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1 **Comparison of solid and liquid-phase bioassays using ecoscores to assess**  
2 **contaminated soils**

3

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15

16 **Abstract**

17

18 Bioassays on aqueous and solid phases of contaminated soils were compared,  
19 belonging to a wide array of trophic and response levels and using ecoscores for  
20 evaluating ecotoxicological and genotoxicological endpoints. The method was applied to

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1 four coke factory soils contaminated mainly with PAHs, but also to a lesser extent by  
2 heavy metals and cyanides. Aquatic bioassays do not differ from terrestrial bioassays  
3 when scaling soils according to toxicity but they are complementary from the viewpoint of  
4 ecological relevance. Both aquatic and terrestrial endpoints are strongly correlated with  
5 concentrations of 3-ring PAHs. This evaluation procedure allows us to propose a cost-  
6 effective battery which embraces a wide array of test organisms and response levels: it  
7 includes two rapid bio-assays (Microtox<sup>®</sup> and springtail avoidance), a micronucleus test  
8 and three bio-assays of a longer duration (algal growth, lettuce germination and springtail  
9 reproduction). This battery can be recommended for a cost-effective assessment of  
10 polluted/remediated soils.

11

## 12 **Capsule**

13

14 Aqueous and solid phases of contaminated soils give similar results in terms of toxicity but  
15 are complementary for the evaluation of environmental hazards by ecoscores.

16

17 *Keywords:* PAHs; Heavy metals; Contaminated soils; Solid-phase bioassays; Liquid-  
18 phase bioassays; Toxicity; Ecoscores

19

## 20 **1. Introduction**

21

22 Some industrial activities can generate hazardous chemicals that may contaminate  
23 soils located in the vicinity of plants. Soil pollutants include polycyclic aromatic

1 hydrocarbons (PAHs) and heavy metals, which represent an important environmental  
2 concern, due to potential adverse ecological and toxicological effects (Bispo et al., 1999;  
3 Boularbah et al., 2006). Hazard assessments of polluted soils are usually performed by a  
4 chemical approach. However, chemical data do not give an exhaustive list of all the  
5 contaminants contributing to toxicity. Indeed, soil pollutant concentrations do not provide  
6 any information on (i) biological effects of toxic compounds, (ii) synergetic or antagonistic  
7 interactions between pollutants, and (iii) bioavailability of pollutants (Maxam et al., 2000;  
8 Juvonen et al., 2000; Vasseur et al., 2008). As a consequence, pollutants, even when  
9 present at weak or undetectable concentrations, can generate negative effects on the  
10 activities of organisms. In order to estimate the actual risk of contaminants, chemical  
11 analyses must be complemented with biological and toxicological assays, including  
12 organisms belonging to a wide array of trophic levels, and living both in solid and aqueous  
13 phases (Hoffman et al., 2002), avoidance responses of motile organisms being  
14 considered as an 'early warning signal', to be added to current environmental risk  
15 assessment (Hellou, 2011).

16 Solid-phase and liquid-phase bio-assays on soils have been compared in order to  
17 assess whether they were complementary, i.e. by addressing different kinds of ecotoxicity,  
18 or redundant, i.e. by classifying soils in the same order of ecotoxicity (Juvonen et al.,  
19 2000; Pandard et al., 2006; Eom et al., 2007; Leitgib et al., 2007; Manzo et al., 2008).  
20 There were clear discrepancies according to the type of soils which were studied and to  
21 the type of pollutant to which each bio-assay was sensitive. It thus appears that there is a  
22 need for a method allowing bulk comparisons between soils, between phases of the same  
23 soil and between response levels of organisms belonging to widely diverging trophic  
24 groups such as plants, protists and terrestrial and aquatic metazoa.

25 The present work complements a previous study by Lors et al. (2010a) who  
26 assessed the toxicity of several contaminated soils issued from a former coal tar distillery

1 by using chemical and ecotoxicological analyses. Studied soils differed by their PAH  
2 concentration and by the occurrence or not of a mixed pollution of heavy metals and/or  
3 cyanides. The toxicity of these soils was determined with solid-phase bioassays based on  
4 the germination and growth of the lettuce *Lactuca sativa*, the survival of the earthworm  
5 *Eisenia fetida* and the avoidance behaviour and the reproduction of the springtail *F.*  
6 *candida*. Ecoscores, a numerical method rescaling results from methods using a variety of  
7 concentration ranges of polluted soils, was used to facilitate comparisons between  
8 different endpoints, and between soils. This study concluded to the higher sensitivity of  
9 behavioural (avoidance) tests compared to toxicological bio-assays based on growth and  
10 reproduction, and to a clear allocation of organism responses to the concentration in 3-  
11 ring PAHs.

