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Bioaccessibility, bioavailability and ecotoxicity of pentachlorophenol in compost amended soils

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ABSTRACT

The influence of compost on the bioaccessibility, bioavailability and ecotoxicity of pentachlorophenol (PCP) as a function of time was studied by means of different chemical and ecotoxicological methods. Experiments were conducted in both sterile and non-sterile microcosms and samplings carried out at 20, 60 and 120 d from initial contamination.

PCP bioaccessibility, assessed by means of Porapak resin extraction, was around 75% of the applied dose with no aging or compost effects. Two different methods were applied to assess the bioavailability of PCP, respectively, to bacteria and earthworms and linked to ecotoxicological assays (biosensor and earthworm coelomocytes assays). Water extraction was applied to assess the bioavailability to bacteria: results showed that this fraction was always below 50%, with significant decreases as a result of aging processes and compost addition. In non-sterile microcosms, compost amendment increased the amount of PCP biodegraded, while the ecotoxicological assay with the biosensor *Pseudomonas fluorescens* pUCD607 indicated a higher toxicity in the most degraded samples, thus suggesting the formation of more toxic metabolites. Earthworm body accumulation results were rather in accordance with water extractions data, with decreasing bioavailable amounts as a result of time and compost addition. No compost or aging effects were instead detected by coelomocytes assay.

Results indicate how different methods must be applied altogether to assess the bioavailability and ecotoxicity of xenobiotics such as PCP in soil. The addition of compost was also proven as an effective strategy for the remediation of PCP contaminated soils, although issues related to the possible formation of toxic metabolites must be taken into account.

1. Introduction

Pentachlorophenol (PCP) is a broad-spectrum biocide firstly introduced in the 1920s. PCP is a widespread environmental contaminant of soil, surface water and groundwater: it is known to bioaccumulate, to be very toxic and it is classified in the priority list of organic micropollutants because of its carcinogenity and toxicity (Reigner et al., 1993; ATSDR, 2001) PCP utilization as a pesticide is widely forbidden, but many countries still use it to prevent fungal attacks on wood (Jenson, 1996).

When organic contaminants such as PCP enter the soil environment they are subjected to a number of processes. A contaminant may be lost at different rates and to different extents through volatilization, leaching and degradation (Stokes et al., 2006). The fraction remaining in the soil is not completely available to living organisms, because part of it is progressively sequestrated by organic and inorganic constituents, and thus less available to microbial attack. Many different and sometimes contrasting definitions have been used to describe this bioavailable fraction of contaminants in soils. A distinction has been recently made between the bioavailable and the bioaccessible fractions of contaminants: bioavailable is the fraction "which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a different time", while bioaccessible is the one "which is available to cross an organism's cellular membrane from the environment if the organism has access to the chemical" (Semple et al., 2004). Bioavailability can be specific to organism and even particular species (Stokes et al., 2006), and it is strongly influenced by actual rates of give (dissolution, desorption and diffusion) and take (degradation), while the bioaccessible fraction may be a relatively defined and robust quantity representing a promising target for the development of chemical proxies (Semple et al., 2007).

Different methods can be used to extract the above defined bioavailable and bioaccessible fraction of contaminants; choice of the appropriate methods must also be based on the molecule studied.

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The fraction of PCP extracted by water at a given time can be considered as an estimation of the bioavailable fraction for microorganisms, especially because at neutral pH the weak acid PCP is mostly present as more soluble phenolate ion (Scelza et al., 2008), while the bioavailability to earthworms can be assessed by employing earthworms that feed on and live in the soil and by measuring the amount accumulated in their body tissue (Edwards and Bater, 1992). Solid phase resin extraction is instead a measure of the bioaccessible fraction of contaminants (Harmsen, 2007; Semple et al., 2007).

In order to achieve a realistic risk assessment, information on the bioavailability and bioaccessibility of pollutants should be related to ecotoxicity assays involving living organisms (Puglisi et al., 2007a; Vernile et al., 2007). Since bioavailability and toxicity of pollutants in soils differ from species to species, it is important to select test organisms with different trophism. Sensitivity, ubiquity, ecological significance and easiness of analysis are other factors to be considered. Bacteria are extremely useful for environmental studies because of their adaptation to different conditions, their capacity to degrade many organic chemicals and their ease of cultivation and genetic transformation. Microbial biosensors are based on bacteria genetically engineered with reporter genes (e.g., luciferase, green fluorescent protein, galactosidase), which are under the control of promoters that are activated by the presence of specific chemicals. Response levels (e.g., amount of light or protein production) are a direct measure of the bioavailability and toxicity of pollutants (Paton et al., 1997). The bacterial biosensor *Pseudomonas fluorescens* pUCD607 has been widely applied for soil ecotoxicity tests (Reid et al., 1998; Tiensing et al., 2002): it is a general toxicity reporter and can thus be used to assess the toxicity for bacteria of several compounds, including their metabolites. Earthworm mortality is a measure of acute toxicity. In addition, the damage degree of lysosomal membrane of earthworm immune cells (coelomocytes), evaluated by the Neutral Red Retention Time (NRRT) method, is a biomarker of exposure (Eason et al., 1999; Reinecke and Reinecke, 1999) and can be used as an ecotoxicological index.

