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Levels of Polychlorinated Biphenyls in Mexican Soils and Their Biodegradation Using Bioaugmentation

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Polychlorinated biphenyls (PCB's) are a mixture of hazardous isomer compounds, which cause health concerns to arise regarding suspected human toxicity, carcinogenicity and endocrine disruption. In addition, they have been shown to accumulate in fat, liver and spleen, kidney and rat brain (Chu et al, 1998). As with most chlorinated compounds, PCB's are somewhat resistant to biodegradation. However, the ability of some microorganisms to degrade PCB's has been demonstrated under aerobic and anaerobic conditions (Abramowicks, 1990).

Since the 1990's, Mexican environmental agencies have been looking to eliminate all wastes containing PCB's, but in some cases this work is too difficult since their distribution in different ecosystems is unknown. Moreover several barriers exist for the implementation of PCB bioremediation, such as their high toxicity, low bioavailability and lack of PCB metabolizing microorganisms (Barriault et al, 1997). Previous work has shown that the native microflora from polluted soil was able to biotransform PCB's contained in a transformer oil, at an extent of 75%, in liquid culture and under aerobic conditions (Rojas-Avelizapa et al, 1999). Successful laboratory PCB biodegradation in soil has been achieved through the use of co-substrates to support cometabolic PCB biodegradation (Rojas Avelizapa et al, 2000).

In Mexico there is no previous evidence related to the type and concentration of PCB's present in soils or their treatment by biological processes. The aim of this work was to evaluate the levels of total polychlorinated biphenyls in sixteen random soil samples and the effect of the augmentation of transformer oil-degrading organisms on PCB biodegradation in a selected soil.

MATERIALS AND METHODS

Since there was no previous information about possible sites polluted with PCB's, sixteen soil samples were randomly selected from suspected PCB-polluted soils. Samples were collected from several regions located in different states of Mexico, from horizon A (0-30 cm depth). Samples were named A1, A2, A3, A4, D1, D2, D3, D4, D5, M1, M2, M3, M4, M5, M6, and M7. Samples A, D and M represent

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samples from different States. Soil samples were evaluated for their PCB content and also for their microbiological status.

PCB's in soil samples (2.5 g of soil) were extracted in a Soxhlet system using hexane-ketone (3:1) during 9-h (Bellar. and Lichtenber, 1982). The extracts were purified through a glass column (1.5 x 30 cm) packed with Florisil mesh 60-100. The extracts were eluted with 100 mL of hexane, and 100 mL were collected and concentrated to 25 mL. Concentrated samples (1 μL) were analyzed by impact electron gas chromatography/mass spectrometry (GC-MS) using a Varian Saturn 3 chromatograph under the following conditions: DB-5 capillary column (0.32 x 30 m, 5% phenyl, 95% methyl silicone) oven temperature 90°C for 3 min, 90-120°C at 6.7°C/min, 120-250°C at 5.8°C/min, and 280°C for 15 min. Helium was used as a carrier gas at a flow rate of 15 ml/min. The temperature of the injector was set to 250°C, and the detector was an electromultiplier. Mass range corresponded from 60 to 500 m/e, based on the fragmentation properties of the tested PCB's.

PCB's were identified on the basis of their fragmentation patterns and validated by comparison of retention times using commercial mixtures of Arochlor 1232, 1242, 1248, 1254 and 1260.

The mixed culture, used throughout all the experiments, was obtained from a sandy and lightly acidic (pH 6.3) soil named D4 with a concentration of 7000 mg PCB's/Kg soil. The culture was maintained at 28°C in a mineral salt medium containing 1% of transformer oil (88% PCB's) as the sole carbon source. Prior to the bioaugmentation, the logarithmic phase of the mixed culture was established and biomass was added to the target soil.

For bioaugmentation studies, a total of three batch experiments were prepared in sealed serum bottles and aerobically incubated for 35 days at 28°C. Each bottle contained 15 g of PCB-polluted soil adjusted to 20% moisture and amended with nutrients and Tween 80 at 0.1%. These culture conditions were optimized in previous work (Rojas-Avelizapa et al, 2000). To avoid anaerobic conditions, bottles were subjected to atmosphere exchange every 24-48 h. Experiments were run in triplicate.

First, treatments were set to evaluate the addition of mixed culture (bioaugmentation) on the extent of PCB degradation in the soil. The soil was inoculated with 1% (w/w) of the mixed culture, which corresponded to 9.23×10^7 CFU/mL. Controls were established, as mentioned above, but avoiding biomass addition to the PCB-polluted soil to eliminate the effect of Tween 80 and nutrients. Other controls were set with polluted soil sterilized with $HgCl_2$ (5% w/w), to eliminate the effect of the native microflora on PCB biodegradation.

Carbon dioxide (CO_2) evolution was monitored daily as mentioned below and PCB transformation was analyzed by HPLC at the beginning and at the end of the treatment.

