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# Diethyl phthalate in compost: Ecotoxicological effects and response of the microbial community

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**Abstract**

There is a great need to understand the environmental impacts of organic pollutants on soil health. Phthalates are widely used in consumables and can be found extensively. We studied the toxicity of diethyl phthalate (DEP), spiked in a compost plant growth substrate, by means of the acute toxicity Flash test and on the basis of the germination and plant growth of radish seedlings. The response of the microbial community to DEP in the growth substrate was studied by PCR-DGGE (denaturing gradient gel electrophoresis). In the acute toxicity test, DEP was found to be less toxic as a pure compound than when mixed with the compost mixture. This suggests the synergistic effect of unknown toxic compounds or the release of compounds due to DEP addition. The same DEP concentration level in compost substrate induced toxic response in both plant test and microbial community analysis. The diversity of the major microbial community was reduced from a broad community to only 10 major species at toxic concentrations of DEP. Several of the identified microbial species are known to be able to degrade phthalates, which means that the suppression of other microbial species might be due to the substrate availability and toxicity. The major species identified included *Sphingomonas* sp., *Pseudomonas* sp., *Actinomycetes* sp.


*Keywords:* Diethyl phthalate; Phytotoxicity; Compost; Microbial community structure; Biotest

## 1. Introduction

Phthalate esters are a group of synthetic chemicals, which are used to plasticize polymers such as polyvinyl chloride (PVC), polyvinyl acetates, cellulosics and polyurethanes. These polymers are widely used in food wraps, plastic tubing, furniture, toys, shower curtains, cosmetics, etc. (Williams et al., 1995; Staples et al., 1997a,b). Dimethyl and diethyl phthalate esters are typically used in cellulose ester-based plastics, such as cellulose acetate and butyrate. As they are not chemically bound to the polymer material, phthalates readily migrate from the plastics to the environment. The release of phthalate esters into the environment during manufacture, use, and disposal has been extensively reviewed (e.g Wams, 1987; Cadogan et al., 1993). Concern has recently been shown about the occurrence and behaviour of phthalate esters in landfill leachates (Bauer and Herrmann, 1998; Jonsson et al., 2003).

The environmental fate and bioaccumulation of phthalate esters have been intensively studied (Staples et al.,

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1997b, 2000). Their low solubility in water is considered to reduce aquatic toxicity, because the bioavailability of phthalates in the environment is considerably lower than the total concentration (Gledhill et al., 1980; Staples et al., 1997a). On the other hand, Staples et al. (1997a) reported that the lower molecular weight phthalate esters are both acutely and chronically toxic at concentrations below their solubility level, and that toxicity increases with increasing alkyl chain length up to four carbon atoms. Several investigators have established that phthalates are teratogenic at high dosages (Lokke and Rasmussen, 1983). Some of the phthalates are suspected to be carcinogenic, and there are indications that low molecular weight phthalates are liver carcinogenic (FDA, 1982; Zhang and Reardon, 1990). The biological effects and environmental fate of phthalates have been reviewed by Thomas et al. (1978) and Staples et al. (1997a,b), who suggested that maximum residue levels would be found at intermediate trophic levels rather than at the top of the food chain. Therefore, phthalates undergo biotransformation in mammalian system.

Great concern about the effects of these compounds in nature has arisen as a result of their endocrine-disrupting properties (Jobling et al., 1995; Zou and Fingerman, 1997). Most of the studies on the endocrine-disrupting effects of chemicals in nature are based on their effects on the reproduction of fish and on changes in their genital structures. A comprehensive reviews of the effect of environmental xenoestrogens on human male reproductive health have been published by Toppari et al. (1996) and Vidaeff and Sever (2005).

Biodegradation of phthalates has been reported in marine and terrestrial environments (Saeger and Tucker, 1976; Engelhardt and Wallnöfer, 1978; Taylor et al., 1981), and in municipal sewage waters (Wang et al., 1998). Several Gram positive and Gram negative bacteria, as well as actinomycetes, can degrade phthalate esters in aerobic and anaerobic conditions. Although some individual microbes are able to completely mineralize phthalate esters, a mixed microbial community, which is typically the case in the environment, appears to have a more efficient phthalate-degrading ability (Aftring et al., 1981; Kurane, 1986). The biodegradable properties of phthalate esters vary depending on the structure of the compound. An increase in molecular weight and alkyl chain length of the molecule decreases the biodegradability. In nature, environmental conditions such as temperature, nutrient composition and the presence of degrader organisms, affect the degradation rate and mechanism (Zhang and Reardon, 1990; Staples et al., 1997b).

