



**HAL**  
open science

## **Biotransformation of nonyphenol surfactants in soils amended with contaminated sewage sludges.**

Jacqueline Dubroca, Agathe Brault, Albert Kollmann, Isabelle Touton, Claude Jolival, Lucien Kerhoas, Christian Mougin

► **To cite this version:**

Jacqueline Dubroca, Agathe Brault, Albert Kollmann, Isabelle Touton, Claude Jolival, et al.. Biotransformation of nonyphenol surfactants in soils amended with contaminated sewage sludges.. Environmental Chemistry: green chemistry and pollutants in ecosystems., Springer-Verlag, 2005, 978-3-540-22860-8. 10.1007/3-540-26531-7\_29 . hal-02832059

**HAL Id: hal-02832059**

**<https://hal.inrae.fr/hal-02832059v1>**

Submitted on 8 Mar 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Biotransformation of nonylphenol surfactants in soils amended with contaminated sewage sludges

Jacqueline Dubroca, Agathe Brault, Albert Kollmann, Isabelle Touton, Claude Jolival, Lucien Kerhoas and Christian Mougin\*

Unité de Phytopharmacie et Médiateurs Chimiques, Institut National de la Recherche Agronomique, Route de Saint-Cyr, 78026 Versailles Cedex, France

### Abstract

The biotransformation of nonylphenol was investigated in an agricultural soil treated with a mixture of [<sup>14</sup>C]labelled and unlabelled surfactant. It was then studied in soil samples amended with sludges spiked with the mixture of chemicals. Nonylphenol amount in all samples of soil and soil/sludge mixtures was 40 mg kg<sup>-1</sup>. In the soil free of sludge, the half-life of nonylphenol was found to be 4 days. When the soil was amended with sludge from the city of Ambares, France, it was about 16 days. In the soil amended with sludge from Plaisir, a 8-day lag phase was observed before the transformation starts, and nonylphenol half-life exceeded 16 days. In each case, nonylphenol transformation resulted in mineralization as well as stabilization of the chemical as bound residues within the soil. Further, some strains of filamentous fungi were isolated from the soil/sludge mixtures and identified to belong to the *Mucor* and *Fusarium* species. Most of them were able to efficiently transform nonylphenol in liquid cultures. In addition, the ligninolytic basidiomycete *Trametes versicolor* was able to catalyze partly the conversion of nonylphenol into carbon dioxide. Laccases purified from *T. versicolor* cultures are enzymes involved in nonylphenol oxidative coupling leading to oligomerization.

Keywords: sewage sludge, soil, alkylphenols, fungi, enzymes, biotransformation, oxidative coupling

### 1. Introduction

The use of sewage sludge to fertilize agricultural land has been recognized in the past decades to be an economic and environmentally acceptable method for municipal sludge disposal. However, these sludges contain, in addition to nutrients, numerous heavy metals and organic contaminants, which can represent serious risks to human health and the environment. Thus, at the end of sewage treatment, sludges contain many substances which are not fully degraded. Among these compounds there are variable amounts of organic endocrine disrupters, whose presence in the environment is currently an increasing concern.

Alkylphenol ethoxylates are non-ionic surfactants used in domestic, industrial and agricultural applications. In wastewater treatment plants, they are partly degraded to nonylphenols, which can amount to about 80% of the ethoxylates. Nonylphenols are released in the environment as a mixture of 18 isomers (nonylphenol), including the well-known 4-*n*-nonylphenol (4nNP) (Figure 1). They comprise both hydrophobic branched nonyl groups and a hydrophilic moiety (Ahel et al. 1994a and b). The mixture exhibits a very high water solubility (5000 mg L<sup>-1</sup>) and high log P value (5.92).

Figure 1

Nonylphenols represent an environmental hazard to the environment because they induce several toxic effects on wildlife and humans. They are strong endocrine disrupters, as well as inducers of breast cancer in women and of prostate in men (Routledge et al. 1996). They also contribute to the feminization of male fish in sewage outflows (White et al. 1994). Endocrine disrupters have been designed to interfere with plant-*Rhizobium* signalling and nitrogen-fixing symbiosis (Fox et al. 2001). They induce also developmental abnormalities among freshwater sponges (Hill et al. 2002). Finally, they are responsible for morphological defects and for multiple physiological effects, partly due to uncoupled respiration in filamentous fungi and yeasts (Karley et al. 1997).

