The impact of *Cinnamomum camphora* on the dissipation of PAHs in diesel contaminated soils from China

El impacto de Cinnamomum camphora en la disipación de los HAPs en suelos de China contaminados con diesel
O impacto de Cinnamomum camphora na dissipação de PAHs em solos da China contaminados com diesel

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ABSTRACT

The ongoing urbanization has led to worldwide increase of diesel consumption resulting in several environmental problems like air, water and soil pollution. Diesel comprises polycyclic aromatic hydrocarbons (PAHs), which, via various vectors like insufficient combustion, accidents, etc., are subsequently deposited in the soil because of their hydrophobicity and low water solubility. Uncontaminated agricultural or urban soils are of great importance as they have a direct impact on food security and human health. Sixteen of the PAHs have been listed by the United States Environmental Protection Agency as 'priority pollutants' because of their mutagenic and carcinogenic properties. The removal of PAHs from the environment through phytoremediation is a growing concern and scientific interest. The main objective of this study was to investigate the phytoremediation capacity of the evergreen tree species *Cinnamomum camphora* for the dissipation and degradation of several PAHs of different chemical structure. A pot experiment was established with artificially diesel contaminated soil from Changsa, China. The experimental design included three different diesel contents with and without tree-plantation. An extraction method and an HPLC separation method with different detectors was developed and applied for the analysis of soil samples. The analytical results revealed that *Cinnamomum camphora* enhances removal of selected PAHs from the contaminated soil with reduction of 91.1% to 98.8% as well as dissipation of persistent PAHs with 4 total and aromatic rings. However a confirmative study is suggested to understand whether the dissipation effect is due to rhizosphere bacteria or combined effect of several factors.

RESUMEN

Las crecientes actividades urbanísticas han incrementado el consumo mundial de diesel, dando lugar a numerosos problemas medioambientales relacionados con la contaminación del aire, el agua y el suelo. El diesel está compuesto de hidrocarburos policíclicos aromáticos (PAHs) que, a través de varios mecanismos como insuficiente combustión, accidentes, etc., se depositan posteriormente en el suelo debido a su hidrofobicidad y su baja solubilidad en agua. Los suelos agrícolas o urbanos no contaminados son de gran importancia ya que tienen un impacto directo sobre la seguridad alimenticia y la saludo humana. Dieciséis hidrocarburos policíclicos aromáticos han sido catalogados por la Agencia de Protección Medioambiental de los Estados Unidos como "contaminantes prioritarios" debido a sus propiedades mutagénicas y cancerígenas. La eliminación de los PAHs del medio ambiente a través de la fitorrecuperación es una preocupación creciente y objeto de interés científico. El principal objetivo de este estudio fue investigar la capacidad para la fitorrecuperación de especies arbóreas de Cinnamomum camphora para la disipación y degradación de varios PAHs con diferente estructura química. Se realizó un experimento en maceta con un suelo de Changsa, China, contaminado artificialmente con diesel. El diseño experimental incluyó tres contenidos distintos en diesel con plantación arbórea y sin ella. Para el análisis de las muestras de suelo se llevó a cabo un método de extracción y un método de separación con HPLC con distintos detectores. Los resultados analíticos revelaron que la Cinnamomum camphora mejora la eliminación de PAHs selectivos del suelo contamiando, con una reducción

comprendida entre 91,1% y 98,8% acompañada de una disipación de PAHs persistentes con cuatro anillos aromáticos. Sin embargo, sería necesario realizar más estudios que permitan comprender si el efecto de disipación se debe a bacterias de la rhizosfera o al efecto combinado de diversos factores.

