Integrating Bioremediation and Phytoremediation to Clean up Polychlorinated Biphenyls Contaminated Soils

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***Abstract*—**This work involved the use of phytoremediation to remediate an aged soil contaminated with polychlorinated biphenyls (PCBs). At microcosm scale, tests were prepared using soil samples that have been collected in an industrial area with a total PCBs concentration of about 250 μg kg^{-1}. *Medicago sativa* and *Lolium italicum* were the species selected in this study that is used as "feasibility test" for full scale remediation. The experiment was carried out with the addition of a mixture of randomly methylated-beta-cyclodextrins (RAMEB). At the end of the experiment analysis of soil samples showed that in general the presence of plants has led to a higher degradation of most congeners with respect to not vegetated soil. The two plant species efficiencies were comparable and improved by RAMEB addition with a final reduction of total PCBs near to 50%. With increasing the chlorination of the congeners the removal percentage of PCBs progressively decreased.

***Keywords*—**contaminated soil, feasibility test, phytoremediation, polychlorinated biphenyls

I. Introduction

POLYCHLORINATED biphenyls (PCBs) are a class of organic compounds widely distributed in the environment. The main cause of their persistence in all environmental compartments is mainly due to their hydrophobicity, a feature that derives from their chemical structure: molecules of biphenyl in which the hydrogen atoms are replaced by chlorine [1]. The number of sites that can be attacked by chlorine atoms are at most 10, so there are 209 different possible isomers. PCBs are synthetic compounds and their industrial production began around 1929 [2]; typically they were produced as complex mixtures of congeners obtained by chlorination of biphenyls up to a certain weight percentage of chlorine, which varied from 40 to 60%. The problem related to organic contaminants of this type is very complex because of their spread, persistence and toxicity in the environment. They were found in water, soils and sediments, fish, birds and other animals and also in human tissues. Their degradation occurs very slowly and, being soluble in fats, they tend to accumulate along the food chain [3, 4]. In 1977 the U.S. Environmental Protection Agency has banned the direct discharge of these pollutants in waterways, and since 1979 has been banned their production and distribution. Mainly the mechanisms of disappearance of PCBs from soils and sediments are volatilization, leaching, degradation by microorganisms and photodegradation. These compounds are characterized by low water solubility and a high octanol water partition coefficient (K_{ow}), therefore, soils with a high content of lipophilic substances and organic material can retain them much more.

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Their mobility and bioavailability in the soil are very important to determine the real hazard and these factors will be influenced not only by the chemical and physical characteristics of the soil, but also by the time of contact, and then by the period of time elapsed from the polluting event. The molecular weight of these compounds, which depends on the degree of chlorination, is also very important to be considered. Heavier compounds will tend to bind strongly to the soil in a way, sometimes irreversible. Mechanisms of loss as leaching and volatilization affect above all PCBs with the lowest molecular weight.

In the soil many microorganisms exist that have the ability to degrade contaminants such as PCBs, producing less hazardous degradation compounds. Also plants play very important role in the degradation of organic contaminants in the soil. Vegetation may enhance biodegradation both with an indirect action by providing carbonaceous substrates and oxygen, and directly by absorbing contaminants. Phytoremediation, the use of plants for the remediation of contaminated soils and waters, is a viable alternative to other chemical and physical techniques since positive results were obtained with both inorganic contaminants [5, 6, 7, 8] such as salts, heavy metals, radioactive elements and organic contaminants including polycyclic aromatic hydrocarbons (PAHs) [9, 10, 11, 12, 13, 14] and polychlorinated biphenyls [15, 16, 17]. The aim of this work was to evaluate the efficiency of two plant species, *Medicago sativa* and *Lolium italicum*, in the remediation of an aged PCBs contaminated soil. The clean up process was carried out with the addition of cyclodextrins. Cyclodextrins are a family of oligosaccharides composed of a-(1,4) linked glucopiranose subunits well known in the environmental field, where are used in many different applications [18]. In this work it was employed a low cost technical grade mixture of randomly methylated-beta-cyclodextrins (RAMEB) that has been repeatedly successfully used to increase the removal of PCBs from soils [17, 19, 20, 21]. The soil has been collected in a contaminated industrial area in Italy characterized by PCBs concentrations of about 250 μg kg^{-1}.