Following sewage treatment, low amounts of nonylphenols (less than 10 µg L<sup>-1</sup>) are discharged in water (Ahel et al. 1994b), and both the acute and chronic toxicity of these compounds to aquatic organisms are well known. By contrast, high amounts of nonylphenol accumulate in sludge (more than 1g kg<sup>-1</sup>, Ahel et al. 1994a). To date, knowledge of their fate and impact is very scarce. This raises two questions of great concern: 1) what is the fate of nonylphenols in soils amended with sludges, and 2) what is their impact on soil (micro)-organisms?

Our objectives are to assess the ecotoxicological risk induced by soil amendment with sewage sludges from urban origin, containing organic pollutants. We are currently involved in exposure assessment of soil organisms to chemicals. In this paper, we investigate the biotransformation of nonylphenol in an agricultural soil, amended or not with nonylphenols-containing sludges. Fungal biotransformation of the pollutants is also reported, as well as transformation by enzymes purified from fungi.

## 2. Experimental

### 2.1 Chemicals

4-*n*-Nonylphenol (4nNP) was obtained from Lancaster, whereas technical nonylphenols (nonylphenol, a mixture of structural isomers of 4nNP through branched nonyl groups) were obtained from Fluka. Other chemicals were available from Sigma. Labelled 4 NP (2 GBq mmol<sup>-1</sup>, radiochemical purity > 99%) was a generous gift of Dr J.-P. Cravedi (INRA, Toulouse).

## 2.2 Characteristics of soil and sludges

We investigated the biotransformation of nonylphenol in a silt loam soil, composed of 25.5% sand, 55.0% silt and 19.5% clay. Its organic matter content was 1.65%. Soil pH was 8.1 and the cationic exchange capacity was 10.2 meq 100 g<sup>-1</sup>. The soil, collected in the 10-20 cm layer from an experimental field in Versailles, was sieved (2-mm) and used immediately.

We used two sludges exhibiting different characteristics (Table 1). The sludge from Ambares was composed of both urban (90,000 equivalent inhabitants) and industrial wastewaters. That from Plaisir mostly contained urban wastewater (42,000 equivalent inhabitants).

Table 1

## 2.3 Biotransformation of nonylphenol in soil and soil/sludge mixtures

Soil samples (27-g dry soil) were incubated in 150-mL Erlenmeyer flasks, in darkness at 25°C, for 96 days. Water was added to obtain a 80% soil moisture holding capacity. The moisture content was kept constant during the experiments by adding sterile water every week. The soil was supplemented with unlabelled and labelled (9 kBq) nonylphenol to give a final concentration of 40 mg kg<sup>-1</sup>. The Erlenmeyer flasks were sealed with cotton plugs and incubated in 1-L sealed flasks with vials containing 10 mL of NaOH 1 N (to trap CO<sub>2</sub>) and 10 mL water (to keep moisture constant), according to Mougin et al. (1997).

We used the same protocol for incubations with soil/sludge mixtures. Sludges containing endogenous nonylphenol were spiked with unlabelled and labelled chemical. After addition of the chemicals, the solvent (acetone) was allowed to evaporate for 30 min, and nonylphenol was sorbed to the sludges for 24 hours at 4°C under nitrogen atmosphere to avoid any biotransformation. 2-g dry sludge samples were then mixed with 25-g dry soil for incubations. Final amount of nonylphenol was 40 mg kg<sup>-1</sup> in all soil/sludge samples.

## 2.4 Soil and soil/sludge mixtures analysis

Soil samples were mixed with 1g hyflosupercel (diatomaceous silica for filtration) and 20 mL water, then nonylphenols were extracted by adding 100 mL acetone-water (80/20; v/v) and shaking for 60 min. The liquid and solid phases were separated by filtration on a Büchner funnel, and the solid fraction was extracted a second time. The extracts were pooled. The radioactivity was measured by counting 500 µL aliquots of the extracts by liquid scintillation.

Extracts obtained from soil/sludge mixtures were purified prior to high performance liquid chromatography (HPLC) analysis. 5-mL aliquots were first evaporated to complete dryness and dissolved in 1 mL hexane. The solution was applied onto a glass column packed with 5 g Florisil (activated with 2% water), then eluted. The first 30-mL fraction of hexane was discarded. The

radioactive compounds were then eluted by 90 mL dichloromethane. Finally, the solvent was concentrated to complete dryness and dissolved in 20 mL acetonitrile for HPLC analysis.

Non-extractable radioactivity in the soil and soil/sludge samples was determined by combustion in a model 307 oxidizer (Packard Instrument, Rungis, France).

