

ABSTRACT Mutagenicity of pentachlorophenol (PCP) contaminated soil was measured by the direct contact version of the Ames test, using a nanotechnology (cyclodextrin - CD) to modify the matrix-effect and to increase mutagenicity of the soil. As a result of cyclodextrin pre-treatment the sensitivity of the test increased. Two cyclodextrins: RAMEB (random methylated β -cyclodextrin) and HPBCD (hydroxy-propyl β -cyclodextrin) were applied and evaluated in comparison with the traditional S9 activation.

1. INTRODUCTION Mutagenicity testing of high K_{ow} (octanol-water partition coefficient) soil contaminants, like PCP, shows high uncertainties due to restricted bioavailability. In the real environment interactions between soil contaminants and exposed organisms may increase accessibility and availability (biotensides, gastrointestinal digestion, root exudates, etc.) and as a consequence the risk of the contaminant. To simulate the worst case of natural availability increasing effects direct contact between contaminated soil and testorganism (Gruiz et. al. 2001) was ensured using cyclodextrin. Cyclodextrin is a molecular-encapsulating agent, which is able to increase mobility (desorption, water solubility and bioavailability) of high K_{ow} contaminants in general and in the contaminated soil as well.

Such an interactive mutagenicity measuring method is an ideal tool for the effect based soil monitoring and for the risk based environmental management, representing a pessimistic, but still biologically realistic situation.

3. RESULTS

TA 1538 strain was the most sensitive (indicating frameshift mutagenicity, White and Claxton, 2004) untreated and S9 mix-treated soil did not show mutagenic effect

Cyclodextrins, both RAMEB and HPBCD significantly increased the number of revertants (positive T-probe)

Significant mutagenicity was measured
 RAMEB_{powder} 4000-5000 revertants/g soil,
 RAMEB_{dissolved} 2000-3000 revertants/g soil
 HPBCD_{powder} 2000-2500 revertants/g soil
 HPBCD_{dissolved} 1000 revertants/g soil

Stoichiometric correlation between PCP and HPBCD

- maximum effect is associated with the maximum concentration of HPBCD
- the correlation between mutagenicity and PCP concentration is above 0.90
- the correlation between mutagenicity and HPBCD concentration is 0.9
- the effect of RAMEB was not proportional with its concentration

2. METHODS

SOIL PRE-TREATMENTS

CD and S9 addition to PCP-contaminated soil:

1. whole soil without additives
2. whole soil with dissolved CD
3. whole soil with CD powder
4. whole soil with S9 enzyme mix solution

We used 2.5; 5 and 10% (related to the dry soil) of CD dissolved in water or in powder form.

TESTING MUTAGENICITY

The applied Salmonella strains: TA 1535, TA 1537 and TA 1538, histidine auxotroph mutants.

The principle of the test is: colonies growing on the histidine-free nutrient media are revertants. Their number is proportional with the mutagenic effect.

Direct contact between the soil and the test microbes was ensured by mixing soil into the agar-medium and spreading Salmonella on it.

After 48 h, 37°C incubation, the revertant colonies were counted.

