Journal of Hazardous Materials 168 (2009) 760–764

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

# Evaluation of the phytoremediation potential of four plant species for dibenzofuran-contaminated soil

Yanxu Wang[a,b,*], Hiroshi Oyaizu[a,b]

[a] Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan
[b] Biotechnology Research Center, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

ARTICLE INFO

*Article history:*
Received 8 September 2008
Received in revised form 17 December 2008
Accepted 16 February 2009
Available online 25 February 2009

*Keywords:*
Phytoremediation
Herbaceous plants
Rhizosphere
Dibenzofuran

ABSTRACT

In this experiment, three grasses, bermuda grass (*Cynodon dactylon*), bent grass (*Agrostis palustris* Huds.), lawn grass (*Zoysia japonica*), and a shallow-rooted legume, white clover (*Trifolium repens* L.) were planted into uncontaminated soil and dibenzofuran (DBF)-contaminated soil. The germination rates of all plants were investigated using contaminated soils to evaluate their sensitivities to DBF. During 2 months of growth, the root biomass and heterotrophic microbial numbers were measured in order to evaluate the potential of remediation. Furthermore, the number of DBF-degrading bacteria was counted to evaluate plants that enhance the microbial DBF degradation potential in contaminated soil. The DBF-removal performance of four plant species was also compared. Regardless of the contamination of DBF, white clover had not only the highest root biomass, but also the highest DBF-degrading bacterial numbers compared to those of the other three grasses. Moreover, white clover-planted contaminated soil exhibited the highest rate of DBF removal among all tested plants. These results suggest that microbial populations capable of degrading DBF were selectively increased by the addition of DBF in the rhizosphere, and also indicate that the presence of plants significantly enhances the reduction of DBF in soils. Based upon these results, white clover was selected for the further investigation of the phytoremediation of dioxin-contaminated soil.



## 1. Introduction

Phytoremediation has been recognized as an alternative for the removal of organic pollutants from soil in comparison with physicochemical remediation technologies due to its potentially lower cost and suitability for applications that require sustenance and low maintenance [1]. The first step in optimizing the phytoremediation of organic contaminants is finding the plant species from a vast array of species. The selection of an appropriate set of plants that are adapted to the exact site conditions and most capable of increasing the contaminant-degradation potential of the soil microbial community is crucial for the successful application of this technology. As an important component of phytoremediation, plant selection should be performed according to the needs of the application and the contaminants of concern. The selected plant species should possess characteristics that enable them to grow on contaminated sites. Simultaneously, they should be able to establish microbial associations that facilitate the degradation of contaminants.

A variety of plant species have been investigated and successfully used for the remediation of organic contaminants. Early indications of the potential use of plants for the managed removal of organic contaminants from soil came from observations of the enhanced dissipation of pesticides under rhizosphere environments compared to dissipation in root-free environments [2]. As the search for remediation alternatives has intensified, the contaminant list has also grown to include industrial chemicals such as total petroleum hydrocarbons (TPHs) [3], polycyclic aromatic hydrocarbons (PAHs) [4,5], polychlorinated biphenyls (PCB) [6], trinitrotoluene (TNT) [7], and trichloroethylene (TCE) [8].

Peterson et al.'s [9] exploration of the potential use of alfalfa (*Medicago sativa* L.) for TNT remediation points to a new focus upon the use of forage crops for addressing organic contamination in soil. Forage crops seem to be an ideal choice for phytoremediation because they have well-established cultural practices, which should facilitate their managed manipulation for accelerated contaminant destruction and removal. Schwab and Banks [10] also reported the enhanced degradation of pyrene in tall fescue- (*Festuca arundinacea*), Sudan grass- (*Sorghum bicolor* L.), Switch grass- (*Panicum virgatum* L.), and alfalfa-planted soils compared to unplanted soils. Plant selection was found to be important in the field study by Qiu et al. [11]. Verde Kleingrass, a selection of *Panicum coloratum*, proved to be superior to 11 other tested warm-season grasses at a

* Corresponding author at: Biotechnology Research Center, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan. Tel.: +81 3 5841 7535; fax: +81 3 5841 2408.
*E-mail address:* awangyan@mail.ecc.u-tokyo.ac.jp (Y. Wang).

0304-3894/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2009.02.082

location in Texas, giving final PAH levels one to two orders lower than those of the other plants at the end of the experiment.

Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are widespread and persistent contaminants in a wide range of ecosystems. Studies have demonstrated that the long-term accumulation of PCDD/F in soils and sediments is related to their physicochemical characteristics [12]. In contaminated soil, most PCDD/F were found to distribute spatially in the top layer (0–10 cm), owing to their high affinity to the soil matrix. As a result, these compounds could be most amenable to enhanced rhizodegradation, because their distribution in soil is usually restricted to the plant root zone depths. However, little work has been done to determine which plant species might optimize the degradation of PCDD/F.

On account of the similarity of metabolism between dibenzofuran (DBF) and dibenzo-*p*-dioxin (DD) in microorganisms, DBF was used as model substrate in our study. We investigated the germination, root biomass, and rhizosphere microbial biomass of four plant species with DBF-contaminated soil, determining the reduction of DBF from soil samples. The main aim of this study was to evaluate the potential of these plant species for the phytoremediation of DBF-contaminated soil.

## 2. Materials and methods

### 2.1. Plants

Four plant species – including two warm-season grasses, Bermuda grass (*Cynodon dactylon*) and lawn grass (*Zoysia japonica*), one cool-season grass, creeping bentgrass (*Agrostis palustris* Huds.), and a shallow-rooted legume, white clover (*Trifolium repens* L.) – were compared in terms of their ability for phytoremediation. The seeds of these plants were purchased from Sakata Seed Co. (Tokyo, Japan).

### 2.2. Soils

Uncontaminated soils (pH 6.17, 66% sand, 31% silt, 3% clay) were collected with a shovel from a garden at the University of Tokyo, Japan. Soils were air-dried and sieved through a 2 mm sieve. A stock solution was prepared by dissolving DBF in acetone to obtain a concentration of 20 g $l^{-1}$. The contaminated soil was prepared by adding the stock solution and mixing thoroughly to ensure uniform distribution of DBF, then was allowed to stand for 24 h, with intermittent stirring to evaporate the acetone completely. The soil was contaminated with DBF to a concentration of approximately 1000 mg $kg^{-1}$.

### 2.3. Seed germination test and pot experiments

A seed germination test was performed in order to investigate the stress tolerance of plants to the contaminant. Prior to their use in the test, the seeds were inspected and any damaged seeds were removed, then seeds of a similar size were selected for the experiments. The seeds were surface-sterilized by soaking in 70% ethanol for 2 min and sodium hypochlorite solution (1% available chlorine) for 5 min, followed by three washes with sterile distilled water. The test consisted of five replicates, and a total of 100 seeds were employed. After 7 days' incubation, the number of germinated seeds was counted and the germination rate was calculated as

$$\text{Germination rate } (\%) = \frac{\text{number of germinated seeds}}{\text{number of sowed seeds}} \times 100.$$

After surface-sterilization, five seeds were planted in a plastic pot as one replicate, containing uncontaminated soil or DBF-contaminated soil, and five such replicates were used for each plant. Unplanted pots were used as controls. Plants were grown in a growth chamber maintained at 26 °C with 16 h of daylight, and harvested at 60 days. Root biomass was evaluated by root fresh weight and dry weight measured after drying at 100 °C for 24 h.

The dilution plate method was used to estimate the microbial number in the soil. Soils were sampled from the rhizosphere and the control pots. A 1 g soil (wet weight) was ground in a mortar and serially diluted (10-fold dilution). Diluted suspensions were spread onto three replicate one-tenth-strength tryptic soy agar (TSA) and incubated at 30 °C. Heterotrophic bacteria were counted after 72 h. To quantify the effect of the rhizosphere on the heterotrophic bacteria and DBF addition, the R/S ratio (rhizosphere/bulk soil populations) was calculated by dividing the microbial number in the rhizosphere soil by the corresponding value for the bulk soil [13].

The number of DBF-degrading bacteria was counted as described previously [14]. Briefly, dilutions were spread onto three replicate Luria–Bertani (LB) plates. After 48 h of incubation at 30 °C, the colonies were assayed for extradiol dioxygenase activity by spraying an aqueous solution of 2,3-dihydroxybiphenyl (DHB) onto the plates, with colonies exhibiting a yellow color selected as DBF-degrading bacteria. For the colony assay, autoclaved soil was used as a control.