$^{14}\text{CO}_2$  production was quantified by counting 500  $\mu\text{L}$  aliquots of NaOH solution by liquid scintillation. HPLC analysis was performed by injecting 100  $\mu\text{L}$  of the organic extracts onto an analytical column TSK ODS-80TM (25 cm x 4.6 mm i.d., Varian, les Ulis, France) set at 30°C. A Varian 9010 pump delivered the mobile phase consisting of a mixture of acetonitrile-water- $\text{H}_3\text{PO}_4$  (50/50/0.05; v/v/v) at a rate of 1 mL  $\text{min}^{-1}$ . It was increased to 70% acetonitrile in one min and maintained at this value during 22 min. Another linear increase to 100% acetonitrile occurred in 2 min, and then followed by a stationary phase of 5 min and a return to initial conditions. Radioactivity and  $A_{224}$  of the column eluate were monitored. Our analytical protocol allowed a high  $^{14}\text{C}$  recovery amounting to 98.0% of initial radioactivity.

## **2.5 Isolation of fungi from soil/sludge mixtures and biodegradation tests**

*Fusarium* and *Mucor* strains were isolated from soil/sludge mixtures by layering soil aggregates onto plates containing malt (20 g  $\text{L}^{-1}$ ), agar (15 g  $\text{L}^{-1}$ ) and yeast extract (1 g  $\text{L}^{-1}$ ), supplemented with a mixture of chloramphenicol, streptomycin, penicillin G and chlortetracycline, each at 50 mg  $\text{L}^{-1}$ . The plates were incubated for one week at 25°C. Then, according to their morphology, fungal strains were picked, individually cultured, and identified. Fungal strains from our collection were also taken into account.

Fungal biotransformation of nonylphenol was then assessed by culturing the strains at 25°C in Erlenmeyers flasks containing 10 mL liquid media as described earlier (Mougin et al. 2000). Glycerol and maltose were used as carbon sources for *P. chrysosporium* and all the other strains, respectively. Nonylphenol was added at 11 mg  $\text{L}^{-1}$  after 3 days of growth.

A detailed study of the biotransformation of 4nNP was achieved in *T. versicolor* cultures using a chemical solution spiked with 5 kBq labelled chemical. It was immediately added to the culture without the 3 days of pregrowing phase. Liquid and solid phases were analyzed separately.

## **2.6 Analysis of fungal cultures**

The remaining nonylphenol was extracted three-times from the biomass with 20 mL hot methanol (60°C). After cooling, the alcoholic fractions were reduced under vacuum, pooled with the medium, and the aqueous solution was extracted twice with 35 mL diethyl ether. Then, the solvent was evaporated and the compounds were dissolved in 1 mL methanol for HPLC analysis. Analysis was performed as described for soil experiments.

## **2.7 Enzymatic assays with purified laccases**

Laccases (*para*-diphenol:dioxygen oxidoreductases, EC 1.10.3.2) were purified according to published procedures (Jolivalt et al. 1999). They were then incubated in aerated 0.1M citrate/phosphate buffer (1 unit enzyme in 1 mL buffer, pH ranging from 4 to 7) under stirring at 30°C, in the presence of 5 mg L<sup>-1</sup> 4nNP. Aliquots (100 µL) of the incubation media were then directly analyzed by HPLC using the procedures described above.

## **2.8 Identification of nonylphenol metabolites**

We determined the chemical structure of the metabolites formed by purified enzymes using a mass-spectrometer Nermag R30-10C. Spectra were obtained by Desorption Chemical Ionization with ammonia (NH<sub>3</sub>) as a reactant gas. The spectrometer was set in positive mode.