Compared to other xenobiotics, relatively little information is available about the impact of phthalates on individual microorganisms, as well as on microbial population dynamics in general (Cartwright et al., 2000b). However, recent investigations have suggested that ecotoxicological studies may also benefit from the determination of changes in the affected microbial communities (Stephen et al., 1999a,b; MacNaughton et al., 1999).

Diethyl phthalate (DEP), the phthalate that we focus on this study, has been found to have diverse acute and chronic toxic effects on several species at different trophic levels, as well as endocrine-disrupting properties (Colborn et al., 1993; Staples et al., 1997b; Zou and Fingerman, 1997). Even though the fate of DEP in aquatic environments has been comprehensively investigated, its effects in soil or compost have not been so well described. There are few recent studies on the degradation pathways and impacts of DEP in soil (Cartwright et al., 2000a,b; Jianlong and Xuan, 2004). While DEP biodegrades relatively rapidly in soil, its membrane-disrupting properties are a potential environmental risk. At concentrations higher than 1 mg $g^{-1}$, DEP has a significant effect on microbial communities and subsequently potential consequences for environmental processes.

Chemicals in the environment and their effect on soil health have led to increasing interest in monitoring acute and chronic toxicity and mutagenicity in soil (Maxam et al., 2000; White and Claxton, 2004; Fernández et al., 2006). ISO Soil Quality 190 is the major standardization body involved in the development of assays for soil monitoring purposes. However, there is a great need to understand the behaviour of multispecies communities, especially the impacts of toxic chemicals on the degrader communities and the growth of plants. In addition, concern has recently been raised about the health effects and tentative transportation of chemicals from soil to plants. Composted municipal sewage sludges are widely used as fertilizer or as growth media for plants in order to improve the chemical and physical properties of the soil. However, sludges are known to contain variable amounts of inorganic and organic contaminants, including phthalates (Abad et al., 2005; Harrison et al., 2006). Among the phthalic acid esters, DEHP has been widely detected in sludge targeted for composting and agricultural use. DEP is formed as a result of the degradation of DEHP during sludge treatment, and it has been detected in composted sludges (Amir et al., 2005).

The present study was performed in order to determine whether a kinetic luminescent bacteria test, the FLASH test (Lappalainen et al., 1999) for measuring acute toxicity, can be used as an indicator of DEP contamination in compost. We have earlier utilized this method to study the toxicity of compost due to the toxic compounds released during the biodegradation of biodegradable plastics (Degli-Innocenti et al., 2001; Kapanen and Itävaara, 2001; Tuominen et al., 2002). In addition, the FLASH test has been used as an indicator of compost immaturity (Itävaara et al., 2002b).

The responses of plants (OECD 208 Terrestrial Plant Growth test) and microbial communities to DEP in the growth substrate were evaluated in order to gain a better understanding of the processes taking place in plant growth substrate contaminated with phthalates. Changes in microbial communities were analysed by PCR-DGGE (Denaturing Gradient Gel Electrophoresis).

## 2. Materials and methods

### 2.1. Compost medium

Fruit and vegetable waste mixed with a bark/peat mixture was composted in controlled composting conditions in insulated 220 l composter bins as earlier described (Itävaara et al., 2002a). After 12 weeks, the compost was mixed (1:2, w:w) with a commercial growth medium (Kekkilän ruukutusmulta, Kekkilä Ltd., Finland) comprising a peat/sand mixture. Diethylene phthalate (SIGMA P5787) was added at doses of 0.01 g, 0.1 g, 1 g, 10 g and 100 g $kg^{-1}$ (fw) to a mixture of air-dried compost (200 g) and commercial growth medium (400 g) and mixed thoroughly by hand. Deionised water was added to give a total fresh weight of 1 kg. The compost medium was used in the plant growth test. Part of the medium was frozen at −20 °C for later determination of acute toxicity in the solid phase luminescent bacteria test and the DEP concentration.

The pH of the prepared compost medium, measured in a compost:water mixture (volume ratio 1:6) was from 7.4 to 7.6 in the control sample and in the samples containing 0.01–10 g DEP $kg^{-1}$. In the compost medium containing 100 g DEP $kg^{-1}$, the pH decreased to 5.9. The dry matter content (105 °C for 24 h) of the compost medium was 42%.

