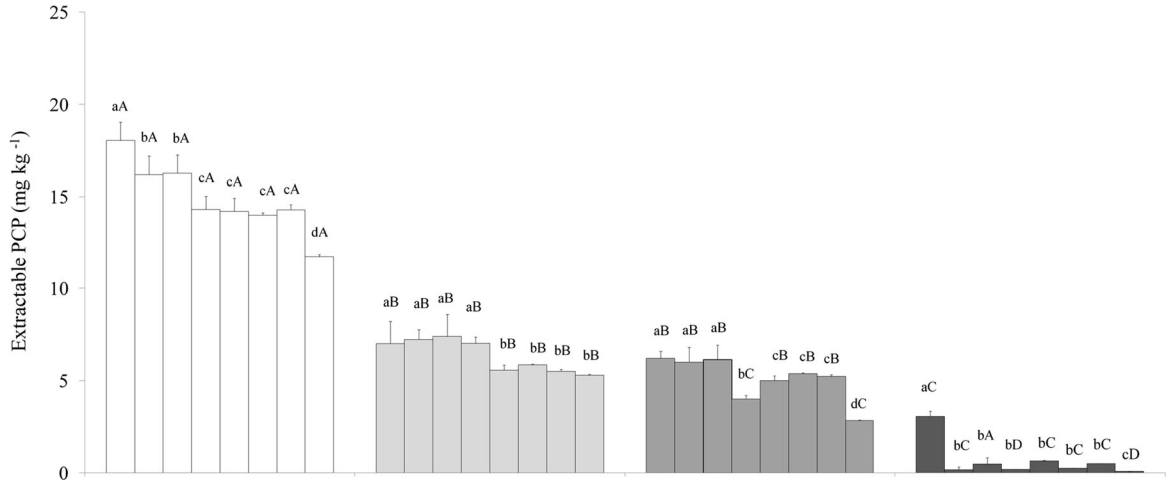


**Fig. 1** Extractable PCP from soil microcosms at different incubation times. *Different small letters* refer to significant differences ($P<0.05$) among different treatments at the same incubation time. *Different capital letters* refer to significant differences ($P<0.05$) among the same treatments at different incubation times. *Differently coloured bars* represent measurements carried out after 1 day (*white bars*), 7 days (*light grey bars*), 14 days (*medium light grey bars*), and 28 days (*dark grey bars*) of incubation. Abbreviations of soil microcosms are explained in Table 1

Scelza et al. 2008). In our experiments, the fresh soil had a very high organic matter value (34.25 g $kg^{-1}$, Table 2), and this can explain the drastic reduction of extractable PCP (32 %) after only 1 day from the spiking of the agricultural soil. Microcosm P, without compost or fungi, was already able to reduce by 88 % the extractable PCP in 28 days of incubation. Although no attempts were made to detect the presence of PCP degradation products