## **3. Results and discussion**

### **3.1 Biotransformation of nonylphenol in an agricultural soil free of sludge**

We studied in that experiment the ability of the endogenous microflora to transform nonylphenol in an agricultural soil. Soil samples were incubated in darkness at 25°C for 96 days in the presence of 40 mg kg<sup>-1</sup> nonylphenol (a mixture of unlabelled and labelled chemicals). Our results showed that the greatest part of the <sup>14</sup>C contained in nonylphenol was associated to the solid fraction of the soil, and about 40.0% of initial <sup>14</sup>C content was measured following soil combustion after 96 days (Figure 2). In addition, 31.4% of the labelled compounds were transformed to carbon dioxide during the same period. The radioactivity present in the organic extract was 30.7% at the end of the experiment. HPLC analysis showed a decrease of the nonylphenol content with time in the extract. They were the main components of the extract during the first week of incubation, and became negligible after 32 days of incubation. Residual nonylphenol was assumed to be the sum of the <sup>14</sup>carbon non extractable from the soil and of the surfactant remaining in the organic extracts analyzed by HPLC. Thus, we calculated a half-life of 4 days for nonylphenols in our incubation conditions. The value corresponded to the time required to transform (by stabilization or degradation) 50% of initial amounts of nonylphenol. The remaining fraction of the radioactivity in the extract was attributed to polar unidentified transformation products. Our results show that nonylphenol is rapidly transformed by the endogenous microflora of the aerated soil, in agreement with the previous study of Topp and Starratt (2000).

Figure 2

### **3.2 Biotransformation of nonylphenol in soils amended with sewage sludges**

This experiment was intended to study the transformation of nonylphenol in soils amended with sludges. Nonylphenol was brought by sludges in order to mimic the upper layer of an agricultural soil

amended with contaminated sludges. The biotransformation of the chemical is supposed to be mediated by soil microflora. Mixtures were incubated in darkness at 25°C for 96 days in the presence of 40 mg kg<sup>-1</sup> of a mixture of unlabelled and labelled nonylphenol. In the soil amended with the sludge from Ambares, the amount of <sup>14</sup>C bound to soil materials represented 21.5% of the initial radioactivity after 96 days of incubation (Table 2). At the opposite, the total <sup>14</sup>CO<sub>2</sub> produced in the incubation vials during the same period amounted to 54.6% of the initial radioactivity. On the other hand, extractable radioactivity was drastically reduced over the incubation period, representing only 5.2% of the initial radioactivity after 96 days. Our results indicated that the kinetics of the transformation of nonylphenol brought by the sludge from Ambares and this observed in the soil free of sludge were different, as its half-life was calculated to be 16 days in the presence of the sludge. The amendment mainly increased the mineralization of nonylphenol whereas its stabilization in soil was decreased. In addition, extracted radioactivity was low in soil/sludge samples. These results suggested that the sludge from Ambares also modified the biotransformation pathways of nonylphenol.

Table 2

Results of nonylphenol incubation were also different in the soil amended with the sludge from Plaisir. Indeed, a 8-day lag phase occurred, during which no transformation could be observed (data not shown). The depressive effects of the sludge on biodegradation can be attributed to several factors: 1) toxicity of the sludges, because of the presence of numerous organic contaminants and high amounts of metal ions, thus requiring the selection of resistant micro-organisms, 2) a high biological oxygen demand, due to the high organic matter content of the sludges, 3) an increase of nonylphenol adsorption in the mixtures by comparison with the soil alone. Nevertheless, relatively few studies have been published, which address the effects of sludge-bound chemicals on microbial processes in soils (Gejlsberg et al., 2001).

After the lag phase, mineralization started rapidly to reach 36.1% of the initial radioactivity after 96 days, a level quite identical to that noticed in the soil incubated alone. Following a similar pattern, non-extractable radioactivity represented 44.9% of the initial radioactivity. Then, extractable radioactivity was also reduced to 7.6% at the end of the experiment. Analysing the organic extract by HPLC allowed estimating a half-life of isoNPs between 16 and 20 days in these conditions. These results clearly demonstrated that the nature of the sludge effect nonylphenol biotransformation in the soil, resulting in an increase of the half-life of the chemical.

### **3.3 Biotransformation of nonylphenol by filamentous fungi**

The aim of that study was to evidence the ability of fungi to transform nonylphenol in liquid cultures. Half-lives of the chemicals is based on their residual content in the cultures with respect to initial amounts (Table 3). Degradation of the 4nNP with half-lives lower than 2 days proceeds in general

more rapidly than that of nonylphenol (> 2days). However, the two white-rot fungi, *T. versicolor* and *P. chrysosporium*, transformed nonylphenol more extensively than 4nNP. In addition, *Fusarium* strains, isolated from soils amended with sludges from both origins, were poor degraders of isoNPs. By contrast, *Mucor* strains, only present in the sludge from Ambares, and *Fusariums*, were able to efficiently transform the mixture. The results demonstrated that the extent of nonylphenol transformation varied according to the fungal taxonomic groups. White-rot fungi have been described for many years as efficient tools for organic pollutant breakdown, because of the secretion of exocellular oxidases (Pointing, 2001). By contrast, no data are available concerning the enzymatic systems from strains belonging to *Mucor* and *Cunninghamella* genera able to transform phenolic compounds.