### 2.2. Plant growth and acute toxicity studies

Forty radish (*Raphanus sativus*; Köpenhavns Torve) seeds were sown in the compost medium described above. Four replicate pots were used at each concentration of DEP. The plants were grown in a phytotron (Weiss Umwelttechnik GMBH, Germany), exposed to light for 16 h (1700 lux) and dark for 8 h at a temperature of 20 °C and 15 °C, respectively at 55% relative humidity. Germination of the seeds was checked daily and the number of germinated seeds counted. The fresh weight of the seedlings was determined after two weeks, and the dry weight of the plant seedlings after freeze-drying. The compost medium was sieved through a 5 mm sieve and frozen at −20 °C until further analysis.

Acute toxicity studies were carried out using a modified bioluminescent bacteria test, the Flash Assay. The measurement was based on the BioTox™ Kit test utilizing bioluminescent *Vibrio fischeri*. Kinetic measurement was carried out on a 1251 Luminometer (Bio-Orbit, Turku, Finland) according to Lappalainen et al. (1999). Luminescence was measured throughout the whole exposure period (30 s), and the peak luminescence value was obtained at $I(0)$ and after exposure time $I(t)$. Results were expressed as inhibition percentage.

$$\text{Inhibition } \% = \left(1 - \frac{I(t)}{I(0)}\right) \times 100$$

$I(0)$ = the maximum value of luminescence during the first 5 s after addition of the *V. fischeri* suspension.
$I(t)$ = the value of luminescence after 30 s exposure.

The acute toxicity of compost medium to *V. fischeri* was tested before and after the plant growth test. Twenty and 40 g (dry weight) of all the samples were suspended in 1 l of 2% NaCl, and the pH was adjusted to 7. The EC50 (effective concentration) and the EC10 values for pure DEP were determined with the Flash Assay and the standard luminescent bacteria test with *V. fischeri* (EN ISO 11348-3, 1998). The dilution series for toxicity analysis with DEP was made with methanol, and the effect of methanol in the sample was taken into account. The DEP concentrations for the Flash and standard tests were 0.35–11.2 and 0.23–1.9 g $l^{-1}$, respectively. EC50 represents the concentration of the tested substance when the amount of light produced by *V. fischeri* is reduced to one half, and the EC10 concentration when the light reduction is 10%.

### 2.3. Diethyl phthalate analysis

The amount of diethyl phthalate in the compost medium was determined before and after the plant growth test. The DEP concentration was determined by high performance liquid chromatography (HPLC-DAD) in order to determine the amounts of DEP biodegraded or volatilized. The samples (1–7 g) were extracted with acetonitrile (ACN, 3 × 10–50 ml). The combined ACN extracts were analysed thereafter by HPLC, and quantitation of DEP was performed using the external standard method. The limit of quantitation was 1 mg $kg^{-1}$, and the uncertainty of the measurement ±30%. The chromatography conditions were as follows. HPLC column: Hypersil ODS (5 μm), length 100 mm, diameter 2.1 mm; eluant: gradient eluation from 20% $H_2O$/80% ACN to 100% ACN/5 min; eluation rate 0.4 ml/min; oven temperature +40 °C; the DAD wavelengths: 205 nm and 227 nm.

## 3. Effects of diethyl phthalate on microbial community structure

### 3.1. Nucleic acid extraction, PCR and DGGE

Samples for PCR and DGGE were taken after the plant growth test. Nucleic acid extractions were carried out in duplicate for native compost and each concentration of diethyl phthalate/compost mixture as described by Stephen et al. (1999a), the only modification being that 0.25 g of compost, rather than 0.5 g, was used. PCR was conducted as described in Muyzer et al. (1993): initial denaturation of DNA at 94 °C for 3 min and amplification in 35 cycles in 94 °C (1 min), 55 °C (60 s), 68 °C (45 s), in 25 μl reactions with 1.25 units of Expand LT polymerase (Boehringer Mannheim, Indianapolis, IN, USA) and 0.3 ng template DNA using a Robocycler thermocycling unit (Stratagene, LaJolla, CA, USA). Amplified 16 S rDNA gene fragments were inspected by agarose gel electrophoresis (1.2% agarose, 0.5 × TBE (0.04 M Tris base plus 0.02% M acetic acid plus 1.0 M EDTA, pH 7.5, 5 μl ethidium bromide)) prior to DGGE analysis. Ethidium bromide stained bands were

quantified by UV fluorescence using an AlphaImager 2000 and manufacturer supplied software (Alpha-Innotech, San Leandro, CA, USA). The DGGE analyses were as described in Muyzer et al. (1993), except that the gels consisted of a 10–65% gradient of denaturant (100% denaturant = 7 M urea, 40% (vol/vol) formamide; Biorad) using a D-Code system (Biorad) at a constant temperature of 60 °C. Strong DNA bands in the polyacrylamide gel were excised, and the DNA re-amplified as described in Kowalchuk et al. (1997) prior to sequence analysis.