12 In the present paper, the toxicity of water extracts obtained from the same soils  
13 (Lors et al., 2010a) was evaluated on organisms representing different trophic levels. The  
14 acute effects of soil water extracts were assessed by Microtox<sup>®</sup> and *Daphnia magna* tests,  
15 and their chronic effects by the growth inhibition of *Pseudokirchneriella subcapitata* and  
16 *Brachionus calyciflorus* populations. The genotoxicity of aqueous phases was analyzed  
17 through the micronucleus test applied on the mouse lymphoma L5178Y.

18 We will ask whether ecoscores, a numerical method rescaling results from  
19 methods using a variety of concentration ranges of polluted soils (Lors et al., 2010a), can  
20 be used to (1) facilitate comparisons between endpoints of aqueous and solid phase bio-  
21 assays, and (2) suggest a restricted battery of tests to perform a cost-effective risk  
22 assessment of polluted soils.

23

## 24 **2. Materials and methods**

25

## 1 2.1. Soil samples

2

3 Soils were provided from three industrial sites located in the North of France. Soil 1  
4 and Soil 2 came from two former industrial sites where coal distillation took place from  
5 1925 to 1971 and from 1925 to 1973, respectively. These soils were fairly polluted with a  
6 mixture of PAHs, cyanides and heavy metals (Lors et al., 2010a). Soil 2 was  
7 bioremediated from October 1995 to June 1997 by landfarming: despite biotreatment it  
8 still contained a high amount of PAHs, similar to that of Soil 3. Soil 3, came from an  
9 industrial site where coal tar distillation took place from 1923 to 1987, it was only  
10 contaminated with PAHs. Soil 3 was treated by a windrow process from August 2003 to  
11 February 2004 (Lors et al., 2010a). The biotreatment process was detailed in Lors et al.  
12 (2010b). Soil 3T, which represented the soil after biotreatment, contained the lowest PAH-  
13 concentration. Data on soil texture, pH, total C, N, P were provided in Lors et al. (2010b).

14 Twenty to 30 kg of Soil 1 and Soil 2 were sampled from Site 1 and Site 2,  
15 respectively, between 1 and 2 m of depth in the non-waterlogged zone and in 10 randomly  
16 chosen plots. Sampling of Soil 1 and Soil 2 took place in February 2002. Soil samples  
17 were then mechanically homogenized and sieved at 4 mm. Soil 3 and Soil 3T were also  
18 sieved at 4 mm after a specific sampling procedure described in Lors et al. (2010b). They  
19 were sampled in August 2003 and February 2004, respectively.

20 Unpolluted soils were also sampled in uncontaminated areas of the three studied  
21 sites. These soils were used as controls in the avoidance test and as a matrix of dilution in  
22 terrestrial ecotoxicity bioassays. Previous chemical and ecotoxicological analyses were  
23 performed on control soils, which did not reveal any ecotoxicity.

24

## 25 2.2. Water extraction of soil samples

1

2 To realize liquid-phase bioassays on soil samples, a first step of extraction by  
3 water was necessary. Soil water extraction was carried out according to ISO (2007)  
4 without any preliminary filtration. Soil water extraction was carried out with a liquid/solid  
5 ratio of 10/1 (170 g of soil in 1.7 l of distilled water) at 20°C in 2 l glass bottles for 24 h at a  
6 stirring rate of 60 rpm. After decantation for 15 min, the soil suspension phase was  
7 centrifuged at 2,000 g during 30 min and stored at 4°C until ecotoxicological analysis.

8

### 9 *2.3. Chemical analyses of soil samples and water extracts*

10

11 Metals, PAHs (list of 16 US-EPA PAHs) and cyanides were measured on the  
12 tested soils and on water extracts of all soils. These chemical analyses were done in  
13 triplicate.

