Cyclodextrin-enhanced mutagenicity of pentachlorophenol

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Abstract

Mutagenicity of pentachlorophenol (PCP) contaminated soil was measured by the modification of Ames test, using a nanotechnology (cyclodextrin) to decrease the matrix-effect and increase mutagenicity of the soil. Increased sensitivity of the whole soil testing method results in a pessimistic biological model for the characterisation of the mutagenic risk of the pollution in soil. Whole soil test was performed by pre-treatment with RAMEB (random methylated ß-cyclodextrin) and compared to usually applied test methods with whole soil and soil extracts.

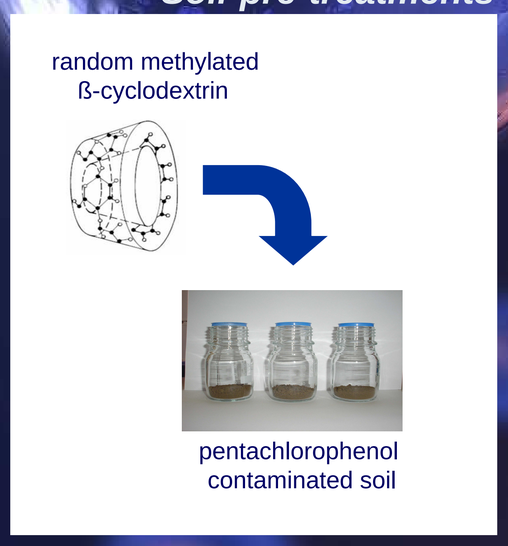
Introduction

Measuring, evaluating and interpreting mutagenicity of polluted environment is one of the important tasks of human and environmental risk assessment. Environmental relevance of soil mutagenicity testing methods is poor at present. The effect of soil contaminants on ecosystem and humans is restricted by their bioavailability and their partition between soil phases. For high K_{ow} (octanol-water partition coefficient) substances, which are hardly available for water, the availability can be increased by decreasing K_{ow}. Decrease of the biological effect is time and microbial activity dependent, and as such, hardly standardisable. Cyclodextrin lowers K_{ow} in a better reproducible way. By the cyclodextrin pre-treatment of the sample the maximum bioavailability can be simulated (pessimistic approach).
Pentacholorophenol's mutagenity is questionable: many authors state that it is mutagenic and carcinogenic, but most of the published test results are negative (Seiler, 1991; Gopalaswamy and Nair, 1992; Sekine et al, 1997).
Cyclodextrin is a molecular encapsulating agent, which is able to increase mobility (desorption, water solubility and bioavailability) of high K_{ow} contaminants in general also in the contaminated soill.
It was shown in our preliminary experiments, that the solubility of PCP in water can be increased by RAMEB, a cyclodextrin with high solubilising potential and of no mutagenic effect. Therefore we used RAMEB also for the soil pre-treatment, supposing that the mobilising effect will appear also in the soil and therefore increases the measured mutagenic effect.

Methods

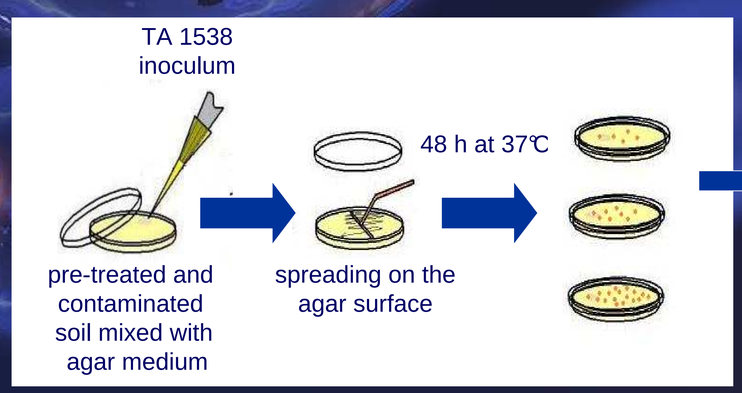
RAMEB addition to PCP-contaminated soil in various forms:
1. whole soil suspended in RAMEB solution (rate 1:1)
2. whole soil extracted with 1:1 of RAMEB-solution
3. whole soil homogeneously mixed with solid RAMEB powder
RAMEB was added into the soil 24 hour before testing. As RAMEB pre-treatment we used 2.5; 5 and 10% RAMEB (related to dry soil) dissolved in water or in powder form. These results were compared with un-treated and S9 enzyme mix treated soils.

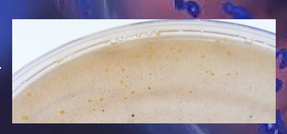
Soil pre-treatments



Testing mutagenicity

We applied three of the frequently used Salmonella strains: TA 1535, TA 1537 and TA 1538, which all are different histidine auxotroph mutants. TA 1538 strain was the most sensitive suggesting that much of the mutagenic activity in PCP-contaminated soil is frameshift-type mutation.
In the applied mutagenicity tests the direct exposure of the Salmonella strains to the contaminated soil samples was ensured by mixing of 0.1 g of soil into agar-medium and spreading the bacteria to the agar surface. After 48 hours at 37℃ incubation, the revertant colonies were counted.
The revertants were grown on agar-plates in Petri dishes. The pre-treated PCP-contaminated soil was mixed into the molten nutrient-agar at 42℃.