The aim of this work was to assess the bioaccessibility, bioavailability and ecotoxicity of PCP in soils amended with compost as a function of time. In particular, the bioavailability to bacteria was studied with water extractions and coupled to luminescent biosensors ecotoxicological responses, while the bioavailability to earthworms was studied by body tissues uptake and coupled to coelomocytes damage assays. Chemical bioavailability data were compared to bioaccessibility data obtained with resin extractions. The effect of compost amendment on the above-mentioned processes was also studied.

2. Materials and methods

2.1. General scheme

A general scheme of the experimental plan is given in Fig. 1, where the processes studied in this paper are linked to the general scheme for bioavailability processes of Semple et al. (2004). The bioaccessibility of PCP was studied by resin extraction, while for bioavailability assessments two different methods were employed: water batch extraction was used as an estimation of the fraction of PCP bioavailable for microorganisms, while body tissues uptake were used to asses the bioavailability of the molecule for earthworms. The two bioavailability measurements were, respectively, linked to biological responses: bioluminescent biosensor response for bacteria and lysosomal membrane stability for earthworms. Experiments were conducted in both sterile and non-sterile microcosms.

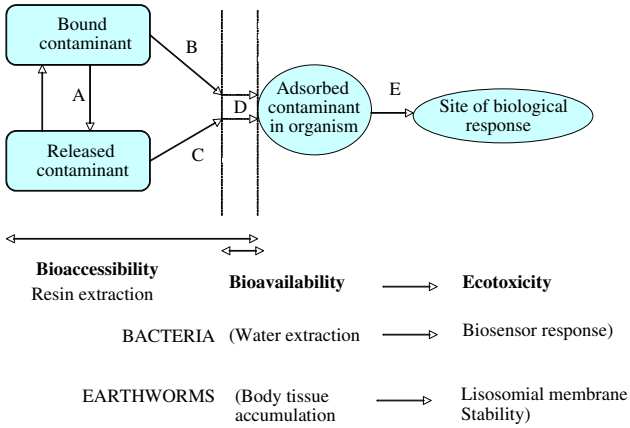

Fig. 1. Schematic representation of bioavailability processes, slightly modified from Semple et al. (2007). Bioaccessibility is the sum of processes A (release of bound/recalcitrant compounds to a more labile state), B (transport of bound compound to biological membranes), C (transport of labile, soluble or dissolved compound to biological membrane) and D (uptake across a biological membrane), while bioavailability is limited to process D. Ecotoxicity is linked to E, the fraction of D that reaches the sites of biological responses. In this work bioaccessibility was estimated by solid phase resin extractions, while different bioavailability and ecotoxicity measurements were carried for bacteria and earthworms.

2.2. Soil amendment and spiking

An organic green compost made from grass cutting, pruning from trees and shrubs, old flowers and houseplants, uncooked vegetables and vegetables peeling, was used in this study. The compost had a water content of 33.7%, pH in water 9.0, EC of 3170 $\mu S\ cm^{-1}$, 2.8% of total Nitrogen, a total organic Carbon content of 31.6% and a C/N ratio of 11.4.

Soil samples were collected from the 5–15 cm layer from a site in South Italy, in the Province of Bari. Soil samples were sieved to 2 mm prior the experiments. The soil had a clay-loam texture, a neutral pH and an organic carbon content of 2.5%.

Soil samples were amended with two different doses of compost, 0.38% and 1.15%. These doses correspond to agronomic doses of 10 and 30 t ha^{-1}, respectively (calculated assuming field application to a depth of 30 cm). Samples were shaken for 24 h in a rotary shaker following compost addition in order to achieve homogeneous blending. Properties of the soil before and after amendment with two doses of compost are reported in Table 1. The compost amendment increased both the quantity of organic carbon and total nitrogen levels. Available phosphorus was increased only by the highest dose of compost.