### 3.2. DNA sequence analysis and phylogenetic inference

Re-amplified rRNA -gene fragments were purified by means of GeneClean Spin columns (BIO-101, Vista, CA, USA) and quantified by fluorometry (Hoefer DyNA-Quant 200$^{TM}$ Fluorometer and Hoechst H33258 dye binding assay; Pharmacia Biotech. Inc, Piscataway, NJ, USA). Double-strand sequencing was carried out on an Applied Biosystems automated sequencer (model 373) with "Prism$^{TM}$" dye terminators. All the clones were sequenced using the primer 519r (Lane et al., 1985), *E. coli* numbering (Brosius et al., 1981), and the sequences were edited using "Seqpup Version 0.6." (Gilbert, 1996). Reference sequences were recovered from the RDP release 7.0 of July 1998 (Cole et al., 2003). Supplemental sequences were retrieved from GenBank *via* the National Institute for Biotechnology Information (NCIB) Internet node using the Entrez facility (Schuler et al., 1996). Crude alignments of recovered sequences were performed using the ALIGN facility of the RDP followed by manual alignment within Seqpup V. 0.6. Ambiguous bases were deleted from the phylogenetic analysis by means of the Genetic Data Environment 2.2 "mask" function operated within ARB (Strunk and Ludwig, 1998). Phylogenetic algorithms (DNA-DIST, NEIGHBOR and SEQBOOT) also operated within the ARB software environment.

### 3.3. Nucleic acid accession numbers

Sequences recovered from DGGE bands were submitted to GenBank under the accession numbers AF187050–AF187056.

## 4. Results

DEP was toxic for radish in the plant growth test at the three highest concentrations studied. Plant growth, determined as seedling dry weight, was 25%, 92% and 100% inhibited by the compost medium with DEP concentrations of 1, 10 and 100 g kg$^{-1}$, respectively (Fig. 1a). Germination of the seeds was a less sensitive indicator, and gave a clear response at only the two highest phthalate concentrations (Fig. 1b). Germination was inhibited totally at the highest DEP concentration. Inhibition of germination by 10 g kg$^{-1}$ DEP was 72%, while most of the seedlings did not show good growth.

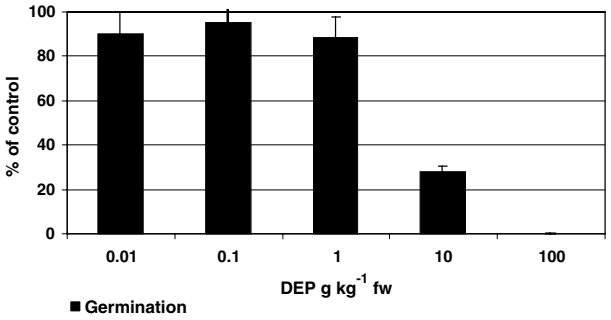


Fig. 1a. Germination expressed as % of the control in the radish (*Raphanus sativus*) germination test at different levels of DEP contamination.

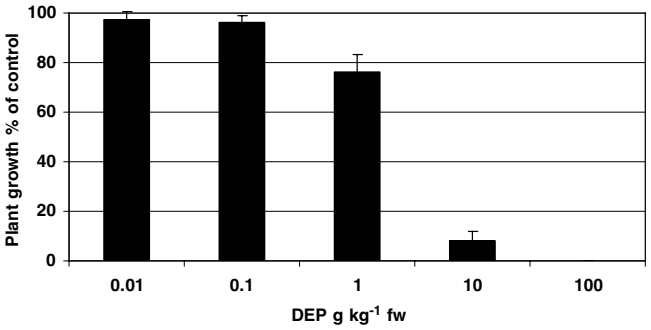


Fig. 1b. Plant growth expressed as % of the control with radish (*Raphanus sativus*) at different levels of DEP contamination.