For CO_2 monitoring, headspace samples (2 mL) were taken from the soil microcosms with a 5-mL gas-tight syringe. The CO_2 content of the samples was determined using a Gow Mac 550 gas chromatograph equipped with a thermal conductivity detector and an Alltech CTRI stainless steel column. The operation conditions were: oven temperature, 30°C, injector temperature, 30°C, and detector temperature, 125°C. Helium was used as a carrier gas at a flow rate of 45 mL/min. Data were processed using Gow Mac Software and integrated to obtain the cumulative CO_2 production.

High performance liquid chromatography was utilized to evaluate PCB degradation after treatment. Soil samples were extracted as described above. Analysis was done under the following conditions: LDC Analytical Constametric 3200 thermo separation products coupled to a detector Spectro monitor 3200 (254 nm) with a C18 column (25 cm) using acetonitrile/water (70:30) as a mobile phase at a flow rate of 1.3 mL/min.

PCB removal in viable treatments was determined after normalization of chromatogram peak areas from non-degradable congeners with those from control samples (Mondello, 1989).

RESULTS AND DISCUSSION

Identification of PCB's in soil samples was performed by peak chromatogram comparison of purified extracts with those obtained from the SATURN Software library, interpretation of their fragmentation patterns and comparison to PCB commercial mixtures (standards). The presence of PCB's was indicated when the pattern of peak chromatograms resembled those of the standards. The results of qualitative analysis demonstrated that three soil samples were not polluted at any extent with PCB's. Five soil samples contained halogenated hydrocarbons whose which retention times did not correspond to any commercial PCB mixtures. Table 1 shows the eight soil samples polluted with PCB's and the type and concentration of PCB's. Analyzed samples showed a concentration range (expressed as total PCB content) from 47 to 11000 mg PCB/Kg soil. As observed in Table 1, PCB mixtures corresponding to Arochlor 1232, 1248 and 1260 were the predominant mixture in most cases. The concentration of individual congeners of the D4 sample is shown in Fig. 1. All analyzed samples contained microorganisms in the range of 10^3-10^4 CFU/g soil, but no correlation was found between the PCB concentration and the CFU/g. This is probably due to the presence of other toxic compounds, such as heavy metals (data not shown).

It is worth mentioning that the results of this work are an important finding, since it is the first time that high amounts of highly chlorinated isomers have been found in several soils. It was also important to observe that 38% of the total soil samples analyzed exceed the permissible limit (50 ppm of PCB's) for the definition of a hazardous waste. These results indicated that contamination in soils with PCB's must be of primary concern to the Mexican environmental authorities reports available (INE-SEMARNAP, 1997) indicate that in 1997 a total of 6,544

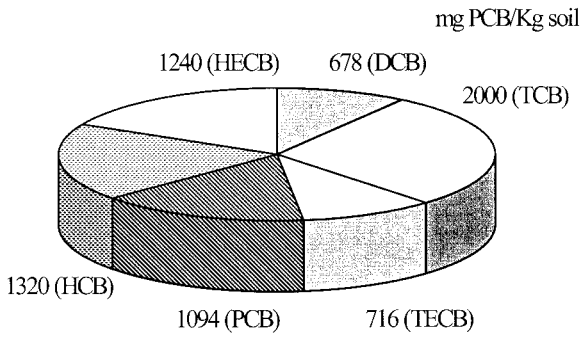

Figure 1. Individual isomer content in the sample of polluted soil (D4); 7000 mg PCB/Kg soil. DCB (dichlorobiphenyl), TCB (trichlorobyphenyl), TECB (tetrachlorobipheyl), PCB (pentachlorobiphenyl), HCB (hexachlorobiphenyl) and HECB (heptachlorobiphenyl).

metric tons of PCB's were in use by parastatal enterprises. In the past decade, the Mexican environmental authorities have developed a program whose aim is to reduce the release of substances into the environment and to substitute their consumption. PCB's have been considered priority substances to be eliminated; thus appropriate technologies must be implemented or developed to eliminate them completely.

For bioaugmentation studies, a sandy and lightly acidic (pH 6.3) soil (D4) polluted with 7000 mg-PCB's/Kg-soil was selected, because it contains a representative PCB mixture (Arochlor 1260) and sufficient microbial population 5 x 10^4 CFU/g soil (Table 1).