Non-amended soil samples (NC) and soil samples amended with the two doses of compost (C1 and C2) were spiked with PCP (Sigma–Aldrich) using a dilution mixing method, with acetone as the carrier solvent (Northcott and Jones, 2000). A portion of each sample (10%) was spiked and mixed with PCP dissolved in acetone. The remaining soil was then added to the spiked portion and mixed. After removing the solvent by volatilization at room temperature, control and spiked soils (250 g) were placed in glass jars, and demineralized water was added to bring the moisture to 80% of field capacity. The final concentration of PCP in all soil samples was 150 mg kg^{-1}. This concentration was chosen according to Heimbach (1984), who detected an LC_{50} of 87 ppm for *Eisenia andrei* exposed to an artificial soil spiked with PCP.

Experiments were performed using both non-sterile and sterile soil microcosms in order to evaluate the fate of PCP in the presence and absence of microbial degradation. Sterilization was carried out using γ-irradiation (5 Mrads).

Table 1
Physico-chemical properties of the soil before (NC) and after compost addition for 0.38% (C1) and 1.15% (C2), corresponding, respectively, to agronomic doses of 10 and 30 t ha^{-1} in the field.

	Control soil (NC)	Soil + 10 t ha^{-1} compost (C1)	Soil + 30 t ha^{-1} compost (C2)
Coarse sand	19	–	–
Fine sand (g kg^{-1})	265	–	–
Lime (g kg^{-1})	336	–	–
Clay (g kg^{-1})	380	–	–
pH (H_2O)	7.81	7.68	7.64
Organic C (g kg^{-1})	2.5	2.8	3.0
Total N (g kg^{-1})	2.4	2.5	2.6
Available P (mg kg^{-1})	40.7	40.7	49.1
Ca (meq (100 g)$^{-1}$)	31.6	32.6	36.2
K (meq (100 g)$^{-1}$)	1.6	1.6	1.7
Na (meq (100 g)$^{-1}$)	1.05	1.06	1.18

In total, 12 experimental treatments were applied: non-amended soils and soils amended with two different doses of compost, all of which were studied under sterile and non-sterile conditions, contaminated or not with 150 mg kg^{-1} of PCP in duplicate. Sterile and non-sterile microcosms were kept sealed in 0.5 L jars big enough to avoid oxygen limitations during biodegradation processes.

In order to examine the aging of PCP, chemical and ecotoxicological analyses were performed after 20, 60 and 120 d from the spiking. Analyses were carried out in triplicate for each microcosm.

2.3. Chemical analyses

2.3.1. Assessment of the bioaccessible fraction

The resin Porapak P (Sigma–Aldrich) was used to assess the PCP desorption kinetics from the soil matrix only in sterile microcosms. At the end of each aging period (20, 60 and 120 d), 0.5 g of soil from all samples were suspended in 8 mL of 0.005 M $CaCl_2$ and 1 mL of NaN_3 (18 mg mL^{-1}) in screw cap centrifuge tubes. Sodium azide was used as bacteria inhibitor. The Porapak resin (100 mg) was added to the soil suspension. Each tube was then incubated in an end-over-end agitator up to 168 h. The resin was separated from suspensions after different times (1, 3, 24, 72, and 168 h) and changed with a fresh resin. The separated resin was then resuspended in 10 mL of acetonitrile and, after agitation for 24 h, the extract was analyzed by HPLC-UV. 24 h cumulative desorption data were taken as a measure of the bioaccessible fraction of PCP, in accordance with literature evidences (Harmsen, 2007) which indicate the first rapid desorption phase as an estimation of the bioaccessible fraction.

2.3.2. Assessment of the bioavailable fractions

Two methods were applied to assess the bioavailable fraction of contaminants: water extraction for the bioavailability to soil microorganisms and body tissues accumulation for earthworms.

For water extractions, samples of soil (1 g) were weighed in polycarbonate centrifuge tubes and 20 mL of distilled water was added. The tubes were sealed, placed on an orbital shaker at 80 rpm for 12 h, and then centrifuged at 5000*g* for 1 h. After centrifugation, the supernatants were filtered and analyzed quantitatively for PCP using HPLC.

Adults of *E. andrei* Bouché were obtained from Compagnoni farm (Lecco, Italy). They were reared in the laboratory at room temperature on a standard diet, for at least 1 month before use in the experiments. After each aging period, 12 mature earthworms (adults with a developed *clitellum* and a weight between 0.3 and 0.6 g) were added to each sterile microcosm and posed for 14 d at 24 ± 1 °C in the dark as recommended by the OECD protocol (2000). At the end of the exposure period, survived worms were collected, rinsed with tap water, left in a Petri dish to reject the content of the gut, and then subjected to biological assays and chemical analysis. Bioavailability measurements of PCP were carried out extracting the worm tissues in acetonitrile for 24 h. Samples were then purified and the extracts were analyzed by HPLC.