### 2.4. Removal of DBF

To evaluate the removal of DBF from the soil, four treatments were performed in this experiment, and each treatment consisted of five replicates:

1. Sterile DBF-contaminated soil without plants;
2. Non-sterile DBF-contaminated soil without plants;
3. Sterile DBF-contaminated soil with plants only;
4. Non-sterile DBF-contaminated soil with plants.

After 60 days of growth, 5 g soils were extracted with an equal volume of ethyl acetate by vortexing for 1 min, and the remaining DBF in the ethyl acetate phases was determined by gas chromatography–mass spectrometry (GC–MS). GC–MS analysis was performed on a Shimadzu GC–MS QP5050 equipped with a 30 m × 0.25 mm × 0.25 μm capillary column J&W DB-1. Three such replicates were used for each pot.

### 2.5. Statistical analysis

In order to examine the significant differences among means, analysis of variance (ANOVA) was performed using the software StatView (Abacus Concepts, Inc. Berkeley, CA). A probability of 0.05 or lower was considered to be significant.

## 3. Results and discussion

### 3.1. Seed germination and root biomass

A pre-test was performed in order to investigate the growth of those plant species with DBF-contaminated soil at three concentrations: 200, 500, and 1000 mg $kg^{-1}$. Non-contaminated soil was used as a control. As a result, no growth inhibition was observed in all plants at DBF concentrations 200 and 500 mg $kg^{-1}$ (data not shown). Hence, the DBF concentration of 1000 mg $kg^{-1}$ was selected for further investigation in our study. In uncontaminated soil, Bermuda grass and lawn grass showed the greatest germination rate (92% and 90% after 7 days) compared to the other species, bent grass (87%) and white clover (88%). There were no significant differences ($p < 0.05$) between these four species of plant (Table 1).

In the DBF-contaminated soil, the germination rates of all three grasses were significantly depressed compared to those in the

**Table 1**
Seed germination of four plant species cultured with uncontaminated and DBF-contaminated soils.

| Plant | Germination (%)[a] | |
|---|---|---|
| | Uncontaminated soil | DBF-contaminated soil |
| Bermuda grass | 92 ± 2.2 | 73 ± 1.1 |
| Bent grass | 87 ± 1.6 | 63 ± 1.3 |
| Lawn grass | 90 ± 2.3 | 77 ± 2.1 |
| White clover | 88 ± 1.3 | 86 ± 1.5 |

[a] Mean value ± S.D.

**Table 2**
Plant root biomass in uncontaminated and DBF-contaminated soils.

| Plant | Uncontaminated soil (g/pot[a]) | | DBF-contaminated soil (g/pot[a]) | |
|---|---|---|---|---|
| | Fresh weight | Dry weight | Fresh weight | Dry weight |
| Bermuda grass | 5.80 ± 0.71b | 1.12 ± 0.30ab | 5.47 ± 0.85c | 1.03 ± 0.27b |
| Bent grass | 4.68 ± 0.73a | 0.93 ± 0.17a | 3.52 ± 0.43a | 0.61 ± 0.13a |
| Lawn grass | 5.01 ± 0.44a | 1.06 ± 0.11ab | 4.38 ± 0.46b | 0.97 ± 0.19ab |
| White clover | 7.12 ± 0.93c | 1.64 ± 0.37b | 7.07 ± 0.88d | 1.60 ± 0.23c |

Values in columns followed by different letters indicate significant differences at $p < 0.05$.
[a] Mean value ± S.D.

uncontaminated soil. Although the germination rate of white clover in DBF-contaminated soil showed a tendency to decline in contrast to the uncontaminated soil, no significant difference was observed. These results indicated that the germination of white clover remained nearly unaffected by the presence of DBF, and white clover showed more tolerance to DBF than those three grasses.

As an important stage in plant growth, germination is particularly sensitive to contaminants [15]. The germination rate possesses several advantages, such as sensitivity, simplicity, and low cost. These advantages made them applicable for developing data on the acute toxicity of chemical substances. On the other hand, the toxicity assessment of chemical substances serves as a tool for evaluating the stress tolerance of plants. Plant toxicity assays are particularly relevant when phytotoxic contaminants are present in soil, and have been designed to evaluate the phytotoxic effects of bioremediated explosives [16].