Table 3

### **3.4 Biotransformation of 4-*n*-nonylphenol by the fungus *T. versicolor***

The biotransformation of 4-*n*-nonylphenol isomer has been investigated in liquid cultures of *T. versicolor*, previously shown as the most efficient alkylphenol degrader. In non-inoculated controls, <sup>14</sup>C amounts in the medium were 50 % lower during the 12 days of incubation, due to adsorption on glass. No mineralization occurred. During the same period, radioactivity partitioned between the medium and the biomass in the inoculated cultures amounted to 29.2 and 23.4% at the end of the experiment, respectively. 4nNP was partly converted by the fungus into labelled carbon dioxide (6%). Using HPLC, we failed to detect any radioactive peaks corresponding to transformation products in analyzing the organic extract obtained from the medium, although it contained significant amounts of <sup>14</sup>C. Direct injection of culture medium into the HPLC system showed that the radioactivity was retained in the analytical column (data not shown). These results suggest that a high-molecular weight compound could be formed by the fungus, and that its ability to mineralize nonylphenol is very reduced. For that reason, such a biodegradation process observed in soils may be rather due to the catabolic activity of bacteria.

Figure 3

### **3.5 Biotransformation of 4-*n*-nonylphenol by laccases purified from *T. versicolor* cultures**

Laccases are the main exocellular enzymes produced by *T. versicolor*. This experiment addresses their ability to transform phenolic compounds such as nonylphenol. For that purpose, purified enzymes have been incubated in the presence of 5 mg L<sup>-1</sup> 4nNP. Concentrations of 4nNP were dramatically reduced in the incubation media in the absence of laccase, and the residual chemical represented only 34% of its theoretical value after only 30 sec of incubation (Figure 4). This apparent loss was due to a strong adsorption of 4nNP on the glass of the incubation vessels. The disappearance of 4nNP was



increased when laccase was added to the media, and more than 90.0% of the chemical were transformed after 5 min of incubation. The reaction was pH-dependant. Higher 4nNP disappearances were observed at acidic pH (4 and 5). Most of 4nNP (80%) was transformed after 5 min at pH 4, 60.0% at pH 5 and only 30% at pH 6. The reaction was totally inhibited at pH 7.

Figure 4

No soluble compounds corresponding to transformation products could be detected after analysis of the medium by HPLC. However, a brown precipitate accumulated on the bottom of the incubation vials, with respect to time. It was not soluble in alcohols, was only slightly solubilized by classical organic solvents, such as dichloromethane, ethyl acetate, or hexane.

### 3.6 Identification of metabolite

We attempted to identify the metabolites formed from incubations of 4-*n*-nonylphenol with purified laccases by mass-spectrometry. The spectrum of the fraction solubilized in organic solvents showed ions at  $m/z$  220, 438 +456, 656 + 674, 874 + 892, 1111 + 1115 (Figure 5). The first ion (220) resulted from the fragmentation of larger molecules, and corresponded to the molecular weight (MW) of 4nNP. The doublets, all exhibiting a difference of 18 amu due to the loss of water, corresponded to  $M^+$  ions and  $MNH_4^+$  adducts. They were attributed to oligomerized 4nNP as dimeric (MW = 438), trimeric (MW = 656), tetrameric (MW = 874) and pentameric (MW = 1092) compounds. For the last compound, the discrepancy between theoretical and experimental data was due to the calibration range of the spectrometer, which was not optimized for high mass values. Additional experiments are under progress to confirm these results using NMR and infra-red spectrometry.

Figure 5

## 4. Conclusions

Nonylphenol is rapidly transformed in aerated soils by mineralization and stabilization (formation of bound residues). The degradation rates are somewhat decreased when the soil is amended with sewage sludges containing similar amounts of nonylphenol. The strongest negative effect was observed in the presence of the sludge from Plaisir. The filamentous fungi isolated from the soil/sludge mixtures and from our collection transformed nonylphenol with different efficiencies. Yet, *T. versicolor* (basidiomycete) and *Mucor* strains (zygomycetes) transformed the chemical, whereas *Fusarium* strains (deuteromycetes or ascomycetes) were poor degraders. Fungal laccase, produced mainly by white-rot basidiomycetes such as *T. versicolor*, are enzymes which catalyze the oxidative coupling of nonylphenol to produce oligomers. The reaction illustrates one of the mechanisms involved in the

stabilization of xenobiotics in soils. Nonylphenol mineralization is mainly attributed to bacterial catabolic activity, whereas fungi are rather involved in the chemical stabilization in the soil through oxidative coupling. Although sludges modify the adsorption of nonylphenol onto soil, our results obtained using soil/sludge mixtures clearly establish that the exposure of soil organisms to the surfactant can be increased by slowing down its biodegradation. This result emphasizes the need to assess the effects of nonylphenol on soil micro-organisms by further investigations.