2.3.3. Assessment of the water:ethanol extractable fraction

Water:ethanol (50:50) batch extraction was applied with the aim of extracting the total PCP content as suggested by Khodadoust et al. (1999), who indicated it as the most exhaustive methods for PCP recovery from soil samples.

Water:ethanol extraction was carried out exactly as described above for water extraction, using water:ethanol (50:50) instead of distilled water.

2.3.4. HPLC analyses

All samples from soil and earthworm extractions were analyzed for PCP by HPLC on a Perkin–Elmer LC 410 high-performance liquid chromatograph and a diode-array detector (Perkin–Elmer, Series 200). Extracts were separated on a reverse-phase Phenomenex Gemini 3 μ C18 column (3.9 × 150 mm, particle size 3 μm, pore volume 1.10 cm^3 g^{-1}, monomeric packing) using a mobile phase of 0.05% phosphoric acid water solution (60%) and acetonitrile (40%). The separation was performed at a constant flow of 1 mL min^{-1}, using an isocratic elution and the wavelength of the detector was set to 220 nm. Concentrations of PCP were quantified from peak areas following linear interpolation of PCP standards injected at increasing concentrations between 0.5 and 5 mg L^{-1}.

2.4. Ecotoxicological analyses

2.4.1. Biosensors

Cultures of *Pseudomonas fluorescens* pUCD607 biosensor (*lux* CDABE from *Vibrio fischerii*, *kan*^{r}, *amp*^{r}; (Hamin-Hanjani et al., 1993) were grown in 250 mL Erlenmeyer flasks containing 100 mL of LB broth at 25 °C, and rotated at 200 rpm. Kanamycin was added at 50 μg mL^{-1} to all cultures. Biosensor assays were carried out on water extract from non-sterile soils at 20, 60 and 120 d of aging. Before exposure to the samples, cells were harvested by centrifugation and resuspended in 5 mL of 0.1 M KCl. After 20 min of contact, light output was measured using a SystemSure luminometer Model 18172 (Nova Biomedical Waltham MA, USA). Data were expressed in terms of % of light reduction compared to the control (i.e., water extracts from non-contaminated soil).

2.4.2. Ecotoxicological assays in earthworms

After each aging period, earthworms were exposed to sterile contaminated samples as described above. For the ecotoxicological assays half of the survived earthworms were subjected to the ethanol extrusion of coelomocytes in a solution composed of PBS (95%), ethanol (5%), guaiacol glyceryl ether (GGE) (10 mg mL^{-1}) (Sigma–Aldrich) and EDTA (2.5 mg mL^{-1}). Samples were then centrifuged at 150*g* for 10 min at 4 °C and coelomocytes were resuspended in *Lumbricus* – balanced salt solution (LBSS Ca-free) (modified by Eyambe et al. (1991)) for two times. Cell viability was evaluated by Trypan blue dye exclusion (0.4%) under light microscope (200× magnification) using a Neübauer slide.

The Neutral Red Retention Time (NRRT) assay indicates the lysosomal stability of coelomocytes. Cell suspension was treated with the neutral red working solution (Maboeta et al., 2002). Slides were examined under a light microscope (400× magnification) every 2 min. Cell counting ended when at least 50% of coelomocytes had fully stained cytosol. The duration of counting represents the NRRT of the lysosomes (Maboeta et al., 2002).

2.5. Statistical analyses

Data were evaluated using analysis of variance (ANOVA) and Tukey's test for comparison of means.

All statistical analyses were performed with SPSS software.

3. Results and discussion

3.1. Assessments of PCP partitioning in sterile microcosms

Results of resin extractions (bioaccessible fraction), water extractions (prediction of bioavailability for bacteria), earthworm body tissue accumulation (bioavailability for earthworms) and water:ethanol extractions in sterilized microcosms are reported in Table 2, together with results of ANOVA and Tukey's test for comparison of means. Results are reported as % of the amount of PCP extracted by water:ethanol immediately after spiking, except from the results of earthworms accumulation which are expressed in mg of PCP per kg of body weight.

Cumulative data of PCP desorbed by Porapak resin in 24 h, representing the fast desorption fraction, showed values ranging from 71% to 84% of the applied dose, but no significant effects of time and compost amendment were found. The exception is represented by the data observed at 120 d, where the lowest compost amendment had a lower PCP recovery than the other two. By extending the desorption time up to 168 h a slower increase of the desorption fraction was obtained in all samples (Fig. 3). However the trends were similar to those observed after the first 24 h.

The values of the bioavailable fraction estimated with water extraction were much lower, ranging from 41.2 ± 2% of the applied dose in the non-amended soil at day 20 to 22.2 ± 8% of the applied dose in the soil amended with the highest dose of compost at 120 d from contamination. A significant decrease between the first two and the last aging period was found for all three treatments ($P < 0.01$), while at 120 d the percentage of PCP extracted in the two compost amended treatments was lower than in the control treatment.