The concentration of DEP in the compost medium decreased during the 14-day plant growth test (Table 1). After the plant growth test, the concentration in the medium that originally contained 100 g kg$^{-1}$ of DEP was only 64 g kg$^{-1}$. Less than 1% of the DEP was left in the medium that originally contained 10 g kg$^{-1}$ of DEP. Furthermore, no detectable amounts of DEP were present after the 14 day-plant growth test in the media that originally contained 1–0.01 g DEP kg$^{-1}$ compost substrate.

Before planting the seeds, the toxicity of the DEP-spiked compost medium was evaluated with the Flash luminescent bacteria test. This test, which measures acute toxicity, showed a clear toxic response at the two highest diethyl

Table 1
DEP concentrations (g kg$^{-1}$ fw) in the compost medium before and after the plant growth test

| Theoretical DEP concentration g kg$^{-1}$ fw[a] (dw[b]) | DEP g kg$^{-1}$ fw[a] before the plant growth test | DEP g kg$^{-1}$ fw[a] after the plant growth test |
|---|---|---|
| Control (0) | 0.0085 | <0.001 |
| 0.01 (0.024) | 0.011 | <0.001 |
| 0.1 (0.24) | 0.080 | <0.001 |
| 1 (2.4) | 0.780 | <0.001 |
| 10 (24) | 8.500 | 0.014 |
| 100 (240) | 100.000 | 64.000 |

[a] fw = fresh weight.
[b] dw = dry weight.

phthalate concentrations (Fig. 2a). Inhibition of the light production of *V. fischeri* with 10 and 100 g kg$^{-1}$ fw DEP in the compost media with dilutions of 40–20 g l$^{-1}$ was 27–51% and 71–75%, respectively. In contrast, the smaller concentrations of diethyl phthalate in the media caused an increase in bioluminescence by 10–20%. After the two-week plant growth assay, the toxicity of the compost media was again studied. In this case, only the highest concentration of DEP gave a toxic response and around 70% inhibition was detected (Fig. 2b). All the other samples increased the amount of light produced by up to 17%.

The toxicity of the 100 g DEP kg$^{-1}$ compost medium did not decrease during the two-week plant growth period, even though the other samples were no longer toxic at the end of the experiment due to the biodegradation or volatilization of DEP.

In order to study the EC50 and EC10 values of DEP toxicity in compost, a dilution series was analyzed by the *V. fischeri* test. The DEP concentrations in the test solutions derived from the compost media are presented in Table 2. DEP concentrations of between 9.4 and 0.47 g l$^{-1}$ in the compost media gave a toxic response in the flash test. The $EC10_{30s}$ value for DEP measured as a pure chemical with the Flash assay was 0.92 g l$^{-1}$ (standard deviation 0.25), and the $EC50_{30s}$ value was 9.40 g l$^{-1}$ (standard deviation 1.28). The standard luminescent bacteria test with a longer contact time gave a stronger inhibition effect for DEP, while $EC50_{30min}$ was 0.50 g l$^{-1}$ (0.02) and $EC20_{30min}$ 0.16 g l$^{-1}$ (0.004).

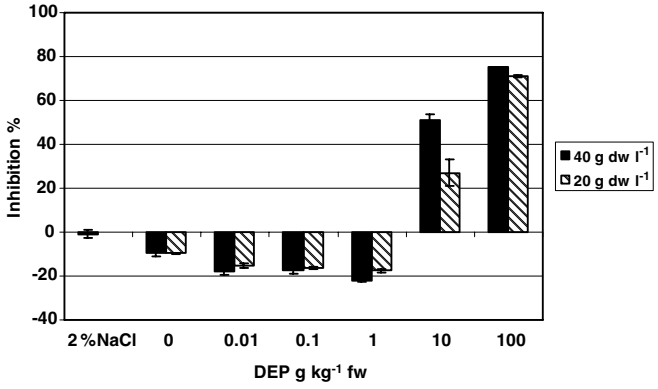


Fig. 2a. Inhibition % in the Flash test with freshly DEP-spiked (0, 0.01, 0.1, 1, 10 and 100 g kg$^{-1}$) compost medium using sample concentrations of 40 and 20 g dw l$^{-1}$. The test control was 2% NaCl.