RESUMO

As crescentes atividades urbanísticas aumentaram o consumo mundial de diesel, dando lugar a numerosos problemas ambientais relacionados com a contaminação do ar, água e solo. O diesel é composto por hidrocarbonetos policíclicos aromáticos (PAHs) que, através de vários mecanismos tais como insuficiente combustão, acidentes, etc., se depositam posteriormente no solo devido à sua hidrofobicidade e baixa solubilidade em água. Os solos agrícolas ou urbanos não contaminados são de grande importância já que têm um impacto direto sobre a segurança alimentar e saúde humana. Dezasseis hidrocarbonetos policíclicos aromáticos foram catalogados pela Agência de Proteção Ambiental dos Estados Unidos como "contaminantes prioritários" devido às suas propriedades mutagénicas e cancerígenas. A eliminação dos PAHs do ambiente através de la fitorremediação é uma preocupação crescente e objeto de interesse científico. O principal objetivo deste estudo foi investigar a capacidade para a fitorremediação de espécies arbóreas de Cinnamomum camphora para a dissipação e degradação de vários PAHs com diferente estrutura química. Efetuou–se um ensaio em vasos com um solo de Changsa, China, contaminado artificialmente com diesel. O delineamento experimental incluiu três conteúdos diferentes de diesel com plantação arbórea e sem ela. Para a análise das amostras de solo utilizou–se um método de extração e um método de separação por HPLC recorrendo a diferentes detetores. Os resultados analíticos revelaram que a Cinnamomum camphora melhora a eliminação de PAHs seletivos do solo contaminado, com uma redução compreendida entre 91,1% y 98,8% acompanhada de uma dissipação de PAHs persistentes com quatro anéis aromáticos. Sem dúvida, que será necessário realizar mais estudos que permitam compreender se o efeito de dissipação se deve a bactérias da rizosfera ou ao efeito combinado de diversos fatores.

1. Introduction

The ongoing urbanization has led to a worldwide increase of diesel consumption resulting with several environmental problems like air, water and soil pollution. Diesel comprises approximately 40% n-alkanes, 40% iso- and cycloalkanes, 2% polycyclic aromatic hydrocarbons (PAHs) and a few percent of N-S- and O- heterocyclics (Zhang and Tao 2009). The major sources of PAHs in the environment arise from various forms of insufficient combustion (Valavanidis et al. 2008); instead of carbon being completely oxidized to carbon dioxide, hydrocarbon fragments are generated which can interact with each other to yield complex polycyclic structures (Sherma 1993).

PAHs are a group of highly lipophilic chemical compounds that are present ubiquitously in the environment as pollutants (Haritash and Kaushik 2009). They rapidly become associated with inorganic and organic suspended particles and are subsequently deposited in sediments and soils because of their hydrophobicity and low water solubility (Yu et al. 2009). Due to their persistent, toxic, mutagenic and carcinogenic properties, determination and quantification of PAHs in the environment have received much attention for over the past three decades (International Agency for Research in Cancer 1983). Sixteen of the PAHs have been listed by the United States Environmental Protection Agency (US EPA) and European Union (EU) as "priority pollutants". Because of their potential toxic effects in human health and environment, several investigations regarding the removal of PAHs is very important and has attracted scientific interests.

Land use and soil properties may influence the accumulation and losses of PAHs in the soils (Maliszewska-Kordybach 1999). Although there is not much information on the relationship between soil characteristics and ad- and desorption of PAHs, it is known that organic matter content is the most important factor for the adsorption of PAHs (Khan et al. 2005).

Phytoremediation, the use of plants to clean up contaminated soil, has been described as a promising approach to remediate soils contaminated with persistent organic pollutants like PAHs (Chen et al. 2003; Huang et al. 2004; Phillips et al. 2009). In the last 20 years phytoremediation of PAHs has been assessed in a number of field experiments and green house studies and much attention has focused on the PAH dissipation and the relationship between plants and microbial communities in the rhizosphere (Aprill and Sims 1990; Binet et al. 2000; Denys et al. 2006; Paul et al. 2006; Vervaeke et al. 2003).

Grasses and leguminous plants have been found to be candidate species for effective rhizoremediation (Adam and Duncan 2003). Some studies also suggested tall fescue (*Festuca arundinacea*) and switch grass (*Panicum virgatum*), both degrading about 1.6 and 8.7% of pyrene in 190 days of incubation (Chen et al. 2003). Researchers have indicated that tropical grasses show special applicability for rhizoremediation most especially since they are tolerant to drought and low nutrient availability (Aprill and Sims 1990).