Bioavailability to earthworms measured by worm body accumulation showed values ranging from 100.3 ± 6 ppm in C2 treatment at 60 d, to 15.4 ± 10 ppm in C1 at 120 d. A decrease in the amount of PCP accumulated in function of aging was shown for all treatments. The presence of compost decreased the average value of PCP bioaccumulated both at 20 and 120 d. Most of these trends were however not confirmed by ANOVA because of the high variability of data.

Water:ethanol immediately after spiking extracted the highest amount of PCP, and was used as a benchmark to express in terms of percentages all other data. At 20, 60 and 120 d from contamination the amount of PCP extracted with this method decreased however to around 70% of the applied dose, in disaccordance with results from Khodadoust et al. (1999). No significant effects of time and compost amendment on water:ethanol recoveries were found.

Differences in PCP recoveries by the different methods can be explained by the different extraction mechanisms and by the fractions of contaminants involved. Water was chosen as a mild solvent for the prediction of the fraction of contaminants bioavailable for bacteria. Other solvents such as cyclodextrin aqueous solution are usually preferred to predict the bioavailability of hydrophobic compounds such as PAHs and PCBs (Reid et al., 2000; Puglisi et al., 2007b). With a weak acid such as PCP, present in ionic form at the neutral pH of the studied soil we preferred however a milder solvent. In the present experiment, PCP recoveries by water were lower than all the other methods but still considerable. Water also showed a significant reduction of the amount extracted with time, thus demonstrating a constant aging effect on the immediately available fraction dissolved in the water phase. The bioavailability of PCP in water was significantly reduced as an effect of the compost treatments, as previously found for other molecules such as phenanthrene (Puglisi et al., 2007b).

Aging and compost effects were found by the results of earthworms bioavailability measurements as well. The bioconcentration factor (BCF), calculated as ratio between the amount of PCP accumulated in earthworms and the PCP concentration in soil, decreased from 0.54 to 0.27 for the NC treatment, from 0.27 to 0.10 for C1 and from 0.33 to 0.18 for C2 after 20 and 120 d of aging, respectively.

Porapak resin extractions indicated that a significant fraction of the PCP added (around 75% of the applied dose) should be considered bioaccessible. This bioaccessible fraction did not change as a result of aging or compost amendment. Water:ethanol extracted similar amounts of PCP, from a maximum of 79% to a minimum of 59% of the applied dose. Water:ethanol batch extraction was chosen as a method for the assessment of the total extractable fraction of PCP after evidences from Khodadoust et al. (1999), Scelza et al. (2008), as well as after preliminary trials which showed recoveries not significantly different from extractions with organic solvents in Soxhlet or Sonicator immediately after spiking (data not shown). The amount of PCP extracted with water:ethanol immediately after spiking was in fact the highest recovery found, but it then decreased in the following samplings, approaching to that obtained by Porapak desorption (bioaccessible fraction). In our case this method failed to be a reliable one for the assessment of the total extractable fraction in contrast with evidences reported by Khodadoust et al. (1999).

Table 2
Average ± standard deviation (df = 2) of PCP recoveries by means of water, Porapak P resin, earthworms and water:ethanol in sterile (ST) soil microcosms at 20, 60 and 120 d after contamination. ANOVA significant differences were indicated by *F* values for comparisons between rows and columns for water, Porapak P resin, earthworms and water:ethanol separately. Data followed by the same minor letter on each column or by the same capital letter on each row are not statistically different from each other (Tukey's test, $P < 0.05$). ns: No significative difference.

Days	20	60	120	*F* value
Bioaccessible fraction (Porapak P resin) in %				
NC	73.5 ± 1 a A	79.2 ± 4 a A	80.6 ± 1 b A	3.82^{ns}
C1	71.1 ± 3 a A	72.8 ± 4 a A	75.8 ± 1 c A	1.61^{ns}
C2	78.9 ± 5 a A	82.9 ± 5 a A	84.4 ± 1 a A	1.06^{ns}
F value	2.89^{ns}	2.79^{ns}	131.97**	
Bioavailable fraction (water) in %				*F* value
NC	41.2 ± 2 a A	36.9 ± 3 a A	30.0 ± 2 a B	19.51**
C1	37.6 ± 9 a A	34.5 ± 2 a A	24.8 ± 2 a B	3.62**
C2	40.9 ± 4 a A	32.7 ± 6 a AB	22.2 ± 8 a B	13.17**
F value	0.15^{ns}	0.83^{ns}	4.89^{ns}	
Bioavailable fraction (worm tissues) in ppm				
NC	80.9 ± 36 a AB	97.9 ± 25 ab A	40.2 ± 36 a B	4.06(*)
C1	40.4 ± 30 a A	53.5 ± 38 b A	15.4 ± 10 a A	2.26^{ns}
C2	49.5 ± 27 a B	100.3 ± 6 a A	26.8 ± 23 a B	9.74(*)
F value	2.29^{ns}	5.01*	0.79^{ns}	
Water:ethanol extractable fraction in %				
NC	79.3 ± 10 a A	58.8 ± 16 a A	68.1 ± 3 a A	2.40^{ns}
C1	75.4 ± 1 a A	70.0 ± 3 a A	70.1 ± 3 a A	3.20^{ns}
C2	63 ± 8 a A	66.6 ± 3 a A	66.8 ± 2 a A	0.52^{ns}
F value	4.02^{ns}	0.99^{ns}	0.92^{ns}	