## References

- Ahel M, Giger W, Koch M (1994a) Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment – I. Occurrence and transformation in sewage treatment. *Water Res* 28:1131-1142.
- Ahel M, Giger W, Schaffner C (1994b) Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment – II. Occurrence and transformation in rivers. *Water Res* 28:1143-1152.
- Fox JE, Starcevic M, Kow KY, Burow ME, McLachlan JA (2001) Endocrine disrupters and flavonoid signal. *Nature* 413:128-129.
- Gejlsberg B., Klinge C., Samsøe-Petersen L., and Madsen T. (2001) Toxicity of linear alkylbenzene sulfonates and nonylphenol in sludge-amended soil. *Environ Toxicol Chem* 20:2709-2716.
- Hill M, Stabile C, Steffen LK, Hill A (2002) Toxic effects of endocrine disrupters on freshwater sponges: common developmental abnormalities. *Environ Pollut* 117:295-300.
- Jolival C, Raynal A, Caminade E, Kokel B, Le Goffic F, Mougin C (1999) Transformation of *N,N'*-dimethyl-*N*-(hydroxyphenyl)ureas by laccase from the white rot fungus *Trametes versicolor*. *Appl Microbiol Biotechnol* 51:676-681.
- Karley AJ, Powell SI, Davies JM (1997) Effect of nonylphenol on growth of *Neurospora crassa* and *Candida albicans*. *Appl Environ Microbiol* 63:1312-1317.
- Mougin C, Pericaud C, Dubroca J and Asther M (1997) Enhanced mineralization of lindane in soils supplemented with the white rot basidiomycete *Phanerochaete chrysosporium*. *Soil Biol Biochem* 29:1321-1324.
- Mougin C, Boyer F-D, Caminade E and Rama R (2000) Cleavage of the diketone nitrile derivative of the herbicide isoxaflutole by extracellular fungal oxidases. *J Agric Food Chem* 48:4529-4534.
- Pointing S.B. (2001) Feasibility of bioremediation by white-rot fungi. *Appl Microbiol Biotechnol* 57:20-33.
- Routledge EJ, Sumpter JP (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environ Toxicol Chem* 15:214-248.
- Topp E and Starratt (2000) Rapid mineralization of the endocrine-disrupting chemical 4-nonylphenol in soil. *Environ toxicol Chem* 19:313-318.
- White R, Jobling S, Hoare SA, Sumpter JP, Parker PG (1994) Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* 135:175-182.



Table 1. Origins and physico-chemical properties of the sludges used in the study.  
 \*fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene; \*\*PCBs 28, 52, 101, 118,  
 138, 153, 180; \*\*\*Fe, Mn

Properties	Ambares (33)	Plaisir (78)
Dry matter (%)	75.71	33.12
Organic matter of dry matter (%)	51.41	45.20
Total nitrogen of dry matter (%)	3.97	2.66
pH water	7.8	12.5
isoNP content (mg/kg d.w.)	200.0	506.0
PAH content* (mg/kg d.w.)	0.51	0.43
PCB content** (mg/kg d.w.)	0.089	0.071
Metal ion content*** (mg/kg d.w.)	8880.71	113.86

Table 2. Distribution of labelled nonylphenol during incubation of soil/sludge mixtures.  
 Note that the transformation kinetics and pathways of nonylphenols differ when the chemicals are in the soil free of sludge or brought by sludges from two origins.

Incubation conditions	% of initial radioactivity					
	bound		extracted		mineralized	
	8 days	96 days	8 days	96 days	8 days	96 days
Soil from Versailles	37.2	40.0	41.9	30.7	15.9	31.4
Soil/sludge from Ambares	26.9	21.5	20.5	5.2	32.9	54.6
Soil/sludge from Plaisir	12.7	44.9	81.1	7.6	0.0	36.1

Table 3. Transformation of nonylphenol compounds in fungal liquid cultures. Half-lives have been calculated on the basis of residual content of the chemicals in the cultures, with respect to initial amounts.