The results of heterotrophic activity (CO_2 evolution) demonstrated that during the bioaugmentation treatment (BAT) a maximum amount of 4 μg CO_2/g of soil was

Table 1. PCB´s and colony forming units (CFU) present in different Mexican soil samples

Soil sample	PCB's mixture type	PCB's concentration (mg/Kg soil)	CFU/g soil
A2	1260	526	N.D.
A4	1260	92	N.D.
M1	1260	167	9.17 x 10^3
M5	1260	90	1.03 x 10_3
M7	1260	149	1.03 x 10^3
D1	1248	11000	9.3 x 10^4
D3	1232-42	47	9 x 10^3
D4	1260	7000	5 x 10^4

N.D. No determined

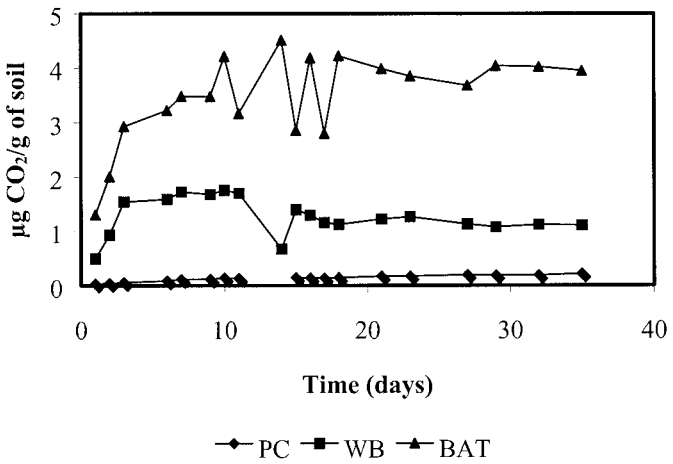

Figure 2. Carbon dioxide evolution during the bioaugmentation treatment of PCB polluted soil (D4) at 28°C, pH 6.3; PC, poison control; without biomass (WB) and bioaugmentation treatment (BAT).

produced after 35 days of incubation (Fig. 2). An increase in CO_2 production was also observed during the first 10 days of incubation, which was maintained over the full incubation period (Fig. 2). Values of 1 and 0.2 µm CO_2/g of soil were obtained for the treatment without biomass (WB) and poison control (PC) respectively.

According to the results of HPLC analysis, after 35 days of incubation the extent of PCB degradation in the BAT treatment corresponded to 39% with respect to the PC treatment, suggesting that the addition of a well acclimated bacteria in a polluted soil plays an important role in enhancing PCB biodegradation (Fig. 3). It is important to mention that the percentage of reported PCB biodegradation is net. PCB degradation in WB treatment was also important. A PCB removal of 20% (over the PC) was observed demonstrating again that Tween 80 added at 0.1 % improves PCB degradation (Rojas-Avelizapa et al, 2000). Additional experiments showed also that Tween 80 supported the growth of mixed cultures (data not shown). Thus the possibility that surfactant addition could have enhanced the activity of PCB degraders can not be excluded.

PCB biodegradation results obtained during this research appear to contradict those studies, assuming that bioaugmentation failed for petroleum hydrocarbons and PCB degradation (Harkness et al, 1993, Phelps et al, 1994) where they did not observe degradation with microorganism addition. Havel and Reineke (1991) mentioned that native microflora affected negatively the degradation of low chlorinated biphenyls, such as Arochlor 1221.

Our results agree with those reported by Hickey et al (1993), who pointed out the success of a bioaugmentation treatment using *Pseudomonas testosteroni* B-356,

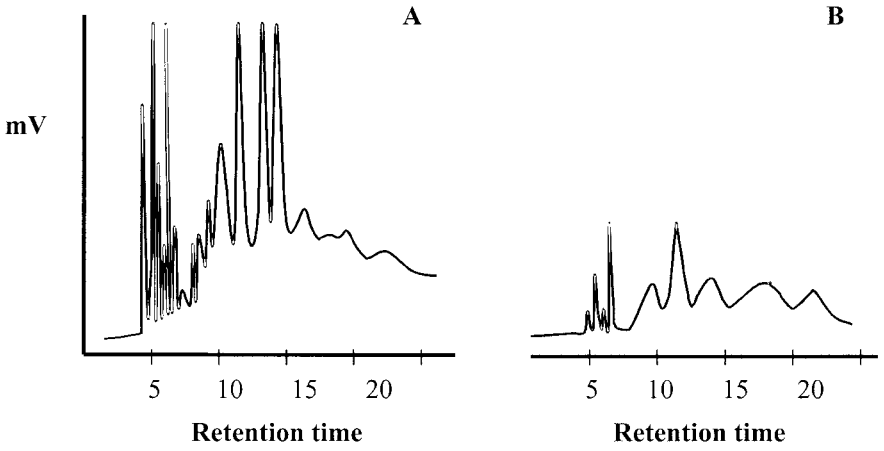

Figure 3. HPLC chromatograms of PCB degradation in treated soil by bioaugmentation A) Poison control and B) bioaugmentation treatment after 35 days.

which was able to degrade Arochlor 1242, to an extent of 30%. Chih et al, (1998) indicated that microorganisms acclimated to Arochlor 1242 and biphenyl could utilize the individual chlorinated biphenyl congeners more effectively. Other authors have performed bioaugmentation studies with good results too. Winningham et al, (1999) augmented soil with chicken manure and exogenous microorganisms, showing that 80 % of PAHs were removed; Tharakan et al, (1999) demonstrated Arochlor 1242 biotransformation in a bioslurry when amended with *R. erythropolys* and byphenyl, showing that 19% of Arochlor 1242 remains after 10 days of treatment.