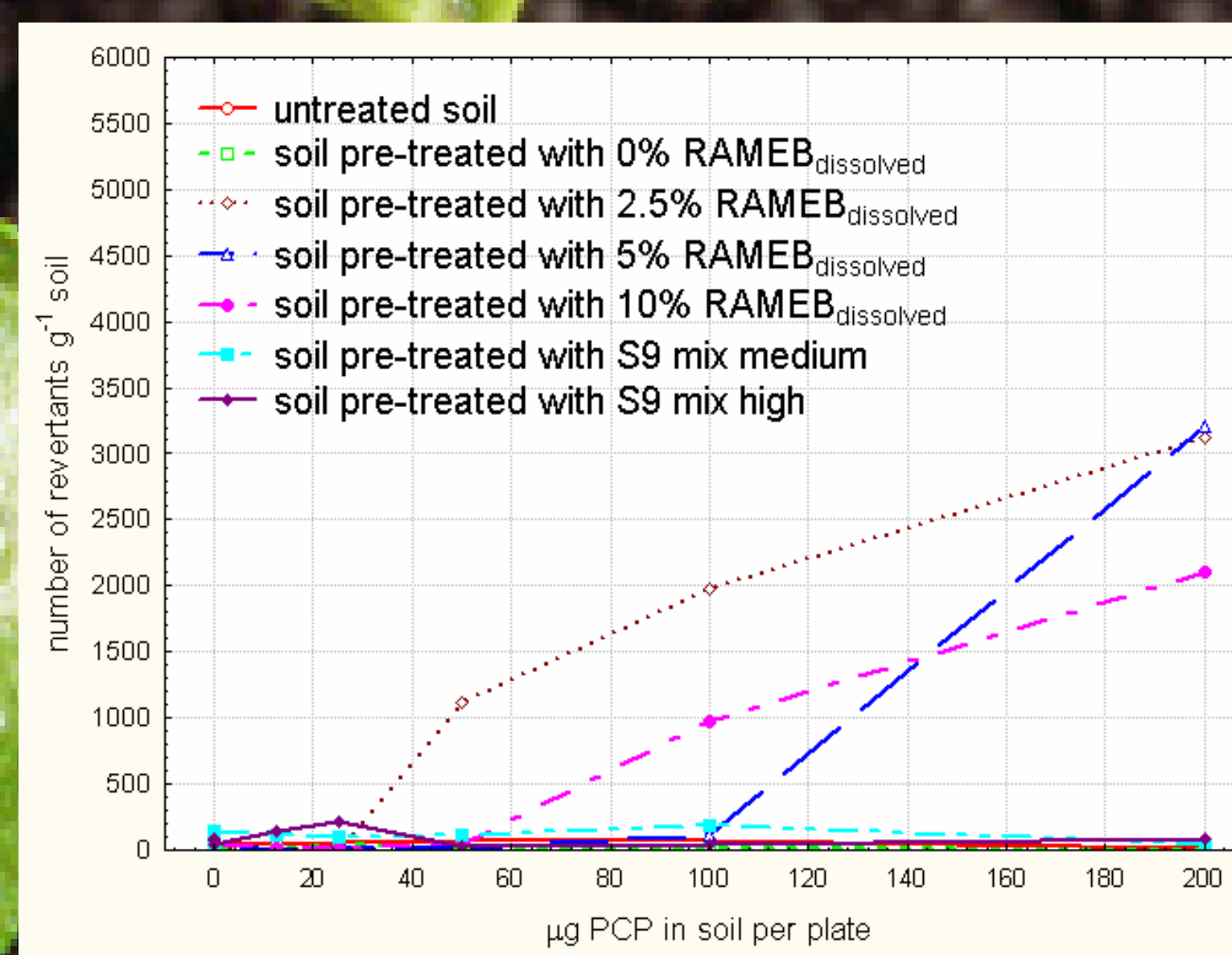


Figure 1. Mutagenic effect of PCP after RAMEB_{dissolved} and S9 mix treatment

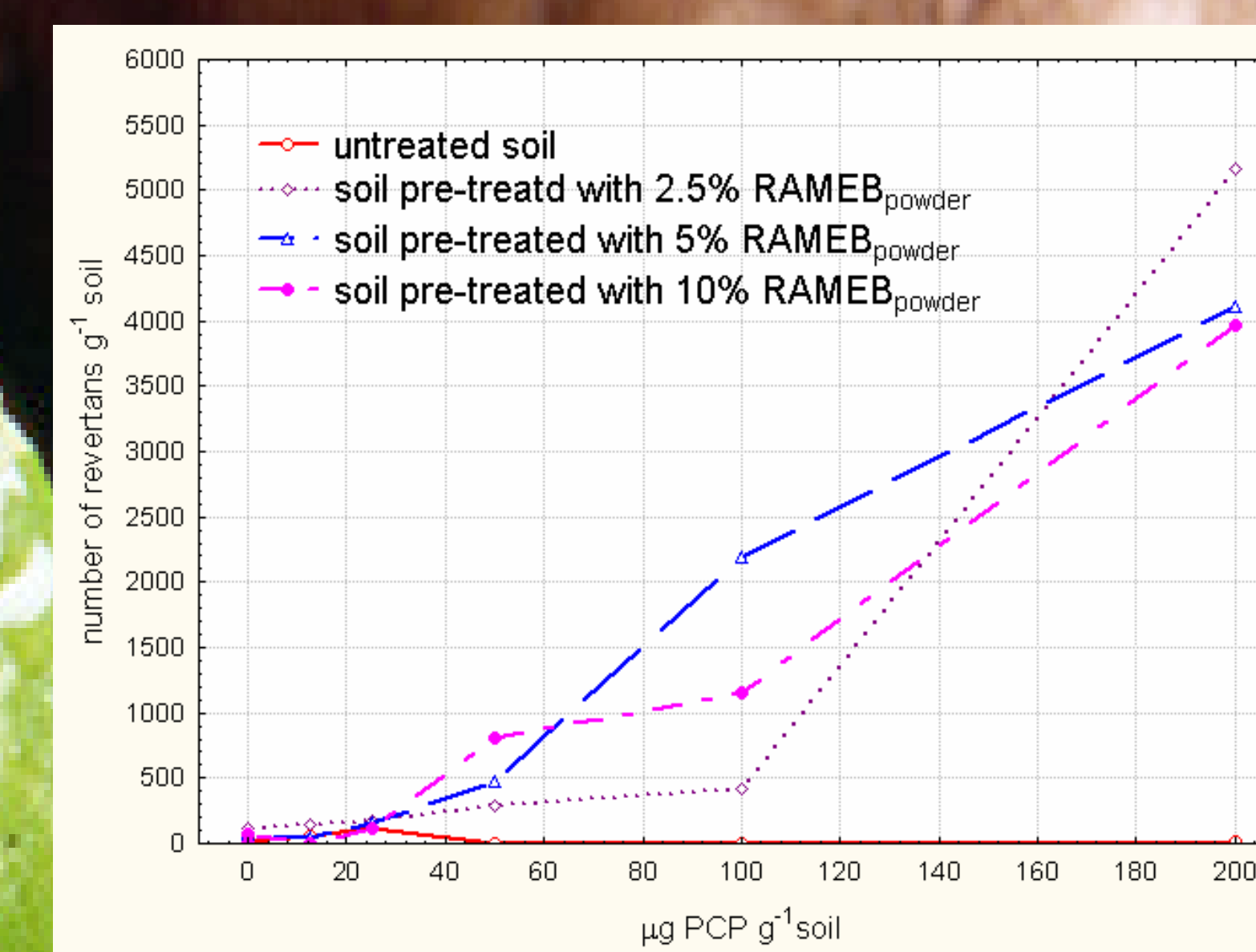


Figure 2. Mutagenic effect of PCP after RAMEB_{powder} treatment

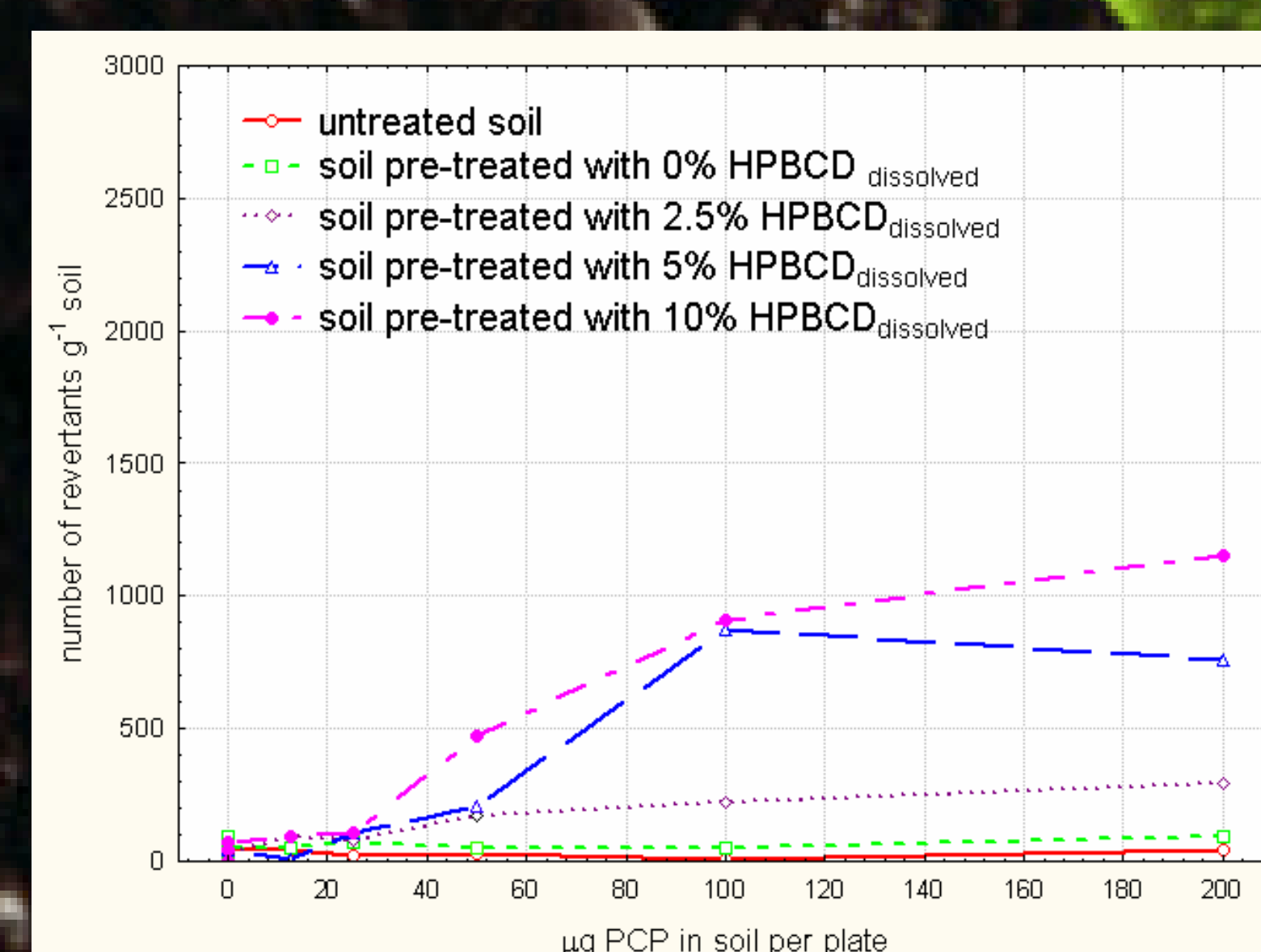


Figure 3. Mutagenic effect of PCP after HPBCD_{dissolved} treatment

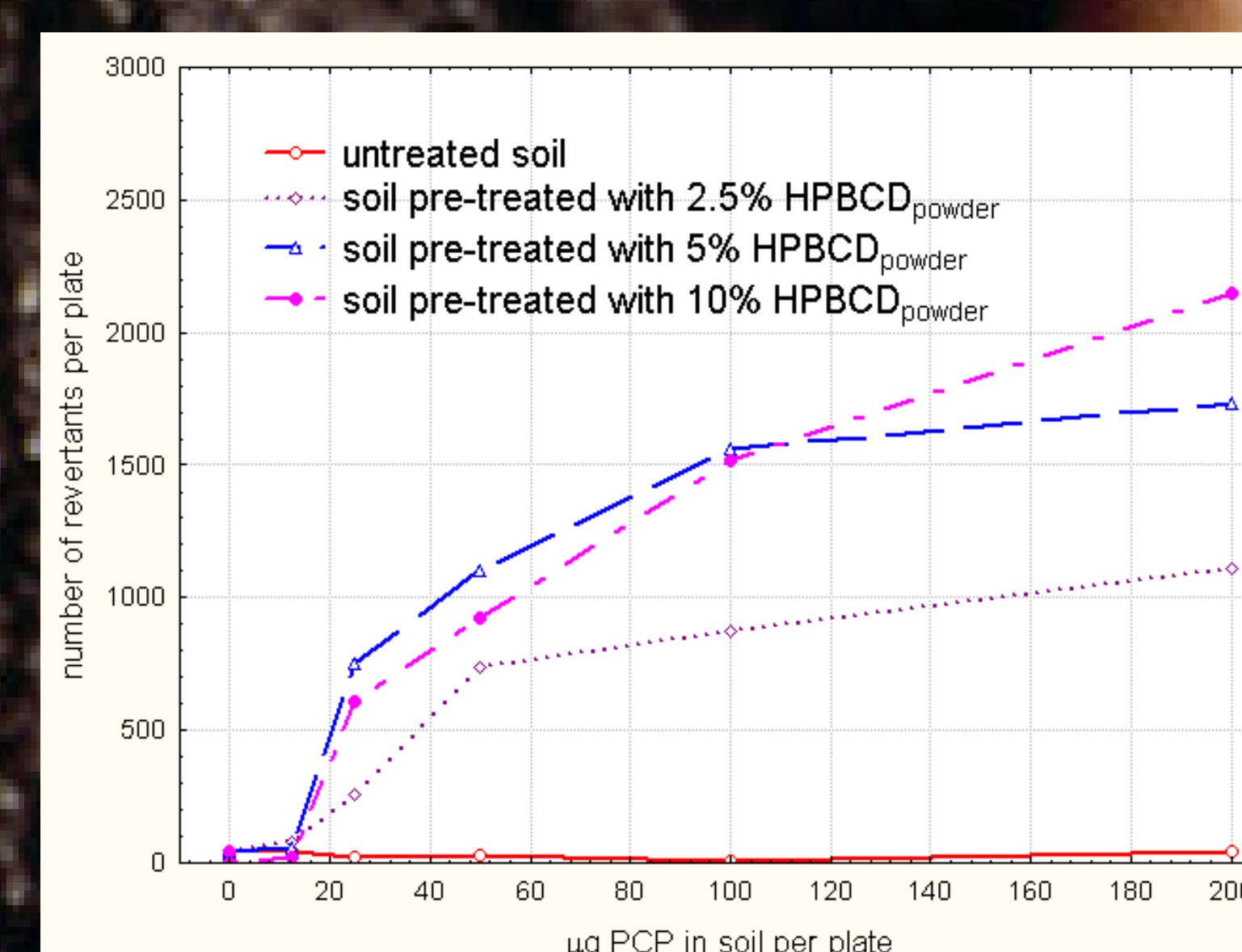


Figure 4. Mutagenic effect of PCP after HPBCD_{powder} treatment

4. CONCLUSIONS