Strains	Half-life (days)	
	4- <i>n</i> -nonylphenol	nonylphenol
Soil amended with sludges from Ambares		
<i>Mucor racemosus</i>	2.0	3.0
<i>Fusarium oxysporum and solani</i>	2.0	>8.0
<i>Mucor hiemalis</i>	1.5	5.0
Soil amended with sludges from Plaisir		
<i>Fusarium solani</i>	1.0	>8.0
<i>Fusarium sp.</i>	1.5	>8.0
<i>Fusarium oxysporum</i>	2.0	6.0
Strains from our collection		
<i>Trametes versicolor</i>	1.0	<1.0
<i>Phanerochaete chrysosporium</i>	6.0	3.0
<i>Cunninghamella elegans</i>	1.0	2.0
<i>Cunninghamella echinulata</i>	1.0	2.0

Figure 1. Structure of the 4-*n*-nonylphenol. Isomers have branched nonyl groups.

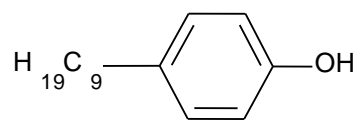


Figure 2. Distribution of labelled nonyphenol during the incubations with the soil free of sludge. Note the increase of both  $^{14}\text{CO}_2$  produced and non extractable radioactivity due to biological activity. On the other hand, extractable radioactivity decreased with incubation time.

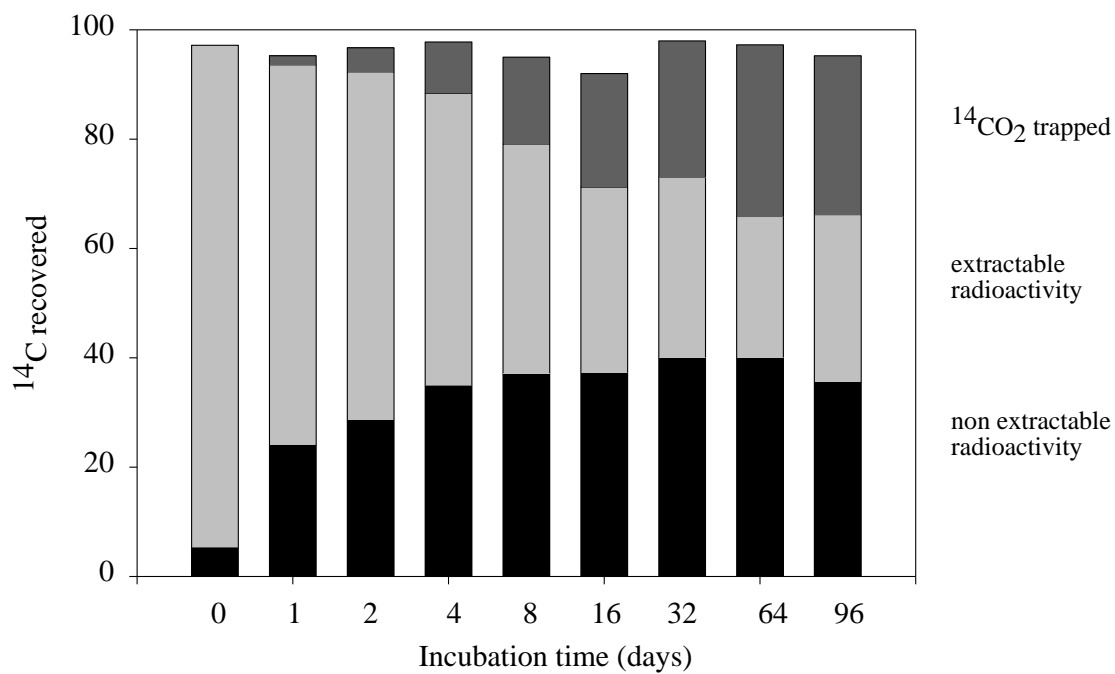




Figure 3. [ $^{14}\text{C}$ ]nonylphenol mass-balance in *T. versicolor* cultures (black symbols) and non-inoculated controls (white symbols). Note the decrease of radioactivity in the control cultures due to adsorption on glass, and its partitioning between medium and biomass in fungal cultures.