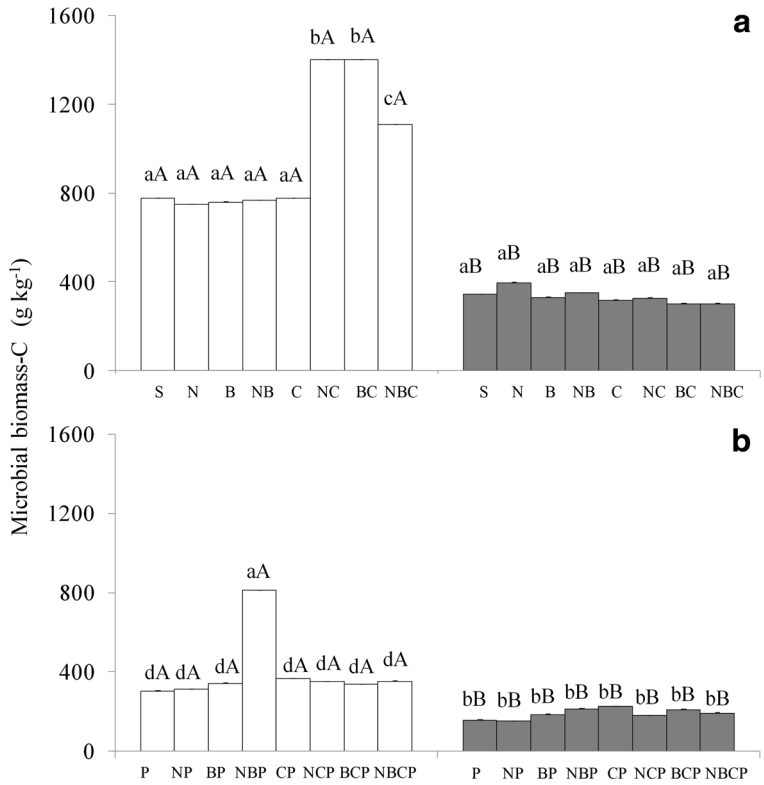


**Fig. 2** Microbial biomass of (**a**) control (without PCP) and (**b**) PCP-contaminated soil microcosms (25 mg $kg^{-1}$). *Different small letters* refer to significant differences ($P<0.05$) among different treatments at the same incubation time. *Different capital letters* refer to significant differences ($P<0.05$) among the same treatments at different incubation times. *Differently coloured bars* represent measurements carried out after 1 day (*white bars*) and 28 days (*grey bars*) of incubation. Abbreviations of soil microcosms are explained in Table 1

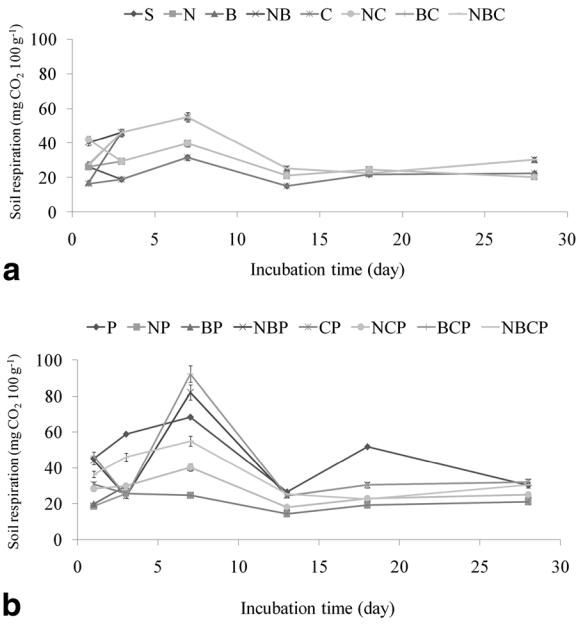


**Fig. 3** Soil respiration of (**a**) not contaminated and (**b**) PCP-contaminated soil microcosms (25 mg $kg^{-1}$). Abbreviations of soil microcosms are explained in Table 1

in the investigated system, the presence of its metabolites may be hypothesized according to Scelza et al. (2008).

By increasing the organic matter in soil, adding compost or DOM, the sorption of PCP to the soil matrix is evidently favoured (Miller et al. 2003; Scelza et al. 2008). Compost provides several microorganisms and nutrients for indigenous degraders when applied to contaminated soils. Compost based strategies have been successfully applied to PCP-contaminated soil, especially because compost accelerates the PCP removal (Semple et al. 2001). In our study, the urban solid waste compost plays an important role to increase the reduction of extractable PCP and to limit the inhibitory effects of the contaminant on soil biochemical activities. After 28 days of incubation, almost 95 % of PCP was removed from the spiked soil (25 mg $kg^{-1}$) treated only with compost.

In relation to fungal strains used in PCP depletion, many studies revealed that diverse members of Ascomycetes and Basidiomycetes are efficient and fast degraders of PCP (McAllister et al. 1996; Bosso and Cristinzio 2014). When *B. nivea* or *S. brumptii* were added to fresh soil microcosms, PCP was reduced by 90 and 92 %, respectively. Higher depletion of PCP (98 %) occurred when fungal strains were simultaneously used.

*B. nivea* and *S. brumptii* do not produce extracellular enzymes such as lignin peroxidases or laccase. It is