- the mutagenic effect of PCP in soil increased on the effect of direct contact with the testorganism: biological enhancement
- the mutagenic effect of PCP in soil increased on the effect of nanoscale inclusion complex forming RAMEB and HPBCD: chemical enhancement

- cyclodextrin pre-treatment is able to increase bioavailability of high K_{ow} contaminants

- cyclodextrin and direct contact together are able to simulate maximum bioavailability of contaminants in the soil

- the sensitivity of the Ames test could be enhanced with direct contact and cyclodextrin pre-treatment together making the test possible to use for measuring mutagenicity of contaminated soil without previous extraction

- direct contact after cyclodextrin pre-treatment resulted a model system which fulfils the requirement of: being a highly sensitive, conservative (worst case) but still biologically realistic and environmentally sound.

- on the basis of the results with PCP our method fits well to the pessimistic risk assessment concept and prevents us from the underestimation of the hazard in the soil due to high K_{ow} of the contaminant, matrix effect of the soil and the dilution of the sample during extraction and testing

REFERENCES

Gruiz K.; Horváth B. és Molnár M. (2001) Environmental toxicology, Műegyetemi Kiadó, Budapest
 White, P. A.; Claxton, L. D. (2004) Mutagens in contaminated soil: a review, Mutation Research, 567 227-345

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