II. Materials and Methods

A. Soils

The PCBs contaminated aged soils have been collected in a former industrial area in northern Italy. Soil samples were air dried and ground to pass through a 2 mm sieve before laboratory analysis. Soil pH was determined using a glass electrode in a soil/water ratio of 1:2.5, cation exchange capacity (CEC) was determined using barium chloride (pH 8.1), and texture by the pipette method according to SSSA methods of soil analysis [22]. Soil dehydrogenase activity was determined by the reduction of triphenyl tetrazolium chloride to triphenyl formazan, according to the procedure described by Chaofeng Shena et al. [17].

B .Microcosm Test

A feasibility test, at microcosm scale, was carried out with the aim to evaluate the efficiency of two plant species, *Medicago sativa* and *Lolium italicum*, in the remediation of this PCBs contaminated soils. Some preliminary tests have showed that these two plant species are characterized by an high tolerance to PCBs contamination. Moreover these species present some special features interesting for phytoremediation. *Medicago sativa* is a leguminous plant able to survive also in presence of reduced nitrogen supply as often it happens in contaminated industrial soil. *Lolium italicum* is particularly interesting because is a spontaneous species in this and many other Italian areas with a deep-rooted plant with high-yield and drought tolerance. Experiments were carried out by planting in 300 g of soil using 0.2 g of seeds per microcosm for both species.

Five replicates of vegetated microcosms for each species have been prepared and daily watered with tap water. Five microcosms without plants were run simultaneously as blanks. Growing period lasted three months, after this period plants started to decay. Experiments were carried out in a growth chamber in controlled conditions: 14 h of light, with a temperature of 24°C, and 10 h in dark conditions at 19°C. Relative humidity was maintained at 70%. Methylated-beta-cyclodextrins (RAMEB) were added at a concentration of 2% w/w on air-dried soil basis.

Soil samples have been analyzed at the beginning and at the end of the experiments in vegetated and non-vegetated microcosms. Analyses were performed after plants were removed, on air dried soil samples.

C .PCBs Analysis

The efficiency of the technology was evaluated by determining the residual PCB soil concentration. The soil samples in this study were extracted by U.S. EPA 3545 [23]. Soil extracts were analyzed by GC/MS, according to U.S. EPA method 8270C [24], using a Thermofinnigan “TRACE DSQ” GC-MS with a quadrupolar analyzer and PTV injector (DB 5 ms capillary column, 30 m x 0.25 mm I.D., 0.25 µm stationary phase film thickness). All reagents were pesticide quality.

Method accuracy and precision were evaluated by analyzing by triplicate one certified reference soil without PCBs spiked at the levels of 10 and 500 µg/g for each compound. The relative standard deviation (RSD) of the replicates on the concentrations of individual compounds ranged from 5% to 15%.

Due to the presence of 209 distinct isomers, it is not possible to determine them all, so the analysis only provide the concentrations of certain congeners which are representative of approximately 1/3 of the total quantity of these contaminants present in the mixtures which were produced.

The polychlorinated biphenyls that has been determined by the analysis of soil samples are PCB 18 (2,2',5-Trichlorobiphenyl), PCB 31+28 (2,4',5- Trichlorobiphenyl + 2,4,4'-Trichlorobiphenyl), PCB 52 (2,2',5,5'-Tetrachlorobiphenyl), PCB 44 (2,2',3,5'- Tetrachlorobiphenyl), PCB 101 (2,2',4,5,5'- Pentachlorobiphenyl), PCB 149 (2,2',3,4',5',6-Esachlorobiphenyl), PCB 118 (2,3',4,4',5-Pentachlorobiphenyl), PCB 153 (2,2',4,4',5,5'-Esachlorobiphenyl), PCB 138 (2,2',3,4,4',5'-Esachlorobiphenyl), PCB 180 (2,2',3,4,4',5,5'-Eptachlorobiphenyl), PCB 194 (2,2',3,3',4,4',5,5'-Octachlorobiphenyl).

III. Statistical Analysis

All statistical analysis was performed using Statistica version 6.0 (Statsoft, Inc.). Treatment effects were analyzed using oneway analysis of variance. Differences among means were compared and a post-hoc analysis of variance was performed using the Tukey honestly significance difference test ($p < 0.05$).