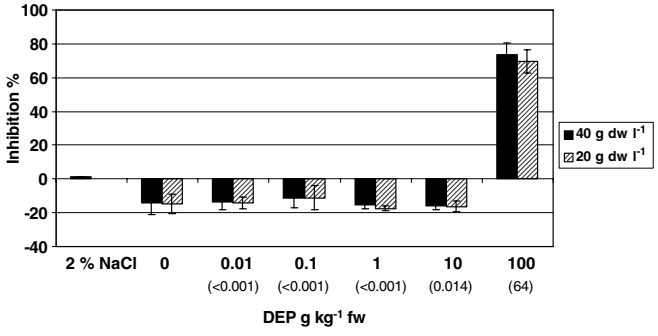


Fig. 2b. Inhibition % in the Flash test with the compost medium at the end of the 14-d plant growth test with DEP-spiked substrates. The test control was 2% NaCl. The concentrations of DEP in the growth media are expressed as nominal (without parentheses) and as measured (in parenthesis) at the end of the plant growth test.

Table 2
Concentration of DEP (g kg$^{-1}$ dw) in the compost media dilutions (40 and 20 g dw l$^{-1}$) for the Flash test

| DEP g kg$^{-1}$ fw in compost medium | DEP g kg$^{-1}$ dw in dilution 40 g dw l$^{-1}$ | | DEP g kg$^{-1}$ dw in dilution 20 g dw l$^{-1}$ | |
|---|---|---|---|---|
| | B | A | B | A |
| 100 | 9.4 | 8.2 | 4.7 | 4.1 |
| 10 | 0.94 | 0.002 | 0.47 | 0.001 |
| 1 | 0.094 | <0.0001 | 0.047 | <0.0001 |
| 0.1 | 0.0094 | <0.0001 | 0.0047 | <0.0001 |
| 0 | 0 | 0 | 0 | <0.0001 |

Samples were taken before (B) and after (A) the plant growth test.

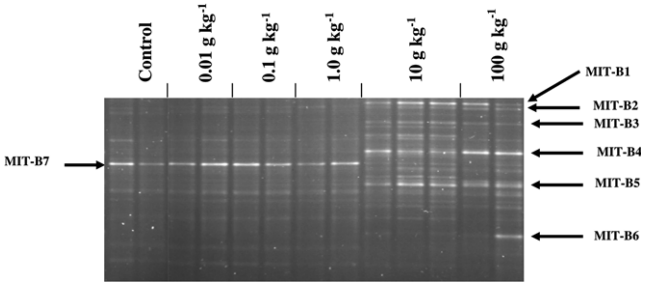


Fig. 3. PCR-DGGE analysis of 16S rDNA fragments recovered from compost. Labels indicate the bands that were excised and successfully subjected to sequence and phylogenetic analysis.

Microbial community analysis by PCR-DGGE revealed a DEP-induced change in the community structure at a concentration of 10 and 100 g kg$^{-1}$ compost medium (Fig. 3). The community structure of the DEP/compost mixtures below these levels was not discernibly different from the control compost (Fig. 3). PCR-DGGE analysis of the control and low-level DEP composts revealed only a single strong band in a highly complex background of minor PCR products. Sequence analysis of this band showed that the source organism was a member of the $\delta$-proteobacteria (Fig. 4). At toxic concentrations, as defined by plant growth and germination, this band was no longer visible. The community profiles of compost containing 10 and 100 g kg$^{-1}$ DEP were dominated by *ca.* 10 major bands, six of which generated legible sequences. Comparison of these sequences to those of cultured organisms suggested association with the genera *Sphingomonas*, *Pseudomonas* and the *Actinomycetes* (Fig. 4).

## 5. Discussion

Phthalates are compounds which are included in the priority list to be monitored in sludge targeted for agriculture