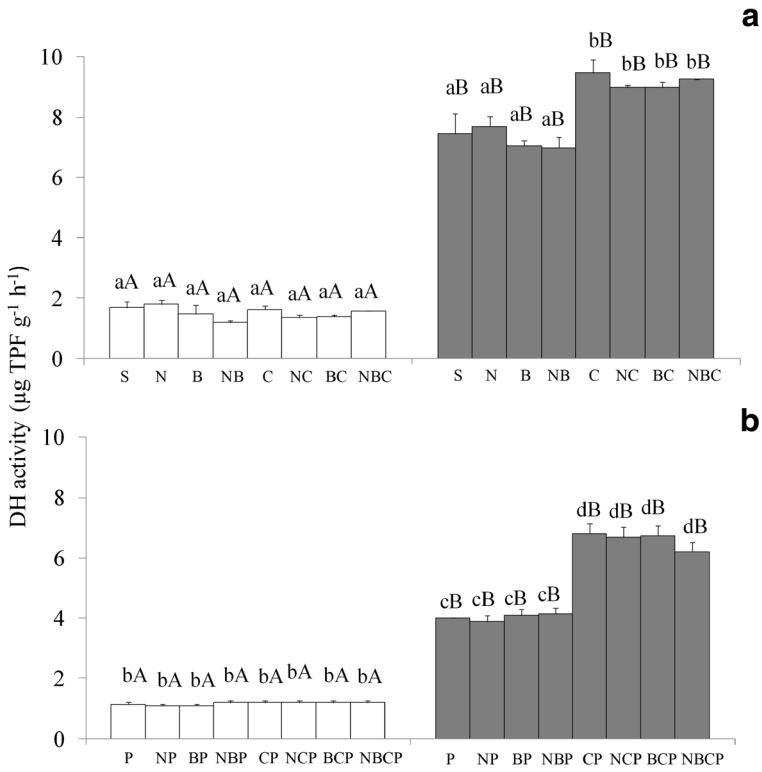


**Fig. 4** Dehydrogenase activity of (**a**) not contaminated and (**b**) PCP-contaminated soil microcosms (25 mg $kg^{-1}$). *Different small letters* refer to significant differences ($P<0.05$) among different treatments at the same incubation time. *Different capital letters* refer to significant differences ($P<0.05$) among the same treatments at different incubation times. *Differently coloured bars* represent measurements carried out after 1 day (*white bars*) and 28 days (*grey bars*) of incubation. Abbreviations of soil microcosms are explained in Table 1

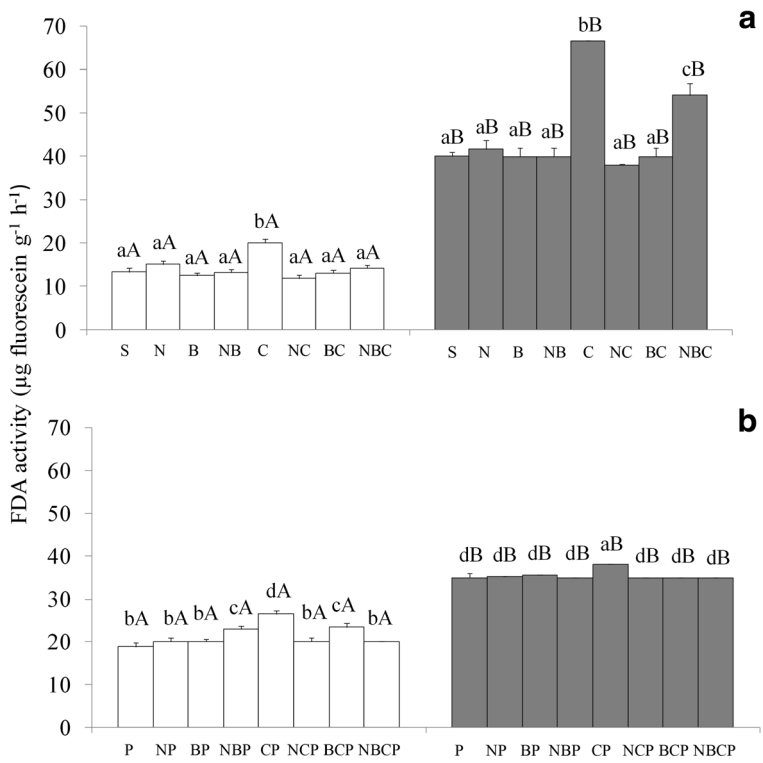


**Fig. 5** Fluorescein diacetate hydrolysis activity of (**a**) not contaminated and (**b**) PCP-contaminated soil microcosms (25 mg $kg^{-1}$). *Different small letters* refer to significant differences ($P<0.05$) among different treatments at the same incubation time. *Different capital letters* refer to significant differences ($P<0.05$) among the same treatments at different incubation times. *Differently coloured bars* represent measurements carried out after 1 day (*white bars*) and 28 days (*grey bars*) of incubation. Abbreviations of soil microcosms are explained in Table 1

widely known that these two enzymes are highly able to degrade PCP in co-metabolism (McAllister et al. 1996; Bosso and Cristinzio 2014). However, *S. brumptii* is a non-ligninolytic fungus that thanks to its phenoloxidase enzymes (Tanaka et al. 2000) can degrade PCP (Gadd 2001). Instead, *B. nivea* can deplete PCP taking advantages of its pectinolytic enzymes, which are widely used in bioremediation strategies (Gadd 2001). Furthermore, in the *Byssochlamys* genus, enzymatic activities involved in the degradation pathway of lignin and other wood constituents have been detected (Furukawa et al. 1999).