All the above cited studies reveal that plants can stimulate microbial activity, thus enhancing the PAH dissipation and PAH degradation can be influenced to a high extent. However, the remediation potential of trees with regard to PAHs has been rarely investigated. As trees provide an extensive root system with a wide range of depth, with large root surface area, planting of trees may be helpful to enhance degradation and removal of PAHs from contaminated soil. Several studies, based on the hypothesis that root exudates increase the rhizosphere microbial community, investigated

the significance of plant microbial interactions for the degradation of PAHs (Binet et al. 2000). According to Vervaeke et al. (2003) and Kevin et al. (2006), a willow stand and several North American tree species like red mulberry, hybrid poplar, sycamore and black locust have ability to improve PAH dissipation and degradation.

Cinnamomum camphora is an evergreen tree species that can be easily grown, is widely distributed and frequently used in urban and peri urban regions of subtropical zone in China. Some studies have shown that *Cinnamomum camphora* has capacity to enhance bioavailability of PAHs and lead to a decrease of persistent PAHs like Pyrene, Benzo(a)anthracene and Chrysene from contaminated soils (Fan et al. 2010). However, the study of Fan et al. (2010) suggested further confirmative studies. Hence the main objective of this study was to investigate the dissipation and degradation of several PAHs of different chemical structure in artificially diesel contaminated soils at three different concentrations and to enhance statistical significance. The present study can be considered as a confirmative study regarding the phytoremediation capacity of *Cinnamomum camphora*.

2. Materials and Methods

2.1. Site description

The study site was located in the southern part of Changsha city, China (E 112º48′, N 28º03′), at a distance of 600 m from the main road. The annual mean temperature of the study site is 16.8 ºC and the annual precipitation is approximately 1400 mm. The site belongs to the subtropical climatic zone.

2.2. Experimental setup

A pot experiment was established under field conditions of the study site. The soil used for the experimental purpose was agricultural top Fluvisol (OENORM L 1061 1986). An Ap horizon from Changsha was collected at a depth of 0 to 5 cm and was free from PAH and other pollutants. For plantation, seeds of *Cinnamomum camphora* were taken from a local nursery. The tree seedlings were selected according to their homogeneity in height and diameter. The tree seedlings were planted in untreated soil for 2 months to ensure their vitality before transplanting them to the pots containing differently PAH treated soil. For the contamination of soil, Chinese diesel No. 0 was used. The laboratory analysis of Chinese diesel No. 0 to determine its composition was not carried out for this study. However, the information was adopted from the study of Yu et al. (2009) that stated that the mass fraction of PAHs in diesel was 13.4% when analyzed by Waters 2695 HPLC. Before diesel treatment, the soil was dried and passed through 5 mm sieve and then contaminated with Chinese diesel No. 0 by mixing it in ratios of 2 g (D1), 10 g (D2) and 50 g (D3) diesel per kg dry soil respectively. Before planting the diesel treated substrates were left for 48 hours so as to ensure the sorption of PAHs to the soil material. A total of four replicates were prepared for each treatment and equivalent numbers of pots of differently contaminated soils (D1, D2 and D3) were left unplanted as control pots. For the control of tree performance and vitality, some tree seedlings were left in PAH free control soil .The pots were arranged completely randomized in a polycarbonate enclosure that permitted good air flow and sunlight penetration, but protected from incidental rainfall. The water content in the pots was checked and adjusted regularly with water to maintain about 50% of the water holding capacity so as to provide optimum growing conditions. 12 months after the experiment, all pots were destructively sampled.

2.3. General soil characteristics

The textural class of the soil used for experimental purpose was Loamy silt Fluvisol. The composition of the soil according to the particle size distribution analysis has been represented in Table 1 as follows.

General chemical characteristics of the soil samples, like pH value, cation exchange capacity, total nitrogen and organic carbon content were determined in accordance with the methods developed by Austrian Standards Institute OENORM L 1083, OENORM L1086, OENORM L 1082 and OENORM L 1080. The pH was analyzed after suspension with deionised water for 2 hours in the ratio of 1:5. The cation exchange capacity was analyzed by using 1M ammonium acetate solution as extractant. The

Table 1. Particle size distribution analysis (OENORM L 1061 1986)

Equivalent Diameter (mm)	Description	% w/w
2 - 0.63	coarse sand	0.1
0.63 - 0.2	medium sand	2.0
0.2 - 0.063	fine sand	15.0
0.063 - 0.02	coarse silt	38.0
0.02 - 0.002	medium silt	32.0
< 0.002	clay	12.9

cations were determined using a simultaneous ICP-AES (Perkin Elmer Optima 8036). The organic carbon content and the total nitrogen were analyzed by using Carlo Erba NA 1500 (CNS) analyzer.