IV. Results and Discussion

The soil characteristics are reported in Table I together with the total PCBs concentration and fertility parameters, determined to evaluate if the condition were suitable to plant growth.

TABLE I
Soil Characteristics for Phytoremediation Tests

pH (H_2O)	7.1
Sand (%)	56.4
Silt (%)	24.1
Clay (%)	19.5
Organic matter (%)	1.9
CEC (cmol(+) kg-1)	22.3
Total N (%)	0.10
Available P (mg kg-1)	12.4
Available K (mg kg-1)	202
Total PCBs (µg kg-1)	250±23

Plant biomass was measured at the end of the experiment. The plant species tested did not exhibit signs of stress or toxicity, therefore it appeared that vegetation establishment in contaminated soil was feasible with these selected plants. The addition of RAMEB did not statistically affect biomass production: 1.25±0.12, and 2.03±0.21 g (dry weight/microcosm) for *Medicago sativa* and *Lolium italicum* respectively. These results could be ascribed to the low uptake of PCBs from plants less than 0.05 % of the original total content in soil (data not reported here).

All planted microcosms showed a significantly decrease of total PCBs concentrations in the respect to unplanted ones (Fig. 1). In non vegetated soil RAMEB addition promoted reduction of 10% of total PCBs. Both plant species increased the PCBs removal percentage to 30% of the original values, while plants with RAMEB reduced to nearly 50% the concentrations of total PCBs.

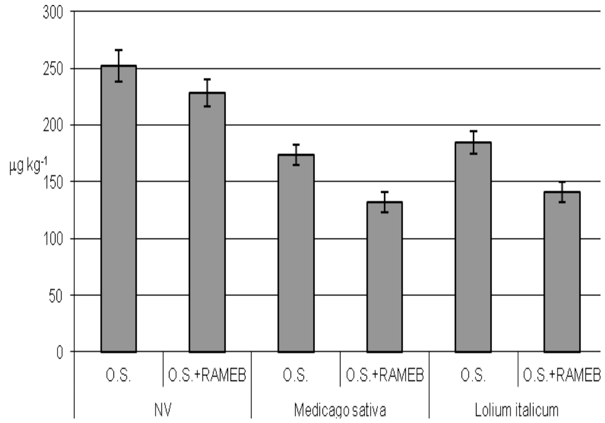

Fig. 1 Total concentration of PCBs congeners (µg kg^{-1}) in soil samples in unplanted (NV) and vegetated microcosms with *Medicago sativa* and *Lolium italicum* with (O.S.+RAMEB) and without (O.S.) RAMEB

The comparison of the removal of different chlorinated PCBs showed that with increasing chlorination, the congeners were more persistent in both soils. Lower chlorinated congeners such as 2,2',5-Trichlorobiphenyl, 2,4',5-Trichlorobiphenyl and 2,4,4'-Trichlorobiphenyl that were present at low concentrations in the original soils, decreased to below the detection limit in both soils.

In the case of tetrachloride PCBs the two plant species had the same effectiveness in promoting the degradation of the contaminants as an example, the concentration of 2,2',5,5'-Tetrachlorobiphenyl was almost halved compared to that present in unplanted soil, dropping to about 0.4 µg kg^{-1}, with a percentage of reduction of 45%, after plant growth. A further reduction to about 57% of the original value vas obtained in RAMEB treated soils.

With increasing the chlorination of the congeners the removal percentage of PCBs progressively decreased. However a relevant efficiency was still obtained in the case of 2,3',4,4',5-Pentachlorobiphenyl. The concentration of this congener, 28 µg kg^{-1} in the unplanted soil, was reduced of about 36% and 41%, after RAMEB addition, in soils grown with *Lolium italicum* and *Medicago sativa* respectively (Fig. 2).