In PCP-spiked soil, the MB-C decreased by 50 % during all incubation times. Scelza et al. (2008) showed that the presence of PCP severely and significantly depressed MB-C in soil contaminated by 50 mg PCP $kg^{-1}$ soil. Drastic MB-C decrement (89 % less than control) with increasing soil PCP concentration was also showed by Megharaj et al. (1998).

In addition, the investigated agricultural soil contaminated with 25 mg PCP $kg^{-1}$ showed an increase of basal respiration due to PCP contamination in the soil treated with compost and fungi already after 7 days of incubation according to Scelza et al. (2008). However, while Scelza et al. (2008) found that the SR increased after 30 days, in this study the SR increased within 7 days and then it maintained approximately similar values. Zelles et al. (1989) detected that PCP concentration of 2 and 20 mg $kg^{-1}$ increased the SR for 90 days, whereas an irreversible inhibition of SR occurred at 200 mg $kg^{-1}$ PCP. Salminen et al. (1995) showed how SR decreased at higher PCP concentration (500 mg $kg^{-1}$) compared to the uncontaminated soil and a soil contaminated with lower PCP concentration (50 mg $kg^{-1}$).

PCP is a wide-spectrum biocide that has a negative impact on microbial diversity and soil microbial activity. Soil enzymatic activities of microcosms such as FDA and DH had similar trend over time. According to Cea et al. (2010), FDA showed a good response to PCP, without strong inhibition, especially during the early days of incubation. Similar results have been obtained also in other studies on indigenous microflora. Kähkönen et al. (2007) found that PCP in a contaminated area with aged chlorophenol contamination did not influence FDA activity. On the other hand, DH activities were negatively influenced by PCP after 1 day of incubation showing 50 % reduction versus the control. The same results were recorded with higher PCP

concentrations than that adopted in our experiment. With 50 and 250 mg $kg^{-1}$ PCP Scelza et al. (2008) and McGrath and Singleton (2000), respectively, found that soil DH activity dramatically decreased and did not recover throughout the experiment (>6 weeks). Conversely, Hechmi et al. (2014) observed that at low concentrations of PCP contamination, the DH activity could slightly increase. Although in uncultivated soils, co-contaminated by Cd and PCP, DH activity was significantly lower (64 %) than the control value, the DH activity tended to decline by increasing PCP and Cd concentration in soil. The low values of biochemical activities, indicative of microbial growth and often used as an index of the PCP metabolite toxicity, indicate that PCP exerted a depressing effect on soil microbial activity although the extracted PCP decreased over time, but the fungal inoculum, better if combined with both fungi, as well as the compost amendment could aid to counteract this negative effect.

Further enhancements of the efficiency of fungal inoculum in contaminated soils could derive from the application of specific nutrients or hormones, useful for biostimulation process, or the bioaugmentation with specific bacterial strains producing a synergic effect with fungi thus on bioremediation approach.

## 5 Conclusions

Our results showed that PCP produced a considerable reduction of the level of some biochemical properties thus suggesting a depressing effect on the soil microbial activity. On the other hand, the addition of solid urban waste compost and the inoculum of fungal strains such as *B. nivea* and *S. brumptii* allowed obtaining a reduction of PCP contamination and an improvement of soil biochemical activities. In contrast with other studies where well-known white-rot or brown-rot fungi (i.e. *T. versicolor*, *Pleurotus ostreatus* and *P. chrysosporium*) are used to deplete PCP, in this work novel Ascomycota fungi and indigenous microorganisms in bioremediation studies were used. The potential of certain fungi in field experiments could be enhanced by several factors including an increase of the inoculum biomass/soil or soil amendment with compost. As fungi and compost were used in controlled experiments in laboratory conditions, further investigations may be helpful to obtain information useful for field experiments closer to natural situations.

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