2.4. Reagents and standards

Ultra residue reagent acetonitrile (ACN), HPLC/MS grade methanol (MeOH) and ultra HPLC/MS grade water (H_2O) were purchased from J.T. Baker. The Oasis HLB extraction cartridges of type HLB 6 cc were purchased from Waters Corporation USA. A Certified Reference Material BCR – 683 (CRM) of Beech Wood was purchased from the Institute for Reference Materials and Measurements (IRMM) which was used for the validation of the entire extraction and analytical procedure. The CRM contained six PAH compounds: Pentachlorophenol, Benzo(a)anthracene, Benzo(a)pyrene, Benzo(e)pyrene, Benzo(b)fluoranthene and Benzo(k)fluoranthene. The PAH mix 9 standard containing 16 PAHs, each of 10 ng/μl was purchased from Dr. Ehrenstorfer with the highest available purity. The 16 different PAHs present in PAH mix 9 were: Acenaphthene, Acenaphthylene, Anthracene, Benzo(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(g,h,i)perylene, Benzo(k)fluoranthene, Chrysene, Dibenzo(a,h)anthracene, Fluoranthene, Fluorene, Indeno(1,2,3,c,d)pyrene, Naphthalene, Phenanthrene and Pyrene . A stock solution of PAH mix 9 of 1000 μg l^{-1} was prepared by dilution in acetonitrile. The stock solution was stored in a dark place at room temperature, the CRM was stored at -20 °C and the calibration standards were prepared fresh on the day of analysis.

2.5. Soil sample preparation extraction and Chromatographic measurement of PAHs

Each soil sample was thoroughly mixed, homogenized and freeze dried for 24 h, by using Christ Alpha 1-2 LD, gently crushed in a ceramic mortar and sieved (< 2 mm). A total of three replicates of each soil sample were made for extraction followed by the analytic determination.

The soil samples were prepared in three different steps as follows:

• *Extraction:* In this step, the soil sample was mixed and was shaken with organic solvent and was ultrasonicated to ensure proper agitation of organic solvent into all relevant surfaces of soil matrix. It was followed by vaporization for the gentle removal of solvents from the soil by evaporation (Figure 1).

• *Solid Phase Extraction (SPE):* This step was carried out for the retention, cleanup and elution of the sample. It included pre-treatment i.e. extraction of samples with polar solvents like methanol; conditioning of SPE sorbent cartridges by using methanol to obtain reproducible results as well as for the penetration of highly aqueous solvent into the hydrophobic surface of the sorbent; loading of samples into SPE cartridges and in this step, matrix interferences are retained on the sorbent; a washing step so as to remove the unwanted matrix components that remain from the loading step and elute step in which a strong solvent (acetonitrile) was introduced to elute the analytes of interest (Figure 1).

• Chromatographic measurement: The chromatographic measurement of the extracted samples was done by the use of High Performance Liquid Chromatography (HPLC) so as to study the chromatograms of different PAHs.