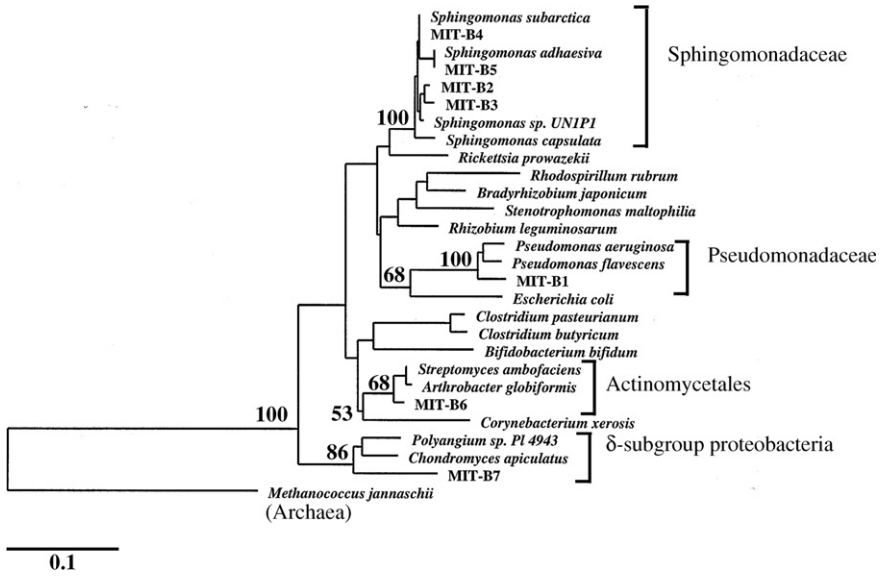


Fig. 4. Neighbour-joining dendrogram with Jukes and Cantor correction of sequences recovered from DGGE bands with reference sequences derived from the Ribosomal Database Project (Maidak et al., 1999). Numbers represent Bootstrap support on 100 replicates for branches immediately to their right and, for simplicity, are shown only for nodes of relevance to the novel sequences described. All analyses were carried out within the ARB sequence management system for the Linux operating system (Strunk and Ludwig, 1998). Scale bar represents a 10% estimated change.

in the 3rd draft of the "Working document of sludge" (EU, 2000; Andersen, 2001). Diethyl phthalate is known to be biodegradable and, in this study, was shown to decrease to nontoxic levels in the plant growth substrate during two weeks' cultivation of the test plants.

Cartwright et al. (2000a) reported that DEP added to soil at concentrations of 10 mg $g^{-1}$ or below was up to 90% biodegraded in 59 days, and Jianlong and Xuan (2004) showed DEP degradation in sludge-amended soils with a half life of 3.7 days. Compared to these values, the degradation in our compost medium was more effective. On the other hand, translocation and absorption of the chemical by the plants has to be taken into consideration. Rapid biodegradation of DEHP (diethylhexyl phthalate) in municipal solid waste and sewage sludge composting has also been reported by Moeller and Reeh (2003).

The concentration of DEP in the compost environment that induced toxic effect in the Flash test and plant assay was in the range of 1–10 g $kg^{-1}$. Lower concentrations stimulated the light production of *V. fischeri*. This phenomenon has earlier been reported by Degli-Innocenti et al. (2001). Aquatic toxicity studies with microorganisms, algae, invertebrates and fish show that EC/LC50 values for acute toxicity for DEP vary from about 10–130 mg $l^{-1}$, and for chronic toxicity from 3.65 to 25 mg $l^{-1}$ (Staples et al., 1997b). There is a lack of information about the toxic levels of DEP in soil and compost ecosystems. In the acute toxicity test (Flash test) used in this study, EC50 for DEP was 9.4 g $l^{-1}$, and in the standard luminescent bacteria test it was 500 mg $l^{-1}$. This seems to be a relatively high value compared to other reported EC50 values (Colborn et al., 1993; Staples et al., 1997a,b; Zou and Fingerman, 1997). Due to the low solubility of DEP, acute toxicity measured with luminescent bacteria is probably not a very sensitive method for determining the toxicity of DEP, especially.

It was assumed that the amount of organic matter in the compost would have reduced the level of toxicity induced by DEP, but our results did not support this. Surprisingly, in the compost matrix, 0.47 g $l^{-1}$ DEP caused 25% inhibition in the Flash test, even though inhibition with the same concentration but with the pure chemical (without compost) was less than 10% ($EC10_{30s}$ 0.9 g $l^{-1}$). This may be due to changes in the compost matrix induced by DEP, and the conversion of certain compounds in the compost into more bioavailable forms, resulting in toxicity to the test organism. According to Cartwright et al. (2000b), DEP is more toxic in soils containing low amounts of organic matter than in soils with high organic matter. In our study, however, DEP in with the presence of the organic matter in the compost did induce higher toxic levels than DEP in pure water. Therefore, the effect of DEP in an organic matter rich environment should be investigated in more detail.