14 Metals (As, Cd, Co, Cr, Cu, Ni, Pb, Zn) were dosed by Inductive Coupled Plasma  
15 Atomic Emission Spectrometry (ICP-AES) in a 138 Ultrace Jobin Yvon® analyser  
16 according to ISO (2008b). For the soil samples, a hot digestion of the solid phase was  
17 carried out with hydrofluoric and perchloric acids, according to ISO (2001).

18 Concentrations of the 16 PAHs of the US-EPA list (Verschueren, 2001) were  
19 dosed in soil and and water extracts according to ISO (1998b). However, PAH  
20 concentration in water extracts did not include acenaphthylene. The separation of PAHs  
21 from water extracts was conducted with dichloromethane. The extraction of PAHs from  
22 soil samples was carried out with the solvent extractor system Dionex® ASE 200 (Dionex  
23 Corporation®, Sunnyvale, CA), allowing a solid/liquid extraction with a solvent mixture  
24 (dichloromethane/acetone ratio 1/1 v/v) for 15 min at 100°C and  $13.8 \times 10^6$  Pa. The

1 solvent was evaporated under an air stream to nearly dryness and the samples were  
2 diluted with acetonitrile before 0.45  $\mu\text{m}$  filtration. The concentrations of the 16 PAHs were  
3 dosed in the extracts by High Performance Liquid Chromatography (Waters<sup>®</sup> HPLC 2690,  
4 Milford, MS), coupled to a UV photodiode array detector (Waters<sup>®</sup> 996). PAHs were  
5 separated in a column (Supelco<sup>®</sup> C18 reverse phase, length 250 mm, internal diameter  
6 2.1 m) using a gradient of acetonitrile/water solvent. PAH analyses were carried out in soil  
7 water extracts by HPLC (Waters<sup>®</sup> 2690, Milford, MS) coupled to a fluorescence detector.  
8 The ratio between PAH concentration in water extract and PAH concentration in soil  
9 allowed to determine the PAH water extraction capacity of the studied soils.

10 Cyanides were dosed in soils and water extracts according to ISO (2003) and ISO  
11 (2002), respectively.

12

#### 13 *2.4. Ecotoxicological analyses*

14

15 Bioassays were performed to assess the direct toxicity of soils and soil water  
16 extracts to terrestrial and aquatic organisms, respectively. A description of the bioassays,  
17 comprising test procedure, type of toxicity measured, selected endpoints, duration and  
18 tested organisms, was given in Table 1. The set of bioassays included bioassays of acute,  
19 chronic and genotoxicity effects, using organisms which were representative of a variety  
20 of trophic levels.

21 Toxicity results were the responses of test organisms according to soils or water  
22 extracts in test media (% w/w). The results were calculated as concentrations producing  
23 no significant effect (NOEC), percent inhibition at the highest concentration of the tested  
24 sample or as concentrations decreasing the measured endpoint by 10%, 20% and 50%  
25 [E(L)C<sub>10</sub>, E(L)C<sub>20</sub> and E(L)C<sub>50</sub>, respectively] compared to controls.



1

2 *2.4.1. Terrestrial toxicity tests*

3

4 The toxicity of soils was evaluated with the same bioassays than those used by  
5 Lors et al. (2010a). Acute toxicity bioassays included phytotoxicity tests on *Lactuca sativa*  
6 (ISO, 2005) and the survival test on *Eisenia fetida* (ISO, 1993). Chronic effects were  
7 evaluated on springtail (*Folsomia candida*) reproduction according to ISO (1999) modified  
8 by Martínez Aldaya et al. (2006). An avoidance test was conducted on *Folsomia candida*  
9 according to Martínez Aldaya et al. (2006) and Lors et al. (2006). Detailed procedures of  
10 these bioassays were described by Lors et al. (2010a). The pH of all soils was compatible  
11 with requirements of test organisms, varying from 7.8 for Soil 2 to 8.3 for Soil 3T (Lors et  
12 al., 2010a).