Calibration of HPLC was carried out by the preparation of five different calibration standards of 20 μg l^{-1}, 50 μg l^{-1}, 100 μg l^{-1}, 200 μg l^{-1} and 500 μg l^{-1} from the stock PAH mix 9 standard of 1000 μg l^{-1} with subsequent dilution with acetonitrile. The chromatographic separation was achieved using an Agilent Eclipse PAH 1.8 μm (solid material), 4.6 × 50 nm column from Agilent Technologies USA was used with an acetonitrile gradient from 60 to 98% at a flow rate of 0.8 ml min^{-1}. The column was thermostated at 30 °C; the injection volume was 5 μl. The detection and quantification was carried out with fluorescence detector (FLD) and UV detector
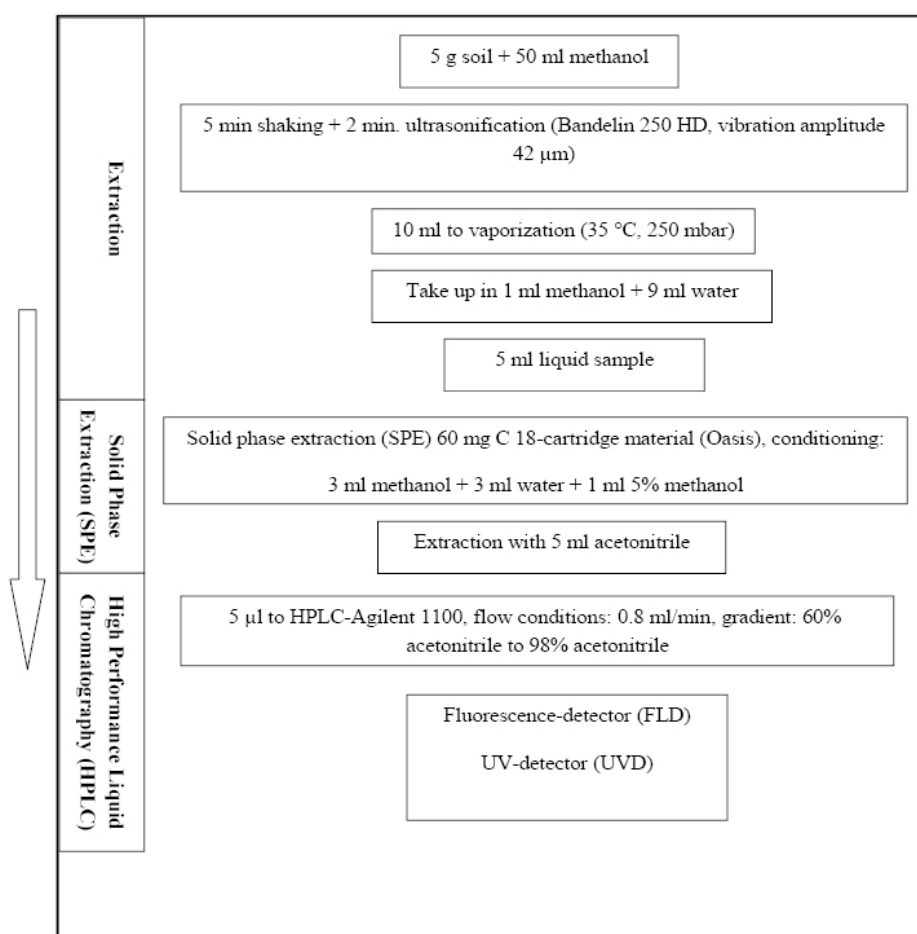
Figure 1. Flow chart for sample extraction, clean up, enrichment and analytical procedure.

connected in line. Figure 1 represents the flow chart for the soil sample extraction, clean up, enrichment and the analytical procedure for the determination of PAHs in soil.

2.6. Recovery experiments

To test the validity and robustness of the entire applied methodology, the recovery was determined by the extraction of Certified Reference Material (CRM) of beech wood by following the same procedure for the extraction of soil samples (Figure 1). As the CRM of soil for PAHs was not available, the nearest possibility of CRM of beech wood was used for the research purpose. However, none of the PAHs present in

the CRM were found in the studied soil samples. The linearity, correlation factors (R^2), relative standard deviation, limit of quantification (LOQ), limit of detection (LOD) were calculated. 3 replicates were analyzed for each concentration.

2.7. Statistical Analysis

The data were analyzed by using SPSS IBM statistics version 21. One way Anova was used for the comparison of the analytical data.

3. Results and Discussion

The recovery of the compounds present in CRM was found to be 47.05-94.6% (Standard deviation between 0.09% and 0.77%). All the compounds under study were found to be linear over five concentrations between 20 µg l^{-1} and 500 µg l^{-1} with correlation factors R^2 between 0.996 and 0.999. Three replicates of each concentration were measured. The LOQ of the analyzed compounds was between 0.15 µg kg^{-1} and 0.43 µg kg^{-1}. The highest LOQ value was 0.43 µg kg^{-1} for Benzo(a)pyrene while the lowest value of 0.15 µg kg^{-1} was found for Benzo(k) fluoranthene. The values of recovery, LOD, LOQ and retention times are represented in Table 2.