There is very little information about the physiological responses of plants to DEPs and about their bioaccumulation in plants. In a related study the toxic response of radish seedlings was studied at the protein level. Toxic concentrations of DEP lead to prominent changes in protein synthesis and the formation of several stress related proteins, such as heat shock proteins (HSPs), when the small aseptically cultivated seedlings were exposed to 222 mg $l^{-1}$ DEP in liquid medium. Novel proteins that might be specific for DEP induction were also detected (Saarma et al., 2003). In the present study, toxic effects were induced in the plant assay and Flash test at higher

concentrations in the range of 1–10 g $kg^{-1}$, probably due to the presence of a solid matrix and lower bioavailability or lower sensitivity of the used methods.

The indigenous microbial communities present in plant growth media are important not only for the degradation and mineralization of organic compounds in the medium and the release of nutrients for the plants, but also because they represent an important suppressive microbial community that prevents the growth of pathogens. In the present study, the microbial community composition in the compost medium changed at the same toxic concentration that was found to be toxic in the single-species toxicity tests. This phenomenon is probably due either to the suppression of sensitive populations by the high concentration of DEP, or to the emergence of a resistant degrader population.

The *Actinomycetes* group, which emerged at higher DEP concentrations, is known to include proficient DEP-degrading strains (Suemori et al., 1993; Chauret et al., 1995). We have not found any report of DEP-degrading *Sphingomonas* sp. but, considering the broad-substrate utilization of this genus and its strong implication in the bioremediation of organic pollutants (White et al., 1996; Kästner et al., 1998; Barkay et al., 1999; Hamann et al., 1999 and references cited therein), its occurrence as a major phthalate-degrader *in situ* is not surprising. However, we did not find any members of the genus *Burkholderia*, for which phthalate degradation has been well described under laboratory conditions (Chang and Zylstra, 1999). The response of the indigenous bacterial community to the addition of DEP was observed at the same level of contamination as was required to elicit an effect in both the single-species phytotoxicity and microbial-toxicity tests described above. This response may have been due to a number of factors, principally to the growth of specific organisms on DEP and to inhibition of the growth of other organisms by DEP. Therefore the response should not be considered as a measure of the toxicity of DEP to the *in situ* microbial community *per se*. Instead, the coincidence of the two ecotoxicological measurements with a clear alteration in the *in situ* bacterial community structure supports the argument that microbial community typing is an effective alternative method for monitoring pollutants and their remediation (Almeida et al., 1998; White et al., 1998).

Cartwright et al. (2000b) reported that pseudomonas were more sensitive to higher concentrations of DEP (10–100 mg $g^{-1}$) than the total culturable bacteria. Lower concentrations stimulated pseudomonas momentarily, but DEP was shown to be inhibitory in both water and soil at concentrations above 10 mg $l^{-1}$. One of the major microbial groups in the samples containing 10–100 g DEP $kg^{-1}$ in our test setup was found to belong to the *Pseudomonas* genera (*sensu stricto*). Concentrations above 1 g $kg^{-1}$ had a significant effect on the microbial community and environmental processes. What are the fundamental agricultural and environmental effects of these changes in the microbial population that have been detected in all contaminated soils? The important questions to be addressed in the future are the effect of organic pollutants on soil health and on the balance of pathogenic and biocontrol microorganisms in agricultural soils.

The recycling of organic matter back to soil is required to maintain the fertility of soils. However, sludge and compost may contain high amounts of organic pollutants such as phthalates, and this poses a potential threat for the contamination of agricultural soils. In order to meet the challenges set by EU legislation for the recycling of sludge and biowaste compost, more information is needed about their bioaccumulation due to continuous application on fields. There is also a need for screening tests (i.e. fast biotests) to evaluate the potential adverse effects of the compost used for soil amelioration, and inexpensive methods for determining the amount of organic pollutants in the environment.

## 6. Conclusions

This study demonstrated that in compost plant growth substrate, microbes and plants responded to the same toxic concentration of DEP. In the acute toxicity test the presence of organic matter in the compost did not, contrary to expectations, reduce the toxicity. DEP was found to be less toxic as a pure chemical than when mixed with the mature compost. The microbial community response exhibited suppression of the most heterotrophic microorganisms at toxic concentrations of DEP. The diversity of the microbial community was reduced to 10 major species at concentrations above 1 g $kg^{-1}$ DEP. In addition to the sensitivity of some microbial species to DEP, the reason for the change in the microbial community was probably due to the increase in the degrader population resulting from abundant substrate availability. The major species identified were *Sphingomonas* sp., *Pseudomonas* sp., and *Actinomyces* sp.

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