13

14 *2.4.2. Aquatic (geno)toxicity tests*

15

16 All aquatic bioassays were performed within 24 h after preparation.

17 The toxicity of water extracts to aquatic organisms was assessed through both  
18 acute and chronic effects. Acute toxicity tests were performed by measuring the inhibition  
19 of bioluminescence of the bacterium *Vibrio fischeri* according to ISO (1998a) and the  
20 immobilization of the crustacean *Daphnia magna* according to ISO (1996). Chronic toxicity  
21 was evaluated on growth of the fresh water alga *Pseudokirchneriella subcapitata*  
22 according to ISO (2004) and the planktonic rotifer *Brachionus calyciflorus* according to  
23 ISO (2008a). The pH of the water extracts was close to 8 for all soils studied, which is  
24 compatible with the validity domain of toxicity tests performed in the present study.

1           The in vitro micronucleus assay was performed according the procedure described  
2 by Nesslany and Marzin (1999). This micro-method used mouse lymphoma cells L5178Y.  
3 The micronucleus assay was performed with and without S9 metabolic activation using  
4 the 9000 g cell supernatant from livers of Aroclor 1254-treated rats.

5

### 6 *2.4.3. Data analysis*

7

8           Toxic effects were calculated as percentages of inhibition at a given concentration  
9 or as  $LE_{Cx}$  values. Percent inhibition was determined with respect to the control soil.  $LE_{Cx}$   
10 values were calculated following adjustment of data to a log-probit logistic model  
11 (Litchfield and Wilcoxon, 1949). NOEC was the highest concentration tested that did not  
12 significantly differ from control at type I error ( $\alpha$ ) of 5%. LOEC was not used and was  
13 replaced by  $EC_{10}$  or  $LC_{10}$ . Toxicity values were also expressed into Toxic Units (TU), using  
14 the formula  $TU = 100/EC(or LC)_{50}$ .

15           Ecoscores were calculated from five ecotoxicological parameters,  $E(L)C_{50}$ ,  $E(L)C_{20}$ ,  
16  $E(L)C_{10}$ , NOEC, and % inhibition, by giving to each endpoint value a score between 0 and  
17 3 as a function of its intensity, according to the scales defined by Lors et al. (2010a):

- 18       • for  $E(L)C_{50}$ ,  $E(L)C_{20}$ ,  $E(L)C_{10}$ , and NOEC
  - 19           ○ 0 = no effect ( $x > 100$ )
  - 20           ○ 1 = weak effect ( $50 < x \leq 100$ )
  - 21           ○ 2 = medium effect ( $20 < x \leq 50$ )
  - 22           ○ 3 = strong effect ( $x \leq 20$ )
- 23       • for % inhibition
  - 24           ○ 0 = no effect ( $x \leq 5$ )
  - 25           ○ 1 = weak effect ( $5 < x \leq 20$ )

- 1           ○ 2 = medium effect ( $20 < x \leq 60$ )
- 2           ○ 3 = strong effect ( $x > 60$ )

3 The five ecoscores were summed up and the total was rescaled to 100 for maximum  
4 intensity of the five endpoints.

5           Correlation analysis, using the Pearson product-moment correlation coefficient ( $r$ ),  
6 was used to explore possible linear relationships between physicochemical parameters  
7 and toxicity endpoints. The probability ( $P$ ) of reaching values higher than the observed  
8 value with the null hypothesis (absence of correlation) was also given, as well as the  
9 coefficient of determination ( $R^2 = r^2$ ) of linear regression, which expresses the part of the  
10 total variance of a parameter which is explained by a linear relationship with another  
11 parameter.

12           All calculations were done with the statistical software XSTAT<sup>®</sup> (Addinsoft<sup>®</sup>, Paris,  
13 France), using Excel<sup>®</sup> (Microsoft Corporation<sup>®</sup>, Redmond, WA).

14

### 15 **3. Results**

16

#### 17 *3.1. Chemical analyses of soils*

18

19           Concentrations of 16 PAHs, cyanides and metals of studied soils (average values  
20 of three replicate measures) were reported in Table 2, together with geochemical  
21 background values and Predicted No-Effect Concentrations (PNEC) for the sake of  
22 comparison. All studied soils exhibited PAH concentrations which were far above PNEC  
23 values, except for acenaphthylene in Soil 3 after bio-treatment (Soil 3T).

