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### ► To cite this version:

J.F. Masfaraud, V. Beaunoir, Y. Perrodin, Eric Thybaud, D. Savanne, et al.. Assessment of soil contamination and ecotoxicity by the use of an ascending flow percolation - bioassay approach. 5. European Conference on Ecotoxicology and Environmental Safety (SECOTOX 1999), Mar 1999, Munich, Germany. pp.NC. ineris-00972192

**HAL Id: ineris-00972192**

**<https://ineris.hal.science/ineris-00972192>**

Submitted on 3 Apr 2014

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# ASSESSMENT OF SOIL CONTAMINATION AND ECOTOXICITY BY THE USE OF AN ASCENDING FLOW PERCOLATION - BIOASSAY APPROACH

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## INTRODUCTION

Soil contamination and subsequent ecotoxicity are to be assessed by the use of appropriate methodologies allowing high repeatability and reproducibility. Non-direct methods consist in preparing either lixiviates or percolates which can be analysed for chemical contaminants and whose toxicities on aquatic organisms may be further determined.

During experimental water percolation through soil columns, the movement of water is generally due to natural gravity. Here we proposed a complementary method in which an ascending water flow through the soil column is created. This system was developed in order to minimise boundary flow during percolation, which may result in improving repeatability and reproducibility of the aqueous extraction.

In this work, the ascending flow percolation method was used for studying soils sampled from 2 contaminated sites. The resulting percolates were then tested for contaminant concentrations, ecotoxicity and genotoxicity.

## MATERIALS AND METHODS

### Percolation

The percolation apparatus comprised a borosilicated glass column ( $\varnothing$  90 mm x 650 mm deep) and a peristaltic pump which delivered pure water at a continuous flow rate of 2.3 ml / minute.

Two soils corresponding to 2 different pollution profiles were studied :

- S<sub>III</sub> : heavy metals, polychlorobiphenyls (PCBs), adsorbable halogenated organics (AOX)

- S<sub>IV</sub> : polycyclic aromatic hydrocarbons (PAHs)

Columns were filled with 5 kg of crushed and 5 mm-sieved soil taking care of damming down the matrix in such a way that successive disks of soils were formed. This procedure was shown to allow a better radial diffusion of water into the experimented matrix. For each soil, 3 columns were prepared in order to permit an evaluation of an inter-column variability. For each column, the 1,2  $\mu$ m-fiberglass filtered percolates were entirely collected after 24, 48 or 109 h, i.e. liquid / solid ratios of 0.66, 1.33 and 3 respectively (encoded P 1, P 2 and P 3). Thus, P 1, P 2 and P 3 corresponded to successive fractions and not to cumulative percolates.

### Percolate analysis

Percolates were analysed for conductivity, DOC, Zn, Pb, PCBs, AOX (S<sub>III</sub>) or naphthalene, fluorene, phenanthrene, chrysene, benzo(a)pyrene, fluoranthene, pyrene (S<sub>IV</sub>). Acute toxicity (Microtox™, 24 h-*Daphnia magna* immobilisation test), chronic toxicity (7 day-*Ceriodaphnia dubia* reproduction test, 72 h-*Pseudokirchneriella subcapitata* inhibition test) and genotoxicity (Mutatox™) were assessed according to references 1,2,3 (modified version),4 and 5 respectively.

## RESULTS

### S<sub>III</sub> percolates

Conductivity, DOC, AOX, Zn and Pb exhibited marked decreases (50-90 %) from percolate P 1 to percolate P 3, with a particular drop between P 1 and P 2. On the contrary, PCB concentrations in the successive percolates remained at similar levels.

The acute toxicity of percolates P 1, P 2 and P 3 were comparable (CE 50  $\approx$  3 % in each case) when measured by the Microtox™ assay. With the 24 h-*Daphnia magna* test, a slight decrease in toxicity was observed : the CE 50 of the P 1, P 2 and P 3 percolates reached 28.3, 36.3 and 46.9 % respectively.

The chronic toxicity of percolates as determined by the 72 h-algae assay and the 7 day-*Ceriodaphnia dubia* assay was shown to decrease markedly from percolate P 1 to percolates P 2 and P 3. Although some variability occurred between columns for the algae assay, a 10-fold increase in either CI 50 (algae assay) or LOEC (*Ceriodaphnia dubia* assay) values was observed.

The results from the Mutatox™ assay led to consider the percolates as «genotoxic» or «suspect genotoxic». Nevertheless, the comparison of the P 1, P 2 and P 3 percolates was made difficult since the results depended on the positivity criteria used.

#### **S<sub>IV</sub> percolates**

The P 1, P 2 and P 3 percolates from the S<sub>IV</sub> soil were analysed for PAHs. Contaminants were extracted from the soil matrix and could be measured all along the experiment from the first percolate (P 1) to the third (P 3). The contaminants exhibited different leaching profiles according to the individual molecules considered. Nevertheless, for most compounds, in particular benzo(a)pyrene and fluoranthene, the highest concentrations were found in the percolate P 2.

No toxicity in the percolates was shown when considered the results from the Microtox™ assay, the *Daphnia magna* assay and the *Ceriodaphnia dubia* assay. Only a slight toxicity was observed in the algae assay but no differences between the percolates could be observed. The genotoxicity of the percolates seemed to decrease from P 1 to P 3.

#### **DISCUSSION - CONCLUSION**

The S<sub>III</sub> soil percolates were monitored for several parameters including AOX, Zn, Pb, DOC and PCBs. While some tracer concentrations exhibited a marked decrease between P 1 and P 2/P 3 (AOX, Zn, Pb, DOC), others (PCBs) were approximately similar in the 3 percolates. The leaching profile of the tracers could be compared to the toxicity of the successive percolates P 1, P 2 and P 3 whose toxicities declined as revealed by chronic assays on algae and *Ceriodaphnia dubia*. On the contrary, acute toxicity tests were not able to detect any change in contaminant load in the successive percolates.

For the PAH-contaminated soil (S<sub>IV</sub>), a leaching of pollutants was observed in the 3 successive percolates but the resulting concentrations were not sufficient enough to induce a toxicity as revealed by the acute and chronic ecotoxicity assays. The genotoxicity declined from P 1 to P 2 and P 3 percolates which is surprising since the highest concentrations in PAHs, particularly benzo(a)pyrene, a well-known genotoxicant, were measured in the P 2 percolate. This suggested that an unidentified and water soluble genotoxic agent was present initially in the soil.

In both cases, the use of ascending flow column for the assessment of soil contamination and ecotoxicity allowed to study the leaching of contaminants as well as the ecotoxicity of successive percolates. For the 2 soils used in this study, the results concerning chemical analysis or toxicity obtained with the 3 columns were generally reproducible for most of the parameters considered. This demonstrates the usefulness of the proposed approach for the hazard assessment of contaminated sites and the risk assessment for potential soil contaminants.

## ACKNOWLEDGEMENTS

The research presented is supported by the « Agence de l'Environnement et de la Maîtrise de l'Energie » (ADEME, contract n°97 93 008).

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