Table 2. The values of recovery, LOD, LOQ and retention times

	Certified value (mg kg^{-1})	Measured value (mg kg^{-1})	Recovery (%)	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Retention time
Benz[a]anthracene	6.5	6.15	94.6	0.07	0.21	10.423
Benzo[a]pyrene	3.4	1.6	47.05	0.14	0.43	13.037
Benzo[b]fluoranthene	5.8	3.15	54.31	0.07	0.21	12.357
Benzo[k]fluoranthene	2.58	1.625	62.98	0.05	0.15	13.211

3.1. Results of the soil analyses

The results obtained from the general soil characteristics study are represented in Table 3 as follows:

Table 3. The values of different soil characteristics parameters

Parameters	Unit	Mean	STDEV
pH		5	0.03
Cation Exchange Capacity	cmol kg^{-1}	7.53	2.52
Total Nitrogen	g kg^{-1}	0.33	0.32
Organic Carbon	g kg^{-1}	17.2	0.21

The PAHs analytical results are presented as mean values of triplicate determinations for each treatment i.e. 2g kg^{-1}, 10 g kg^{-1} and 50 g kg^{-1} and at each time interval: after 48 h, after 12 months without plantation and after 12 months with plantation.

The analytical results revealed that the diesel contaminated soil samples contained the following seven PAHs: Acenaphthene, Anthracene, Fluoranthene, Fluorene, Naphthalene, Phenanthrene and Pyrene. According to the analytical results, the residual PAH contents in soils at each contamination

level i.e. 2g kg^{-1}, 10 g kg^{-1} and 50 g kg^{-1} were found to be significantly (P < 0.01) lower in planted soils as compared to the unplanted ones. However, due to time, a significant loss of PAHs in the three different contamination levels was observed in the soils without plantation. A similar result was obtained in a pot experiment that was conducted to investigate the dissipation of PAHs in the rhizosphere of ryegrass where significant dissipation of PAHs was observed in pots without plants (Binet et al. 2000). The authors believed that the reason for this dissipation could be due to growth chamber effect. Another study carried out to assess the rhizoremediation of long term PAH contaminated soils by the plantation of *Miscanthus giganteus* showed that there was significant dissipation of 3, 4 and 5 rings PAH in unplanted soils. The authors believed that the reasons for this dissipation could have been due to leaching of some PAHs during the handling of soil samples and microbial activity of soil microflora (Techer et al. 2012). Figure 2 represents the dissipation of total PAHs calculated as the sum of mean values seven PAHs at three different diesel contamination levels.
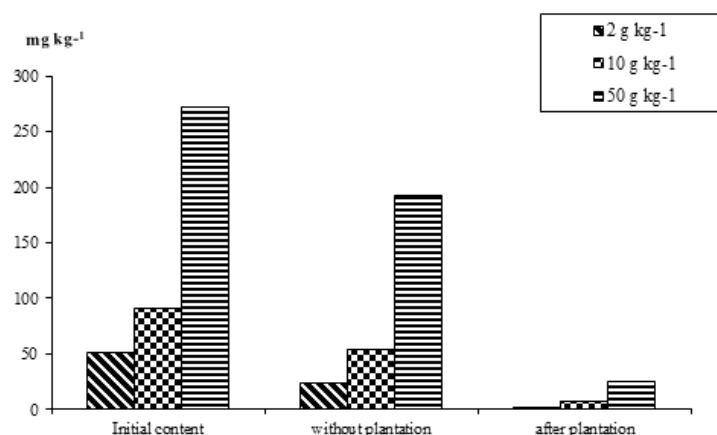

Figure 2. Dissipation of total PAHs at three different contamination levels after different time periods.

The total PAH dissipation for the soil at the contamination level 2 g kg^{-1} was 55.3% for unplanted and 99.1% for planted, at contamination level 10 g kg^{-1} it was 40.4% for unplanted and 92.0% for planted and at contamination level 50 g kg^{-1} it was 29.4% for unplanted and 91.1% for planted soils. According to these data, there was a decrease of PAHs in planted soils at all three contamination levels when compared to the initial diesel treatment level. This shows that the microbial population in the rhizosphere of *Cinnamomum camphora* is successful in detoxifying PAHs. A selective enrichment of the bacterial populations that degraded organic compounds in the rhizosphere of *Medicago sativa* and *Sesleria varia* was observed by Nichols et al. (1997) as well. However, these higher percentage of PAH losses could have been attributed by the combined effect of volatilization, leaching, microbial biodegradation and other factors.