24           Soil 1 and Soil 2 showed a dual organic and inorganic contamination. Soil 1

1 contained low amounts of PAHs, cyanides and heavy metals. Soil 2 was highly  
2 contaminated with PAHs, despite landfarming treatment and was contaminated with PAHs  
3 of higher molecular weight than Soil 1. It was also contaminated with heavy metals, in  
4 particularly Zn, Pb, Cu and Cd, contents of which were 7 to 12 times the geochemical  
5 background and all above PNEC values. Cyanides were also in considerable amount in  
6 Soil 2.

7 Contrarily to Soil 1 and Soil 2, Soil 3 was mainly contaminated with organic  
8 compounds, and particularly with PAHs which amounted to the same level as Soil 2.  
9 Despite similar global amounts of PAHs in Soils 2 and 3, their distribution was different,  
10 Soil 3 containing mainly 2-, 3- and 4-ring PAHs. Conversely, heavy metals and cyanides  
11 were not important contaminants of Soil 3, with concentrations close to the geochemical  
12 background, and equal or below PNEC values. It should be noted that the geochemical  
13 background for Ni in the study region was somewhat above PNEC values. Soil 3T, which  
14 corresponded to Soil 3 after six months of windrow biotreatment, contained a much lower  
15 PAH concentration (nearly 10 times less) than Soil 3. In particular biotreatment led to a  
16 strong degradation of 2-, 3- and 4-ring PAHs. As Soil 3 contained low concentrations of  
17 heavy metals, no evolution of their content was observed after biotreatment.

18 Control soils sampled in each site displayed very low contents of PAHs ( $< 10 \text{ mg}$   
19  $\text{kg}^{-1}$  dry soil), metals (around geochemical background) and cyanides (content below 0.1  
20 to  $0.2 \text{ mg kg}^{-1}$  dry soil), like already shown by Lors et al. (2010b).

21

### 22 *3.2. Chemical analyses of water extracts*

23

24 The chemical characteristics of water extracts of studied soils were presented in  
25 Table 3. Metals and cyanides (data not shown) were at concentrations lower than

1 detection limits, except for Cu and Pb in Water extract 2 (0.06 and 0.04  $\mu\text{g l}^{-1}$ ,  
2 respectively).

3 Water extract 1 contained a very low PAH content (although above PNEC values),  
4 3- and 4-ring PAHs being the most easily leached (Fig. 1). Among them, the major  
5 compounds in solution were anthracene and pyrene (Table 3). Their occurrence in Water  
6 extract 1 was related to their concentration in Soil 1, where 3- and 4-ring PAHs were the  
7 most represented among the 16 PAHs of the US-EPA list (Table 2).

8 Water extract 2 was distinguished from Water extract 1 by higher amounts of  
9 PAHs, the water extraction capacity of Soil 2 being three times that of Soil 1 (Table 4).  
10 Moreover, 5- and 6-ring PAHs occurred in major proportions, (Fig. 1), and reached higher  
11 concentrations than in Water extract 1 (Table 3).

12 Water extract 3 was highly contaminated with PAHs, the soil water extraction  
13 capacity of Soil 3 (ratio water extract/soil in PAH concentration) being near ten times that  
14 of Soil 2 (Table 4). All concentration values of individual PAHs in Water extract 3 were  
15 well above corresponding PNEC values. Water extract 3 mainly contained 3-ring PAHs  
16 (Fig. 1). Their high concentration in solution was linked to their high amount in Soil 3  
17 (Table 2). Four-ring PAHs were also present but in a smaller proportion (Fig. 1). Five- and  
18 6-ring PAHs were in weaker amounts (Fig. 1). Naphthalene, which was in a high amount  
19 in Soil 3, was surprisingly present at a small concentration (0.2  $\mu\text{g l}^{-1}$ ) in the corresponding  
20 water extract. This was probably due to its volatilization during water extraction, given its  
21 high vapour tensile strength (37 Pa). The  $\Sigma$ -PAH concentration of Water extract 3T was  
22 clearly weaker than that of Water extract 3. The biotreatment of Soil 3 thus allowed a  
23 considerable reduction (84%) of PAHs extracted by water, which mainly concerned 3-ring  
24 PAHs (99%). Three-ring PAHs were present in a very small proportion, not exceeding 5%.  
25 Five- and 6-ring PAH concentrations measured in Water extract 3T were double those of  
26 Water extract 3 (Table 3), contrary to what was observed in the corresponding soil

1 matrices, for which a reduction in their content was detected (Table 2).