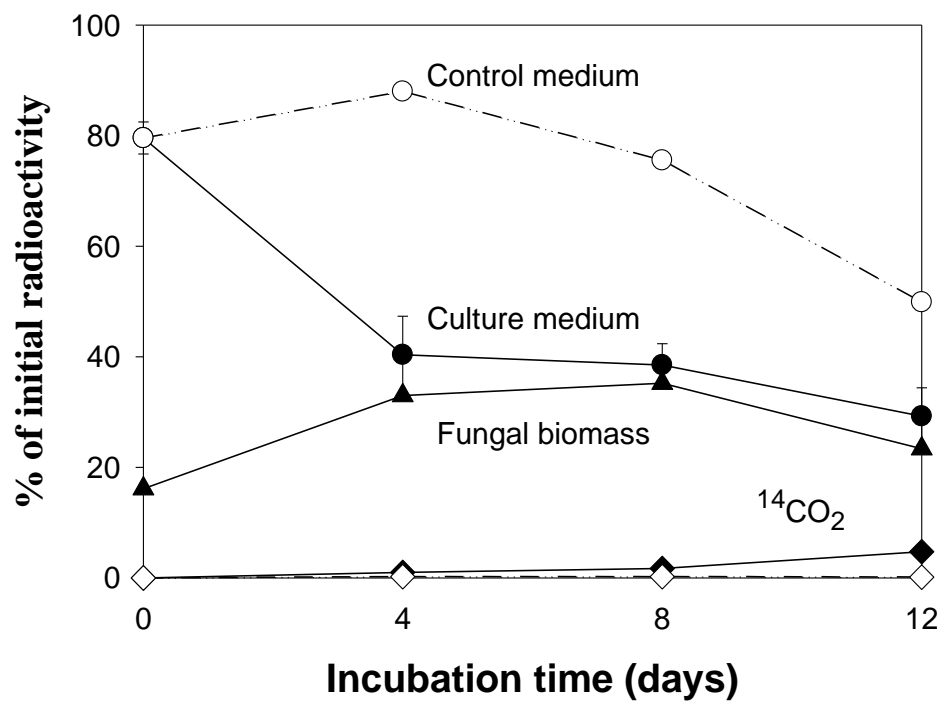


Figure 4. Transformation of 4-*n*-nonylphenol by purified laccases. Note the more rapid decrease of nonylphenol content in the buffer containing the enzymes than in the control medium.

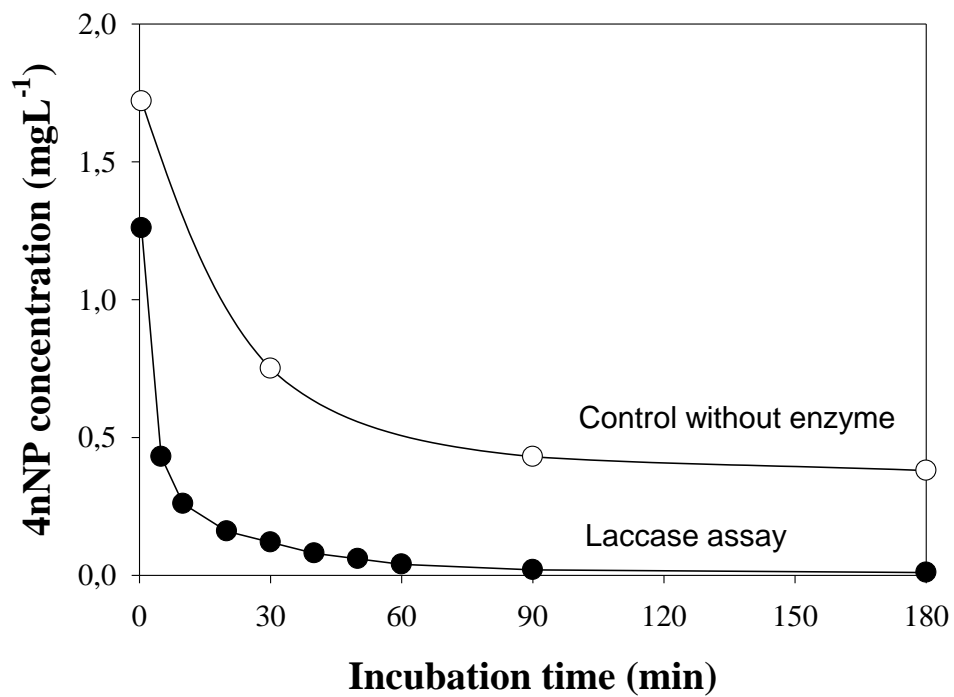


Figure 5. Mass-spectrum and structure of oligomers formed from 4nNP by fungal laccases.

