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# Contribution of ecotoxicological tests in the evaluation of soil bioremediation efficiency

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## INTRODUCTION

Clean-up of contaminated soils became a high priority only recently. Several techniques have been developed for this purpose such as chemical, physical, thermic or microbiological methods.

Efficiency of the remediation can be estimated using two approaches : a chemical specific approach and a toxicity-based approach. So far, the efficiency of the decontamination process was based essentially on chemical analyses which does not integrate the toxicity of all the soil contaminants and does not give a response on effects caused by the bioavailable fraction of these contaminants as the toxicity-based approach. In the present study, bioremediation efficiency of a soil contaminated by 4-chlorobiphenyl was evaluated using chemical and biological analyses. Experiments were carried out in microcosms contaminated at a rate of 1 g/kg. Control microcosms without specific degrader were performed simultaneously. Acute toxicity to earthworms and inhibition of growth of barley roots were selected, from previous work, as relevant ecotoxicological tests.

## METHODS

### Microcosms

Experiments were performed in microcosms : 30 kg of OECD artificial soil contaminated at a rate of 1 g/kg. 4-chlorobiphenyl was dissolved in acetone before addition to the soil. After mixing and evaporation of the solvent, deionised water (control microcosm) or bacterial suspension in saline were added to give an overall moisture content of 30 % of dry weight.

Microcosms were incubated at 20°C in the dark.

### Bacterial strains

In the first experiment, microcosm was inoculated with *Pseudomonas sp. B4*, a biphenyl and a chlorinated biphenyl degrader ( $10^7$  bacteria per g of dry soil). In the second one, two bacterial strains were introduced successively : *Pseudomonas sp. B4* and *Pseudomonas sp. CBS3*, a chlorobenzoate degrader ( $10^7$  bacteria per g of dry soil). These strains were inoculated at the beginning of the experiment and after 3 weeks respectively. During the last experiment, the two strains were inoculated simultaneously.

Total and adapted bacterial populations (plating techniques, acridine orange epifluorescence counting, quantitative PCR after total DNA extraction) were followed within the experiments.

### Analytical methods

#### Experiment n°1

Soil samples (20 g wet weight) were extracted for 30 minutes in an hexane/acetone mixture (1/1) and resuspended in methanol (1.5 ml) after filtration and evaporation. Soil extracts were analysed by GC/ECD.

#### Experiments n°2 and 3

In addition, concentrations of 4-chlorobenzoate (intermediate metabolite of the degradation of 4-chlorobiphenyl) were determined. Both compounds were analysed by HPLC with UV detection (254 nm). The same extraction method was used in these experiments.

### Ecotoxicological tests

Bioremediation efficiency of the contaminated soil was evaluated during the two experiments using the following tests :

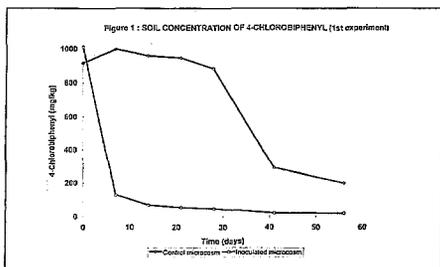
- inhibition of root growth of barley (ISO 11269-1),
- acute toxicity to the earthworm *Eisenia fetida* (ISO 11268-1).

Both tests were carried out on 100 % soil (inoculated and control microcosms).

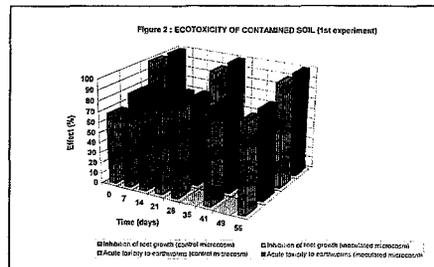
## RESULTS AND DISCUSSION

### *Pseudomonas sp. B4* alone

While 4-chlorobiphenyl was rapidly degraded by *Pseudomonas sp. B4* : 87 % in less than one week, the toxicity of contaminated soil remained comparable to the control one.



Notwithstanding a lower concentration of 4-chlorobiphenyl than the no observed effect concentrations for earthworms and barley (127 and 135 mg/kg respectively), no detoxification was observed at the end of the experiment with regards to the initial toxic effects.



## → RESULTS AND DISCUSSION

Further experiments demonstrated that the residual toxicity could be attributed to the 4-chlorobenzoate : a dead-end metabolite of 4-chlorobiphenyl degradation by *Pseudomonas sp.* B4. This compound was found to be as toxic as the parent compound towards earthworms and barley ( table 1).