After 60 days of growth, the root biomass was measured in order to evaluate the efficiency of remediation. Both with and without the contamination of DBF, white clover had the highest root fresh biomass and dry biomass compared with those of the other three grasses (Table 2). This suggests a fast growth rate of white clover. The growth rate of a plant will directly affect the rate of remediation. A fast growth rate will minimize the time required to reach the desired root density, lateral extension, and root biomass. For rhizodegradation, a large root biomass is desired for an increased production of exudates and enzymes. Compared with the deep root system of wood plants, herbaceous plants may be more appropriate in order to clean up the PCDD/F pollution due to their root morphology.

**Table 4**
The R/S ratios (rhizosphere/bulk soil) for microbial populations.

| Plant | R/S ratio[a] | |
|---|---|---|
| | Uncontaminated soil | DBF-contaminated soil |
| Bermuda grass | 3.1 ± 0.4a | 2.5 ± 0.2a |
| Bent grass | 2.5 ± 0.5a | 1.9 ± 0.3a |
| Lawn grass | 2.7 ± 0.3a | 2.4 ± 0.3a |
| White clover | 8.3 ± 0.7b | 7.6 ± 0.5b |

Values in columns followed by different letters indicate significant differences at $p < 0.05$.
[a] Mean value ± S.D.

Huang et al. [17] showed that the germination was severely depressed after investigating the effect of total petroleum hydrocarbons in soil upon the germination of *F. arundinacea*, in agreement with our results. Smith et al. [18] evaluated different grasses and legumes in freshly PAH-contaminated soil and observed that the germination was unaffected. Reynoso-Cuevas et al. [19] studied the effect of a hydrocarbon mixture (HCM) of three PAH on the germination, growth, and survival of four grasses. Their results showed that germination was not affected for any assayed concentration; however, the length of the stems and roots decreased when HCM increased and the survival of the four species also decreased. The differences found could be attributed to the fact that the growth of plants is dependent upon many environmental factors such as soil conditions, the availability of nutrients, water, etc. Also, studies of plant root growth have shown that they can only survive contamination up to a certain critical toxic concentration in the soil, which varies according to plant species [20].

### 3.2. Microbial enumeration

Rhizosphere heterotrophic microbial numbers were measured after 60 days of growth. Both with and without the contamination of DBF, white clover had the highest rhizosphere microbial population compared with those of the other three grasses (Table 3). As a legume, white clover can fix atmospheric nitrogen and improve the nutrient status of the soil. Consequently, the rhizosphere microbial populations were stimulated. Further, in both uncontaminated soil and DBF-contaminated soil, the heterotrophic microbial numbers in the rhizosphere soil of white clover were about one order of magnitude higher than those in bulk soil. Higher microbial populations were found in the rhizosphere soil than in the bulk soil, presumably in response to the presence of readily available carbon sources and growth factors found in the form of root exudates and sloughed root cells [21].

A vast number of species of microorganisms are present in the rhizosphere, and their numbers generally decrease as the distance from the root increases. The R/S ratio provides an estimate of how strongly the rhizosphere affects a particular microorganism. This relationship can also differ with plant species. The R/S ratio is especially helpful in determining the rhizosphere competence, which is the ability of a microorganism to colonize the rhizosphere. A microorganism with good rhizosphere competence is a good

**Table 3**
Microbial numbers in bulk and rhizosphere soils (mean value ± S.D).

| Plant | Uncontaminated soil (log CFU/g soil[a]) | | DBF-contaminated soil (log CFU/g soil[a]) | |
|---|---|---|---|---|
| | Bulk | Rhizosphere | Bulk | Rhizosphere |
| Bermuda grass | 6.59 ± 2.11a | 7.08 ± 1.96ab | 6.62 ± 0.74a | 7.02 ± 1.80a |
| Bent grass | 6.53 ± 1.73a | 6.93 ± 2.37a | 6.76 ± 1.22a | 7.05 ± 2.64a |
| Lawn grass | 6.48 ± 2.42a | 6.91 ± 1.81a | 6.73 ± 2.16a | 7.01 ± 2.37a |
| White clover | 6.57 ± 1.83a | 7.29 ± 2.17b | 6.83 ± 1.77a | 7.68 ± 2.75b |

Values in columns followed by different letters indicate significant differences at $p < 0.05$.
[a] CFU = colony-forming unit.