2

### 3 3.3. Toxicological analyses of water extracts

4

5 Water extract 1 did not exhibit any genotoxicity, as estimated by the micronucleus  
6 test (Table 5). Acute and chronic toxicity tests displayed contrasting results according to  
7 methods in use. Among acute toxicity tests, the Microtox<sup>®</sup> test was more sensitive than  
8 the *Daphnia* mobility test, as shown by ecoscores. Conversely, among chronic toxicity  
9 tests, the algal growth test was more sensitive than the rotifer test. On the basis of  
10 Microtox<sup>®</sup> and algal growth tests, Water extract 1 presented moderate acute and chronic  
11 toxicities as shown by EC<sub>10</sub> and % of inhibition compared to control.

12 Water extract 2 did not show any genotoxicity as estimated by the micronucleus  
13 test (Table 6). Moreover, it did not exhibit any acute toxicity with respect to *Vibrio fischeri*  
14 (Microtox<sup>®</sup>) and *Daphnia magna* (micro-crustacean) tests. Conversely, algal and rotifer  
15 tests revealed chronic toxicity, according to ecoscores. The chronic toxicity of Water  
16 extract 2 was higher than that of Water extract 1. As observed for Water extract 1, the  
17 algal growth test was more sensitive than the rotifer test.

18 Water extract 3 displayed a pronounced acute toxicity whatever the test used  
19 (Table 7). Indeed, this water extract showed a significant effect on the inhibition of the  
20 luminescence of *Vibrio fischeri* and the mobility of *Daphnia magna*. Moreover, it presented  
21 a high chronic toxicity and a high genotoxicity (only without S9).

22 Water extract 3T showed that after six months of biotreatment, acute toxicity  
23 decreased to a great extent, as ascertained by Microtox<sup>®</sup> and *Daphnia* mobility tests, while  
24 chronic toxicity displayed contrasting results according to the test used (Table 8). The

1 genotoxicity of Water extract 3T was nil according to the micronucleus test.

2

### 3 3.4. Comparison of liquid and solid bio-assays by ecoscores

4

5 The two acute toxicity tests on soil water extracts (Microtox<sup>®</sup> and *Daphnia* mobility)  
6 did not exhibit the same sensitivity. While both of them revealed a pronounced toxicity in  
7 the water extract of Soil 3 and did not detect any acute toxicity in the water extract of Soil  
8 2 (Table 9), only Microtox<sup>®</sup> revealed a toxicity in the water extracts of Soils 1 and 3T  
9 (despite biotreatment for the latter), pointing to a better sensitivity of Microtox<sup>®</sup> when  
10 compared to *Daphnia* mobility.

11 Except for Soil 3, where the two chronic toxicity tests revealed a pronounced  
12 toxicity of its aqueous phase (Table 9), the algal growth test was more sensitive than the  
13 rotifer test. In particular the former was the only test able to reveal a chronic toxicity in the  
14 aqueous phase of Soil 1 and Soil 3T.

15 The micronucleus test did not show any genotoxicity in the aqueous phase of  
16 studied soils (Table 9), to the exception of the most contaminated Soil 3.

17 Results of solid phase bio-assays have been already detailed in Lors et al.  
18 (2010a). In short, the behavioural test (avoidance by *Folsomia*) showed a better sensitivity  
19 than ecotoxicity tests. Nevertheless, inhibition of lettuce (*Lactuca*) germination and  
20 springtail (*Folsomia*) population growth tests were more sensitive than earthworm  
21 (*Eisenia*) mortality and lettuce growth inhibition tests. Moreover, the springtail reproduction  
22 test was the only bio-assay which showed some remaining toxicity in Soil 3 after bio-  
23 treatment (Soil 3T). These results seem to show a risk of long-term toxicity of Soil 3T.  
24 However, this response must be confirmed by other chronic bioassays.