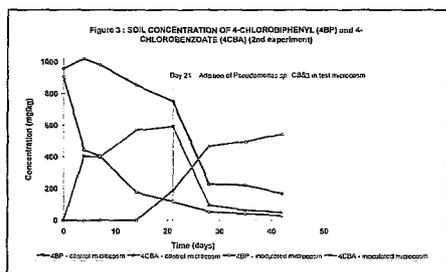
After day 28, a decrease of the 4-chlorobiphenyl concentration was observed in the control microcosm (fig1). Quantitative PCR analysis and biochemical test showed a presence of *Pseudomonas sp.* B4 that could explain this decrease.

Species	Effect	Compound	NOEC (mg/kg)*	EC50 (mg/kg)*
Earthworm <i>Eisenia fetida</i>	Mortality	4-chlorobiphenyl	127	127 < LC50 < 159
		4-chlorobenzoate	128	162 (154-170)**
Barley <i>Hordeum vulgare</i>	Inhibition of root growth	4-chlorobiphenyl	135	560 (441-715)**
		4-chlorobenzoate	50	226 (198-256)**

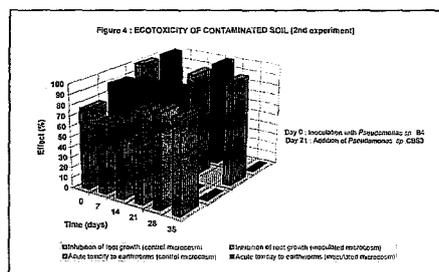
\* : mg/kg of soil dry weight ; \*\* : 95% confidence limits  
Table 1 : NOEC and EC 50 values for 4-chlorobiphenyl and 4-chlorobenzoate

### *Pseudomonas sp.* B4 and *Pseudomonas sp.* CBS3 successively

The experiment was repeated with a successive inoculation of two bacterial strains : *Pseudomonas sp.* B4 and *Pseudomonas sp.* CBS3, a 4-chlorobenzoate degrader. Inoculation with those strains occurred respectively at the beginning of the experiment and after 3 weeks. A reduction of the 4-chlorobiphenyl concentration and a corresponding production of 4-chlorobenzoate was observed during the first 3 weeks (fig 3). No decrease of the soil toxicity was recorded within this period (fig 4) as shown in the first experiment.



Detoxification of soil was effective one week after *Pseudomonas sp.* CBS3 inoculation in the microcosm as shown in figure 4,

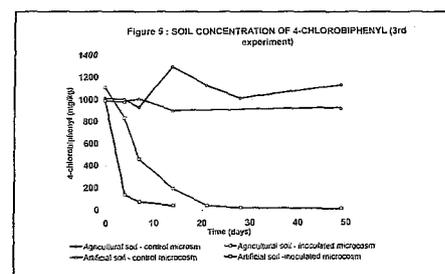


concomitantly with the disappearance of 4 chlorobenzoate (as observed in figure 3). A decrease of the 4-chlorobiphenyl concentration has also been observed in the control microcosm as in the first experiment,

probably due to an aerial contamination of this control microcosm by *Pseudomonas sp.* B4.

### *Pseudomonas sp.* B4 and *Pseudomonas sp.* CBS3 simultaneously

When the two strains are inoculated at the same time, a reduction of the 4-chlorobiphenyl concentration was noticed in both soils : artificial and agricultural (fig 5). No concomitant production of 4-chlorobenzoate was detected. Concerning control microcosm, the 4-chlorobiphenyl concentration remained stable during all the experiment (Test conditions were fitted in order to avoid aerial contamination between test and control microcosms). Ecotoxicological tests confirmed the efficiency of the remediation : one week after the beginning of experiment using an artificial soil, two weeks after using an agricultural soil.



## CONCLUSIONS

These results underline the need of taking biological effects into account in order to assess remediation efficiency of contaminated areas. In the first experiment indeed, the toxicity of contaminated soil remained stable towards earthworms and barley in spite of a degradation of 4-chlorobiphenyl higher than 98 %. With chemical analyses only, we could come to the conclusion that the biological clean-up was effective at the end of the experiment.

In that case, biological tests interest is to take toxicity of degradation metabolites into account. More generally, such tests allow to measure aggregate toxicity of all constituents of a contaminated soil including additive, synergistic and antagonistic effects.

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