**Table 5**
Microbial numbers of DBF-degrading bacteria in bulk and rhizosphere soils.

| Plant | Uncontaminated soil (log CFU/g soil[a]) | | DBF-contaminated soil (log CFU/g soil[a]) | |
|---|---|---|---|---|
| | Bulk | Rhizosphere | Bulk | Rhizosphere |
| Bermuda grass | ND[b] | ND | 2.02 ± 0.89b | 3.37 ± 0.940b |
| Bent grass | ND | ND | 1.57 ± 0.68a | 2.16 ± 0.77a |
| Lawn grass | ND | 1.37 ± .069a | 1.81 ± 0.70b | 3.33 ± 1.01b |
| White clover | ND | 1.68 ± 0.57b | 2.27 ± 0.92c | 4.13 ± 0.98c |

Values in columns followed by different letters indicate significant differences at $p < 0.05$.
[a] CFU: colony-forming unit.
[b] ND: not detected.

candidate for use as a microbial inoculant. A comparison of R/S ratios (Table 4) shows a lower R/S ratio in the DBF-contaminated soil than the uncontaminated soil. This might suggest that due to the addition of DBF, the microbial population that could not degrade DBF was inhibited. However, in Table 3, higher microbial numbers in DBF-contaminated soils compared to those in uncontaminated soils also imply a most effective selective enrichment of DBF-degrader populations in white clover rhizosphere. The data of the DBF-degrading bacteria enumeration (Table 5) strongly support that the number of DBF degraders increased in contaminated soil planted with tested plant species. This suggests that the selective enhancement of DBF degraders occurs in the rhizosphere.

### 3.3. Reduction of DBF from soils

Fig. 1 shows that the concentrations of DBF remained in the contaminated soils after 60 days' incubation. In soils, hydrophobic chemicals (logarithm of octanol–water partition coefficient, $\log K_{ow} > 3.0$) such as PCDD/F are very immobile, and strongly bound to the soil and the surface of roots. Therefore, PCDD/F transport within plants is considered to be unlikely [22,23]. The vapor pressure for dibenzofuran is 0.0175 mm/Hg at 25 °C, and its $\log K_{ow}$ varies between 3.18 and 4.12 [24]. Compared with the unplanted sterile soil, a slight depletion of DBF was observed in grass-planted sterile soil, mainly due to adsorption by the roots accepting the evaporation. This suggests that the grasses in the present study did not play a direct role in the dissipation of DBF. Interestingly, a significant reduction of DBF was observed in the sterile soil planted with white clover. To our knowledge, only a few plants have been reported to uptake dioxins from the soil using their roots, such as zucchinis (*Cucurbita pepo*), etc. [25]. The result presented here shows that white clover has the potential to remediate the contaminated soil.

In contrast with the unplanted sterile soil, the concentration of DBF in the unplanted non-sterile soil was significantly reduced by about 200 mg $kg^{-1}$ over a period of 60 days, suggesting active degradation by DBF-degrading microbes. The planted non-sterile soil showed a significant reduction in DBF compared to the unplanted non-sterile soil, with the exception of bent grass. Among these grass-planted non-sterile soils, the DBF residues of Bermuda grass-, lawn grass-, and bent grass-planted non-sterile soils were about 544, 560, and 676 mg $kg^{-1}$, respectively. There was no significant difference observed between Bermuda grass and lawn grass. However, the DBF remaining in the bent grass-planted non-sterile soil was significantly high compared to Bermuda grass- or bent grass-planted non-sterile soils. After 60 days' incubation, the DBF residue in the white clover-planted non-sterile soil was about 333 mg $kg^{-1}$, exhibiting the most significant decrease of DBF among these four plant species.

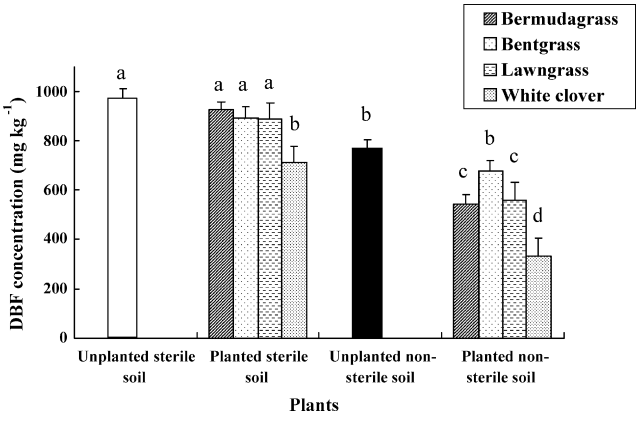


**Fig. 1.** Dibenzofuran removal in planted and unplanted soils at the end of 60 days' cultivation. Columns denoted by different letters are statistically different at $p < 0.05$.