revertant colonies on agar surface

Results

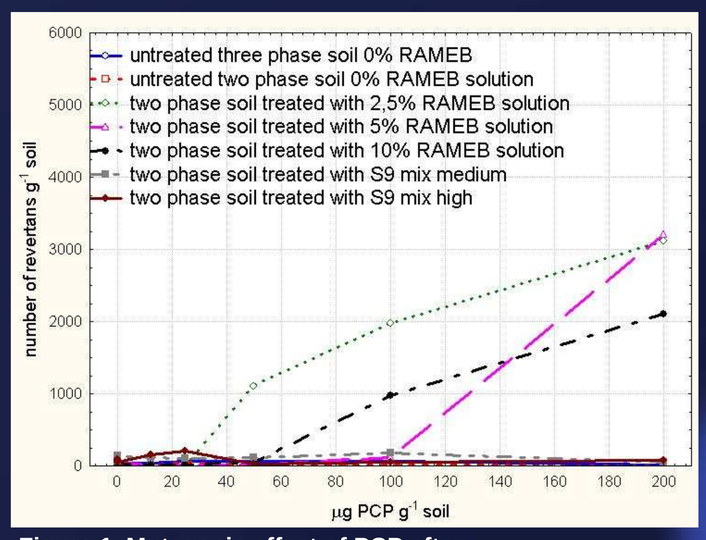


Figure 1. Mutagenic effect of PCP after pre-treatment with RAMEB solution and S9 mix

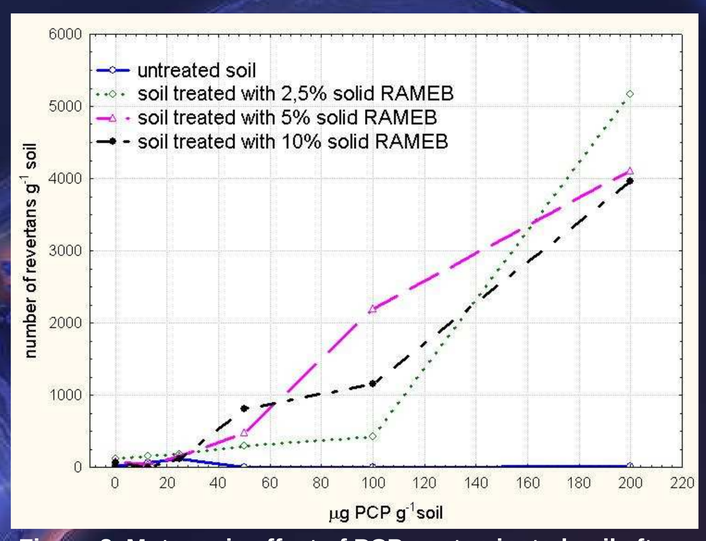


Figure 2. Mutagenic effect of PCP contaminated soil after RAMEB-powder pre-treatment

We found that RAMEB increased the number of revertants in a large scale (Figure 1 and 2). RAMEB itself has no mutagenic effect as it is shown at each curves at 0 µg g^{-1} PCP concentration.

For the whole soil contaminated with PCP significant mutagenic effect was obtained by both RAMEB solution and solid RAMEB addition to the soil. These results prove that RAMEB increases biological availability of the soil contaminant.

As the inclusion complex formation is a dynamic process, solid phase-bounded PCP serves as a continuously available source.

The non pre-treated soil was compared with S9 and cyclodextrin pre-treated ones. For untreated and S9 mix-treated soil no mutagenic effect was observed.

There is high relevancy between PCP concentration in the soil and mutagenic effect in case of both solid and dissolved RAMEB addition. Solid RAMEB treatment gave 1.5 times more revertants. RAMEB is more effective in the air dried three phase soil, where the moisture content is very low: the concentration of the active inclusion complex is higher. The lack of free water-phase changes the partition between soil moisture, soil solid and the testorganism, resulting in increased mutagenic effect.

Table 1 Correlation between PCP concentration, soil pre-treatment and mutagenic effect

Tested soil phase	Type of the treatment		Correlation factor (p>0.05)
three phase soil	without treatment		No observed mutagenic effect
two phase soil			
water extract			
three phase soil	solid RAMEB pre-treatment	2.5 %	0.90
		5%	0.99
		10%	0.98
two phase soil	RAMEB solution pre-treatment	2.5 %	0.98
		5%	0.96
		10%	0.97
supernatant of two phase soil	RAMEB solution pre-treatment	2.5 %	No observed mutagenic effect
		5%	
		10%	
two phase soil	S9 enzyme mix pre-treatment	with Medium concentration S9	No observed mutagenic effect
		with High	
		concentration S9	

Conclusions

☞The mutagenic effect of PCP in soil is increased by the presence of inclusion complex forming agent, RAMEB.
☞There is an evidence that insufficient availability led to negative results both in soil suspension and air dried soil.
☞The results of this study suggest that cyclodextrin could be a proper agent for pre-treatment of soil samples, making possible the direct testing of whole soil. It is able to simulate maximum bioavailability both in water and whole soil.
☞The application of RAMEB is a good possibility to increase the biologically available fraction of the contaminant, to reach a better estimate for a „realistic worth case" situation: applying a pessimistic model, with a biologically relevant maximum.
☞The sensitivity of the Ames-test could be enhanced with RAMEB making possible the use of whole soil test for measuring mutagenicity. On the basis of our first result with PCP our method fits well into this risk assessment concept and prevents us from the opposite: underestimation of the hazard in the soil due to high K_{ow} of the contaminant and the dilution of the sample during testing.

References

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Acknowledgements

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