Results of this investigation suggest that transformer oil-degrading microorganisms have the ability to degrade PCB's in soil conditions. However, these conditions must be appropriate to develop a good microbial activity, which can be improved by the amendment of the surfactant Tween 80, an appropriate C/N/P ratio and moisture, as was demonstrated in previous work. It is also important to point out that major investigations have been performed with cosubstrates, thus in this particular case other compounds different to PCB's present in soil could be used as cosubstrates to support microbial growth and degradative activity.

To summarize, bioremediation of PCB-polluted soil by bioaugmentation using a well-adapted mixed culture could be a promising method of enhancing the degradation of PCB's in highly polluted soils.

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REFERENCES

Abramowicks DA (1990) Aerobic and anaerobic biodegradation of PCB's: a review. Crit Rev Biotechnol 10:241-251

Barriault D, Pelletier C, Hurtubise Y,, Sylvestre M (1997) Substrate selectivity pattern of *Comamonas testosteroni* strain B-356 toward dichlorobiphenyls. Int Biodeter Biodeg 39:311-316

Bellar TA, Lichtenber JJ (1982) The determination of polychlorinated biphenyls in transformer fluid and waste oils. EPA/600/4-81/045; U.S. Environmental Protection Agency: Cincinnati, OH

Chih JL, Chi ML, Kuang CS (1998) Effects of acclimation on the removal of polychlorinated biphenyls. Proceedings of the 4th International Symposium In situ and On site bioremediation San Diego, CA USA Vol. 2 pp 433-437

Chu I, Poon R, Yagminas A, Lecavalier P, Hakansson H, Valli VE, Kennedy SW, Bergman A, Seegal RF, Feeley M. (1998) Subchronic toxicity of PCB 105 (2,3,3',4,4'-pentachlorobiphenyl) in rats. J Appl Toxicol 18:285-292

Harkness MR, McDermatt JB, Abramowicks DA, Salvo JJ, Flanagan WP, Stephens ML, Mondello FJ, May RJ, Lobos JM, Carroll KM (1993) In situ stimulation of aerobic PCB biodegradation in Hudson River sediments. Science 22:503-507

Havel J, Reineke W (1991) Total degradation of various chlorobiphenyls by co-cultures and in vivo constructed hybrid Pseudomonads. FEMS Microbiol Lett 78:163-170

Hickey DB, Seabs, Focht DD (1993) Enhanced mineralization of PCB's in soil inoculated with chlorobenzoate degrading bacteria. Appl Environ Microbiol 39:1194-1200

Mondello FJ (1989) Cloning and expression in *Escherichia coli* of *Pseudomonas* strain LB400 genes encoding polychlorinated biphenyl degradation. J Bacteriol 171:1723-1732

INE-SEMARNAP. (1997) Programa de gestión ambiental de sustancias tóxicas de atención prioritaria. Gaceta Ecológica 45:58-65

Phelps TJ, Segrist RL, Korte NE, Pickering DA, Strong-Guderson JJM, Palumbo AV, Walker JF, Morrissey CM, Mackowski R (1994) Bioremediaton of petroleum hydrocarbon in soil column lysimeters from Kwajalein island. Appl Biochem Biotechnol 45-46:835-845

Rojas-Avelizapa NG, Rodríguez-Vázquez R, Martínez-Cruz .J, Enríquez Villanueva F, Poggi Varaldo HM (1999) Transformer oil degradation by an indigenous microflora isolated from a contaminated soil. Resour Conserv Recycl 27:15-26

Rojas-Avelizapa NG, Rodríguez-Vázquez R, Saval-Bohorquez S, Alvarez PJJ (2000) Effect of C/N/P ratio and nonionic surfactants on PCB biodegradation. World J Microbiol Biotechnol 16:319-324

Tharakan JP, Ebenezar S, Raycharn L, Chawla R (1999) Transformation of Aroclor by indigenous and inoculated microbes in slurry reactors. In Andrea Leeson and Bruce C. Alleman In Bioremediation of nitroaromatic and haloaromatic compounds, Battelle Press Columbus Ohio. USA

Winningham J, Britto R, Pael M, McInturff F (1999) A Landfarming field study

of creosote-contaminated soil In Andrea Leason and Bruce C. Alleman In bioremediation technologies for polycyclic aromatic hydrocarbon compounds Battelle Press Columbus Ohio. USA