* $P < 0.05$.
** $P < 0.01$.

3.2. Ecotoxicological assays in earthworms exposed to sterile microcosms

A most exact evaluation of the risk assessment of contaminated soils should be accomplished by joining chemical tests

(bioaccumulation and bioavailability) to biological analyses by biomarkers (Lanno et al., 2004).

The damage degree of earthworm immune cells (coelomocytes) is a biomarker of exposure and expresses the lysosomal membrane stability which have been evaluated by the Neutral Red Retention Time (NRRT) for pentachlorophenol (Vernile et al., 2007). Regarding PCP effects, a significant decrease of the NRRT index between earthworms reared in contaminated soil and the untreated group was found ($P < 0.001$). In particular, in NC soil (Fig. 2), after 20 d of aging, the NRRT index was equal to 71.0 ± 8.5 min in the untreated and 19.7 ± 7.4 min in contaminated soil. After 120 d of aging, it was 84.3 ± 8.5 min in the untreated and 12.2 ± 3.3 min in spiked soil. A similar trend was recovered in amended soils (Fig. 2). No aging effects or amendment influences were observed up to 120 d.

After extrusion, the earthworm cell viability was assessed too. No significant differences were detected from earthworms exposed to soils spiked and/or amended with different compost amounts, compared to the untreated group. Furthermore, the aging of soils did not affect cell viability (data not shown).

3.3. Fate of PCP in non-sterile soil microcosms: bioavailability, biodegradation and ecotoxicity

Further measurements on selected parameters were also carried out in non-sterile soil microcosms, in order to assess the fate of PCP in presence of the native soil microflora and identify possible effects of compost addition. The bioavailable fraction extracted with water was firstly compared to the results of water–ethanol extraction (Table 3). The amount of PCP extracted using both methods decreased over time. This effect was significant for all treatments (F values of ANOVA between columns). Amounts extracted with water:ethanol were always higher than the ones extracted with water, and they decreased as a function of time for all treatments. At both 60 and 120 d from contamination, water:ethanol recoveries were significantly lower ($P < 0.05$) in the two compost treatments.

A similar trend but with lower values was found for the bioavailable fraction estimated by water extraction. In the unamended samples, the fraction decreased from 46.6 ± 12% at 20 d to 20.5 ± 2% at 60 d, and it remained almost the same (21.2 ± 5%) at 120 d. In the two compost amended treatments there was instead a progressive reduction at 60 and 120 d, where only

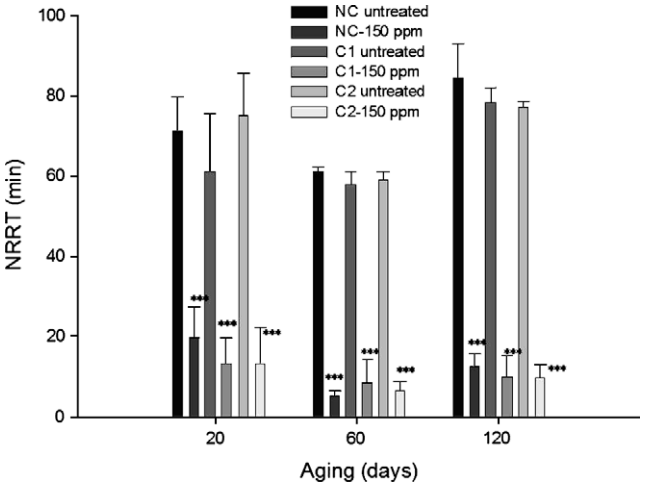

Fig. 2. Retention time of lysosomal membrane in earthworms exposed to soils unamended and amended. *** Indicates a significant difference of lysosomal stability in earthworm reared in spiked soil compared to the relative untreated soil, at the $P < 0.001$ level (parametric post-hoc Tukey's test).