Although there was a clear difference in the content of seven individual PAHs in soils related to the contamination levels, only the four main compounds that contributed a large extent to soil contamination are presented in Figures 3a to d.
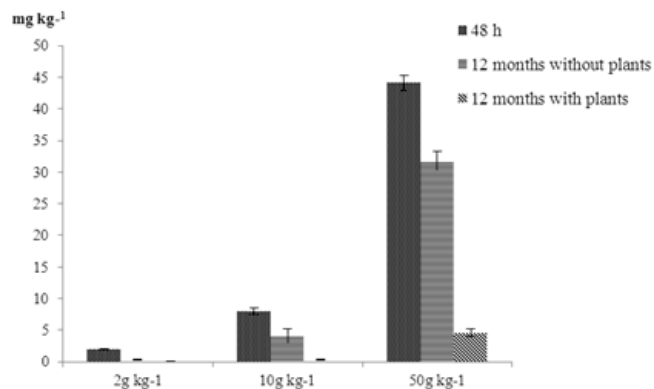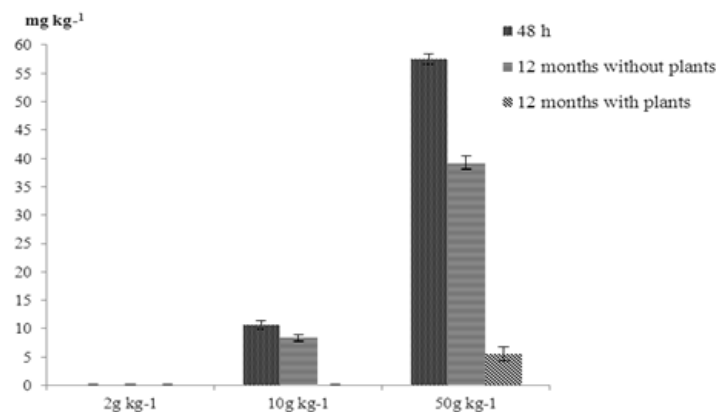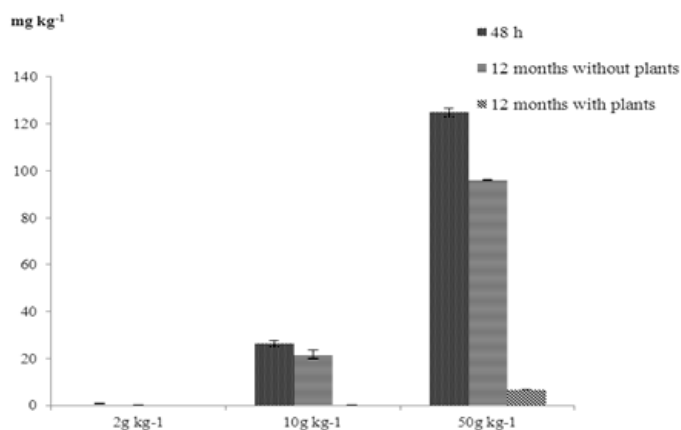
Figure 3a. Acenaphthene.

Figure 3b. Phenanthrene.

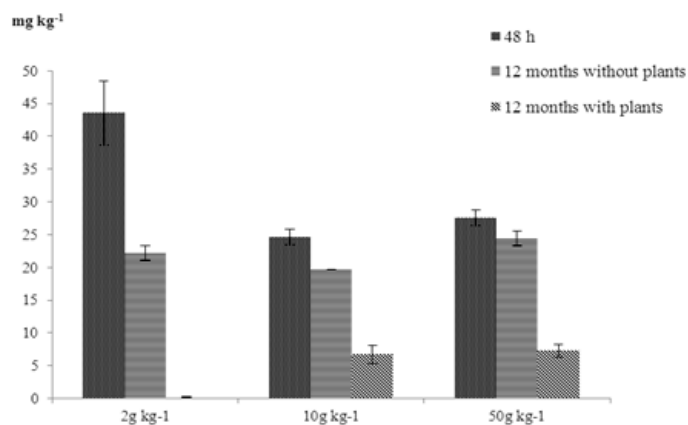
Figure 3c. Fluoranthene.