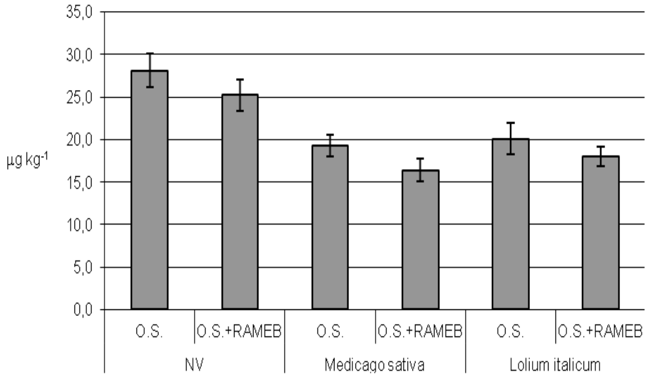

Fig. 2 Concentration of 2,3',4,4',5-Pentachlorobiphenyl (µg kg^{-1}) in soil samples in unplanted (NV) and vegetated microcosms with *Medicago sativa* and *Lolium italicum* with (O.S.+RAMEB) and without (O.S.) RAMEB

In the case of the congener 2,2',4,5,5'-Pentachlorobiphenyl originally present in the soil with a concentration of about 7 µg kg^{-1} the plants promoted the degradation of this contaminant with reduction percentages of 26.3% and 18.6% respectively for *Medicago sativa* and *Lolium italicum*, after the addition of RAMEB.

Similar results were obtained for 2,2',4,4',5,5'-Esachlorobiphenyl , with a reduction of 25% and 14,7% of the original concentration in microcosms planted with *Medicago sativa* and *Lolium italicum* respectively. In the case of the compound 2,2',3,4,4',5,5'-Eptachlorobiphenyl the concentration present in the not vegetated soil was around 4 µg kg^{-1} the plants of *Medicago sativa* promoted a reduction of about 10%. However a reduction of only about 2% was found in soil samples vegetated with *Lolium italicum* even after RAMEB treatment. Finally, there was no significative reduction in the concentration of congener 2,2',3,3',4,4',5,5'-Octachlorobiphenyl even after the addition of RAMEB.

Being very low the amount of PCBs uptaken by plants, the removal of PCBs can be ascribed to the greater activity of microorganisms in planted soil.

The increased biological activity, due to the presence of plants, was confirmed by the increasing of dehydrogenase activities in vegetated microcosms compared with those of non vegetated controls (Table II).

TABLE II
DEHYDROGENASE ACTIVITIES (µG TPF/G SOIL) IN VEGETATED AND NON-VEGETATED MICROCOSMS WITH (O.S.+RAMEB) AND WITHOUT (O.S.) RAMEB AT THE END OF THE EXPERIMENT.

Plant	O.S.	O.S.+RAMEB
Medicago sativa	17.5±1.2 aB	27.8±3.5 bB
Lolium italicum	18.8±2.1 aB	24.1±2.0 bB
Unplanted soil	3.71±0.23 aA	5.68±0.6 bA

Values in each row followed with different lower case letters indicated significant ($p<0.05$) differences following RAMEB treatment. Values in each column followed with different capital letters indicated significant ($p<0.05$) differences due to plants growth.

Dehydrogenase activities are commonly used as indicator of microbial activity being present in all microorganisms [25]. The increased dehydrogenase activities significantly correlate with the higher PCBs removal that can be ascribed to biodegradation processes.

V. CONCLUSIONS

Different plants have different reactions when grow in contact with contaminants based on their tolerance, ability to accumulate and metabolize them, and also to the chemical and physical properties of soils. Obtained results showed that the selected species have promoted higher PCBs biodegradation, even in the short period, compared to the non vegetated microcosms. In the adopted experimental conditions *Medicago sativa* and *Lolium italicum* showed nearly the same efficiency in reducing PCBs concentration in soil. Both species have strong potential for use in the remediation of organic contaminants and can be suggested as effective for the treatment of PCBs contaminated soils by phytoremediation, being the less chlorinated PCBs the more easily degradable. Addition of RAMEB further contributed in removing PCBs due to the ability to increase the bioavailability of the organic contaminants, and to stimulate microbial communities. Moreover β Cyclodextrins accelerated the degradation of PCBs promoting higher microbial biomass and a better utilization of the organic contaminants as a carbon and energy source [26]. Following the choice of combining phytoremediation and bioremediation, it was possible to promote a functioning microbial community able to degrade PCBs molecules, producing at the end of the remediation a better functioning soil ecosystem.

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