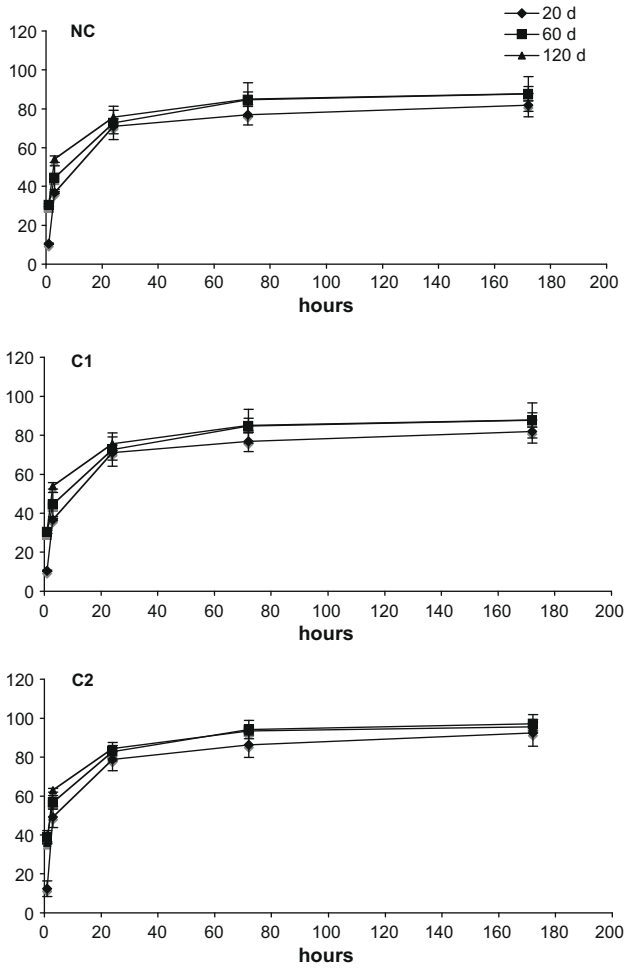

Fig. 3. Cumulative desorption kinetics of PCP assessed by means of Porapak P resins at different times of contact (1, 3, 24, 72 and 168 h) in non-amended (NC) and amended with two different doses of compost (C1 and C2) at 20, 60 and 120 d from initial contamination with 150 ppm of PCP.

1.2 ± 1% of the applied dose was still available in the lowest dose treatment and no PCP was available in the highest dose treatment.

The amount of PCP biodegraded in the non-sterile microcosms during the experiments was estimated as the difference extracted

Table 3
Average ± standard deviation (df = 2) of PCP recoveries by means of water, and water:ethanol in non-sterilized (NST) soil microcosms 20, 60 and 120 d after contamination. Data are expressed as percentages of applied dose. ANOVA significant differences were indicated by F values for comparisons between rows and columns for water and water:ethanol separately. Data followed by the same minor letter on each column or by the same capital letter on each row are not statistically different from each other (Tukey's test, $P < 0.05$).

Days	20	60	120	
Bioavailable fraction (water) in %				
NC	46.6 ± 12 a A	20.5 ± 2 a B	21.2 ± 5 a AB	8.56*
C1	47.1 ± 4 a A	25.9 ± 9 a A	1.2 ± 1 b B	21.13**
C2	47.3 ± 11 a A	29.1 ± 1 a A	0 b B	33.13**
F value	0.06^{ns}	0.80^{ns}	164.14***	
Water:ethanol extractable fraction in %				
NC	84.7 ± 7 a A	61.7 ± 2 a B	54.9 ± 2 a B	39.93***
C1	74.2 ± 4 a A	49.8 ± 3 b B	36.1 ± 8 b B	34.91***
C2	72.5 ± 4 a A	55.1 ± 5 ab B	31.4 ± 4 b C	70.92***
F value	3.22^{ns}	8.30*	15.35*	

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.005$.

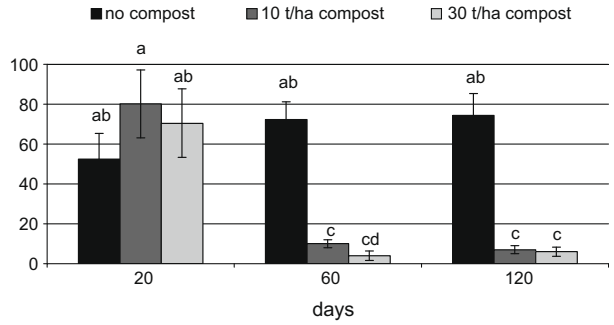

Fig. 4. Ecotoxicity of PCP evaluated with the lux-marked microbial biosensor *Pseudomonas fluorescens* pUCD607. Data are expressed as percentage of light emission compared to the control. Results of Tukey's test for comparison of means are reported: bars labeled by the same letter are not statistically different.