It has long been known that microorganisms exist in a variety of tissue types within numerous plant species. Plant–microbe symbioses are ubiquitous in natural and most anthropogenically influenced soils. Previously, we have reported *Comamonas* sp. strains isolated from white clover roots capable of utilizing DBF as the sole carbon source. Members of the genus *Comamonas* are well known for their unavailability to utilize D-fructose and D-glucose as a sole carbon source. It is noteworthy that the *Comamonas* sp. strains isolated from white clover roots were able to utilize D-fructose and D-glucose, the major components of reducing sugars in the root exudates, suggesting an environmental adaption of root-colonizing bacteria [14].

The microbial transformation of organic compounds is usually not driven by energy needs, but it is necessary to reduce toxicity, for which microbes may have to suffer an energy deficit. Thus, the processes may be helped and driven by the abundant energy that is provided by root exudates. Such stimulation of soil microbial communities by root exudates also benefits plants through the increased availability of soil-borne nutrients and the degradation of phytotoxic soil contaminants. These results also indicate that plants and their associated rhizosphere microorganisms could be important in the remediation of soils contaminated with organic chemicals.

## 4. Conclusion

In conclusion, white clover grows fast and spreads easily due to the creeping stem system. Furthermore, white clover rhizosphere soil contained the highest microbial population compared to the other three grasses. Our results showed that microbial populations capable of degrading DBF were selectively increased by the addition of DBF in the rhizosphere. Based upon the results of this study, white clover was selected for further investigation of the degradation of chlorinated dioxins. Although the process of DBF degradation in clover or plant–microbe combination has not been revealed, we are confident that this study provides an alternative approach to remediating the soils contaminated with organic pollutants. The performance will be further evaluated, and the exact mechanisms involved in this interaction required further elucidation.