Figure 3d. Pyrene.

Figure 3. Dissipation of PAHs (**a**) Acenaphthene (**b**) Phenanthrene (**c**) Fluoranthene (**d**) Pyrene at different treatment levels.

As presented in Figure 3 (a-d), for all the four compounds a significant reduction of PAHs at all treatment levels was observed in planted soils (P < 0.01). However some reduction can also be seen in soils without plants but the effect of *Cinnamomum camphora* is more pronounced and highly significant in the planted soils. In the lowest contamination level of 2 g kg^{-1}, the degradation of PAHs in planted and unplanted soil was almost the same for the compounds Acenaphthene, Phenanthrene and Fluoranthene. An effect of *Cinnamomum camphora* was not detectable for these compounds. For the lowest PAH treatment level no such enhancement was detectable. However, a drastic reduction was observed in the compound Pyrene (Figure 3d) even at the lowest contamination level of 2 g kg^{-1}.

The percentage reduction of the four selected PAHs in planted and unplanted soils at three contamination levels are listed in Table 4.

Table 4. Percentage reduction of four selected PAHs in planted and unplanted soil
12 months after the experiment

| | Percentage reduction of PAHs in soils | | | | | |
| | With plants | | | Without plants | | |
	2 g kg^{-1}	10 g kg^{-1}	50 g kg^{-1}	2 g kg^{-1}	10 g kg^{-1}	50 g kg^{-1}
Acenaphthene	93.9±1.35	96.6±1.14	89.6±3.35	82.2±3.54	48.6±0.22	27.9±0.72
Phenanthrene	48.5±3.26	98.5±0.56	90.4±1.12	30.8±2.68	21.1±1.21	31.7±1.13
Fluoranthene	99.4±1.12	99.5±0.15	94.6±2.01	86.9±3.12	17.3±0.86	22.9±1.51
Pyrene	99.5±0.14	72.9±3.05	73.7±3.10	49.0±4.20	20.3±1.13	11.4±0.53

A significant reduction of the four selected PAHs was observed at all treatment levels. However at treatment level 2 g kg^{-1}, a significant reduction of Acenaphthene and Fluoranthene was observed in planted and unplanted soils. As Acenaphthene is a PAH with 3 aromatic rings, the decomposition could have been relatively high in unplanted soil as well because of volatility of light PAHs. Higher dissipation rates were observed for persistent PAHs with 4 total rings like Fluoranthene and 4 aromatic rings like Pyrene for the planted soil, thereby showing the efficiency of *Cinnamomum camphora* in phytoremediation. This is in agreement with a study of Erickson et al. (1993), where light PAHs with 3 aromatic rings were degraded easily while heavy 4 ring PAHs were more persistent and degraded slowly without phytoremediation. A drastic difference in the dissipation rates have been observed in planted and unplanted soils at contamination levels 10 g kg^{-1} and 50 g kg^{-1} for all four selected PAHs. The reason for low dissipation rate in unplanted soil at these levels could be due to inhibition of activity of degrading microorganisms present in soil at too high PAH contents (Huang et al. 2004).

4. Conclusions

The present study showed that *Cinnamomum camphora* was able to dissipate the studied PAHs, showing that this tree species is able to accelerate the dissipation of several PAHs including persistent ones with 4 total and aromatic rings. Hence, our result agrees with the outcome of Fan et al. (2010) and suggests that the remediation of diesel contaminated soils can be attributed by plantation of *Cinnamomum camphora* for the remediation of some PAHs. Plants may contribute to the dissipation of PAHs by an increase in microbial numbers, improvement of physical and chemical soil conditions, increased humification and adsorption of pollutants in the rhizosphere, but the impact of each process needs to be clearly investigated. Hence, the authors suggest for a continual study to confirm whether the removal efficiency of PAHs is due to the rhizosphere effect or combined effect of several factors including the detailed study of contaminated soil, roots and shoots of *Cinnamomum camphora*.

5. Acknowledgements

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