by water:ethanol between sterile and non-sterile samples. This approach allows separating the biological degradation assessed in the non-sterile microcosms from eventual abiotic dissipation processes. No degradation was found for all treatments at 20 d from contamination. At 60 d 8 ± 3% of the applied dose was degraded in NC, 20 ± 4% in C1 and 11 ± 6% in C2, and at 120 d from contamination, the degradation remained almost constant for NC (13 ± 3%) and it increased, respectively, to 30 ± 5% in C1 and 35 ± 5% in C2. Tukey's test for comparison of means highlighted how the degradation at 120 d was significantly higher in the two compost treatments, with no differences between the two studied doses.

A lux-marked bacterial biosensors, *Pseudomonas fluorescens* pUCD607, was applied to water extracts from non-sterile soil samples in order to assess their ecotoxicity. The biosensor reports on general toxicity effects by a quantitative reduction in light emission, and, thus, gives indications on the toxicity of the parent compound as well as on the eventual effects of metabolites formed during the degradation process. Results are reported in Fig. 4 as percentages of light emission compared to the untreated, i.e., water extracts from non-contaminated soil samples. In the unamended soil, light emission was 50% after 20 d and above 75% of the untreated after 60 and 120 d, indicating a slight ecotoxicity of the PCP extracts that reduced over time. Different results were obtained for the two compost treatments: emission of light after 20 d of aging was above 75% in both amendment levels showing a very low toxicity towards the bacteria, but after 60 and 120 d from contamination it reduced, respectively, to 10.2 ± 2% and 7.2 ± 2% of the untreated for C1 and to 4.1 ± 3 and 6.0 ± 2% of the untreated for C2.

Considering degradation and ecotoxicity (Fig. 4) altogether, it emerges clearly how the samples with higher degradation rates, i.e., C1 and C2 at 60 and 120 d from contamination, where the ones which displayed also the highest toxicity for the lux-marked biosensor. This effect may be due to the formation of metabolites more toxic than PCP such as chlorohydroquinones and by synergistic effects of PCP and other chlorophenols on the strain metabolism as highlighted by Tiensing et al. (2002).

4. Conclusions

In this work we have analyzed the fate of PCP in sterile and non-sterile soil microcosms, assessing as well the effects of compost amendment. Bioaccessibility and bioavailability were studied according to the definitions of Semple et al. (2004); results of bioavailability studies were also linked to ecotoxicological assays based on luminescent bacteria and earthworms. Compared to other hydrophobic organic molecules such as PCBs and PAHs usually studied in bioavailability works, PCP is a weak acid (pK_a 4.75) that in neutral soil conditions as the ones studied here is almost present in anionic form (Hong and Zeng, 2002). For this reason a water extraction was chosen as a method to estimate its bioavailable fraction, while the bioavailability to earthworms was assessed by body accumulation. Bioaccessibility of the molecule was instead assessed by resin desorption (Harmsen, 2007; Semple et al., 2007). A Porapak resin was preferred to the more used Tenax and XAD resins because of its higher affinity for PCP ($K_d = 1.47 \times 10^4$). Water:ethanol shake extraction was initially chosen as an exhaustive extraction method as indicated by literature evidences (Khodadoust et al., 1999), but in our case recoveries from 20 d on were quite low.

Results showed that a considerable fraction of PCP (around 75% of the applied dose) is bioaccessible according to the resin extraction regardless time and compost addition. Bioavailability for bacteria and earthworms is instead much lower, and it decreased because of aging and compost amendment effects. Both measurements were linked to ecotoxicological assessment, using, respectively, coelomocytes NRRT measurements for earthworms and luminescent *Pseudomonas fluorescens* response for bacteria. In sterile microcosms the NRRT method applied on earthworms coelomocytes showed a strong PCP dose dependent effect without any compost and aging effect. Experiments in non-sterile microcosms showed that at 120 d from contamination the degradation of PCP is quite limited, since it did not reach more than 40% of the applied dose. Taking into account the bioavailability data, the low rate of disappearance of the molecule is not due to a low chemical availability but to a high recalcitrance. Addition of compost represented a way to partly overcome this recalcitrance and thus to increase PCP degradation, as represented by the highest degradation rate at both 60 and 120 d from contamination in the two compost treatments. Biosensors based ecotoxicological assessment highlighted a possible drawback of a higher degradation, i.e., the possible formation of more toxic metabolites. Results of the work show the importance of applying different chemical and biological methods to study the fate of environmental pollutants in soil. A positive effect of compost amendment for the remediation of soils contaminated by PCPs was also demonstrated.

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