## References


[1] S.D. Cunningham, W.R. Berti, J.W. Huang, Phytoremediation of contaminated soils, Trends Biotechnol. 13 (1995) 393–397.
[2] E. Sandman, M.A. Loos, Enumeration of 2,4-D degrading microorganisms in soils and crop plant rhizospheres using indicator media: high populations associated with sugarcane (*Saccharum officinarum*), Chemosphere 13 (1984) 1073–1084.
[3] M.K. Banks, A.P. Schwab, R.S. Govindaraju, P. Kulakow, Field demonstration, in: S. Fiorenza, C.L. Oubre, C.H. Ward (Eds.), Phytoremediation of Hydrocarbon Contaminated Soil, Lewis Publishers, Boca Raton, FL, 1999, pp. 3–88.
[4] E.J. Joner, A. Johansen, A.P. Loibner, M.A. Dela Cruz, O.H. Szolar, J.M. Portal, C. Leyval, Rhizosphere effects on microbial community structure and dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked soil, Environ. Sci. Technol. 35 (2001) 2773–2777.
[5] S.D. Siciliano, J.J. Germida, K. Banks, C.W. Greer, Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial, Appl. Environ. Microbiol. 69 (2003) 483–489.
[6] K. Asai, K. Takagi, M. Shimokawa, T. Sue, A. Hibi, T. Hiruta, S. Fujihiro, H. Nagasaka, S. Hisamatsu, S. Sonoki, Phytoaccumulation of coplanar PCBs by *Arabidosis thaliana*, Environ. Pollut. 120 (2002) 509–511.
[7] P.L. Thompson, L.A. Ramer, J.L. Schnoor, Uptake and transformation of TNT by hybrid poplar trees, Environ. Sci. Technol. 32 (1998) 975–980.
[8] B.T. Walton, T.A. Anderson, Microbial degradation of trichloroethylene in the rhizosphere: potential application to biological remediation of waste sites, Appl. Environ. Microbiol. 56 (1990) 1012–1016.
[9] M. Peterson, G.L. Horst, P.J. Shea, S.D. Comfort, Proceedings of 10th Annual Conference on Hazardous Waste Research, 1995.
[10] A.P. Schwab, M.K. Banks, Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone, in: T.A. Anderson, J.R. Coats (Eds.), Bioremediation Through Rhizosphere Technology, American Chemical Society, Washington, DC, 1994, pp. 132–141.
[11] X. Qiu, T.W. Leland, S.I. Shah, D.L. Sorenson, E.W. Kendall, Chapter 14 field study: grass remediation of clay soil contaminated with polycyclic aromatic hydrocarbons, in: E.L. Kruger, T.A. Anderson, J.R. Coats (Eds.), Phytoremediation of Solid and Water Contaminants, ACS Symposium Series 664, American Chemical Society, Washington, DC, 1997, pp. 186–189.
[12] R.A. Hites, L.P. Brzuzy, Estimating the atmospheric deposition of polychlorinated dibenzo-*p*-dioxins and dibenzofurans from soils, Environ. Sci. Technol. 29 (1995) 2090–2098.
[13] H. Katznelson, The "rhizosphere effect" of mangels on certain microorganisms, Soil Sci. 62 (1946) 343–354.
[14] Y.X. Wang, A. Yamazoe, S. Suzuki, C.T. Liu, T. Aono, H. Oyaizu, Isolation and characterization of dibenzofuran-degrading *Comamonas* sp. strains isolated from white clover roots, Curr. Microbiol. 49 (2004) 288–294.
[15] M.K. Banks, K.E. Schultz, Comparison of plants for germination toxicity tests in petroleum-contaminated soils, Water Air Soil Pollut. 167 (2005) 211–219.
[16] T. Frische, Ecotoxicological evaluation of in situ bioremediation of soils contaminated by the explosive 2,4,6-trinitrotoluene (TNT), Environ. Pollut. 121 (2003) 103–113.
[17] X.D. Huang, Y. El-Alawi, J. Gurska, B.R. Glick, B.M. Greenberg, A multiprocess phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils, Microchem. J. 81 (2005) 139–147.
[18] M.J. Smith, T.H. Flowers, H.J. Duncan, J. Alder, Effects of polycyclic aromatic hydrocarbons on germination and subsequent growth of grasses and legumes in freshly contaminated soil and soil with aged PAHs residues, Environ. Pollut. 141 (2006) 519–525.
[19] L. Reynoso-Cuevas, M.E. Gallegos-Martínez, F. Cruz-Sosa, M. Gutiérrez-Rojas, In vitro evaluation of germination and growth of five plant species on medium supplemented with hydrocarbons associated with contaminated soils, Bioresour. Technol. 99 (2008) 6379–6385.
[20] H.-H. Liste, I. Prutz, Plant performance, dioxygenase-expressing rhizosphere bacteria, and biodegradation of weathered hydrocarbons in contaminated soil, Chemosphere 62 (2006) 1411–1420.
[21] E.A. Curl, B. Truelove, Rhizosphere in relation to plant nutrient and growth, in: D.R. Bommer, B.R. Sabey, G.W. Thomas, Y. Vaadia, L.D. Van Vleck (Eds.), The Rhizosphere, Springer, Berlin, Heidelberg, New York, 1986, pp. 167–190.
[22] A. Reischl, M. Reissinger, H. Thoma, O. Hutzinger, Uptake and accumulation of PCDD/F in terrestrial plants: basic considerations, Chemosphere 19 (1989) 467–474.
[23] E. Bacci, M.J. Cerejeira, C. Gaggi, G. Chemello, D. Calamari, M. Vighii, Chlorinated dioxins: volatilization from soils and bioconcentration in plant leaves, Bull. Environ. Contam. Toxicol. 48 (1992) 401–408.
[24] U.S. Environmental Protection Agency, Health Effects Assessment for Dibenzofuran, EPA/600/8-88/026. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, Cincinnati, OH, 1988.
[25] A. Hülster, J.F. Müller, H. Marschner, Soil–plant transfer of poly chlorinated dibenzo-*p*-dioxins and dibenzofurans to vegetables of the cucumber family (*Cucurbitaceae*), Environ. Sci. Technol. 28 (1994) 1110–1115.