1 Mean ecoscores for liquid (5 bio-assays) and solid (5 bio-assays) phases allowed  
2 to compare the sensitivity of both groups of tests (Fig. 2). Both phases were highly  
3 correlated ( $r = 0.996$ ,  $P = 0.004$ ), indicating that water extracts reflected fairly well the  
4 toxicity of solid phases, at least within the limits of selected bio-assays. However, the use  
5 of a restricted number of soils (four in the present study) does not allow concluding  
6 definitely to the existence (or not) of strong relationships between liquid and solid phase  
7 bio-assays. In the same way, when pooling ecoscores, the four soils were classified in the  
8 same increasing order of toxicity for liquid phase bioassays than for solid phase  
9 bioassays: Soil/Water extract 3T << Soil/Water extract 2 < Soil/Water extract 1 <<  
10 Soil/Water extract 3. However, ecoscores calculated on solid-phase bioassays were  
11 always somewhat higher (mean ratio 1.2) than those calculated on liquid-phase  
12 bioassays, indicating a better sensitivity to contamination. In addition, bioassays on both  
13 phases showed that the best correlation of mean ecoscores (pooled over five bioassays)  
14 was obtained with the concentration of 3-ring PAHs:  $r = 0.954$  and  $0.957$ , with  $P = 0.046$   
15 and  $0.043$  for solid and liquid phases, respectively.

16 No significant relationship was observed with heavy metals, which was coherent  
17 with their weak amount and their poor solubility in the studied soils.

18

#### 19 **4. Discussion**

20

21 The use of water extracts of soil samples for the assessment of environmental  
22 hazards of water-mobilised soil pollutants has been critically examined (Leitgib et al.,  
23 2007; Manzo et al., 2008; Pablos et al., 2009). Toxicity endpoints on aquatic organisms  
24 need a previous soil water extraction which does not mimic exactly what happens when a  
25 soil matrix contaminated with PAHs is in permanent exchange with soil solution, in



1 particular when organic or inorganic contaminants are adsorbed on clay surfaces  
2 (Degryse et al., 2009; Changchaivong and Khaodhiar, 2009) and microbial biofilms (Wicke  
3 et al., 2007). Lixiviation tests are cheap and of a short duration, and the water extraction  
4 process does not allow exchanges between matrix and solution to reach equilibrium, even  
5 after 24 h (Gamst et al., 2007), and thus they may underestimate PAH concentrations in  
6 the soil solution according to solubility (Hitchcock and Smith, 1998). Bioassays applied to  
7 soil water extracts allow predicting groundwater hazards of contaminants, but are poorly  
8 predictive when the whole soil is considered as an environmental element (Leitgib et al.,  
9 2007). In the present study we showed on a restricted array of industrial soils mainly  
10 contaminated by PAHs that aquatic bio-assays give the same information (although at a  
11 lower level of sensitivity) as solid phase bio-assays in the evaluation of bulk soil toxicity.  
12 The sensitivity of aquatic bio-assays was expressed by mean of ecoscores, a method  
13 applied by Lors et al. (2010a) on the same soils but to terrestrial organisms only. Both  
14 phases consider different kinds of organisms, not necessarily living in the same micro-  
15 environment (Vannier, 1987) and endpoints are calculated on quite different scales, but  
16 the use of ecoscores in place of original endpoint values facilitates comparisons to be  
17 made between them. The observed convergence of aquatic and terrestrial bio-assays has  
18 been already mentioned in the evaluation of PAH contamination, although not purposely  
19 tested, by several authors (Juvonen et al., 2000; Pandard et al., 2006; Eom et al., 2007;  
20 Manzo et al., 2008). It could be explained by common metabolic routes for PAHs once  
21 contaminants are at the inside of an organism, whether ingested with soil or directly  
22 absorbed from the outside, in particular for low molecular weight PAHs (Leaner and  
23 Mason, 2002; Van de Wiele et al., 2004). We also showed that both aquatic and terrestrial  
24 endpoints were strongly correlated with the concentration of 3-ring PAHs, which are  
25 common to aquatic and solid phases, and cross easily cell membranes (Kang et al.,  
26 2010).

27 The lower sensitivity of liquid-phase bioassays can be explained by (i) the time

1 needed for equilibrium to be reached between the soil matrix and the water extract  
2 suspension, more especially for PAHs with a high molecular weight, and (ii) the lower  
3 concentration of PAHs in the water extracts compared to the bulk soil (Table 4). It should  
4 be noticed that the studied soils are old coke factory sites, where industrial activities  
5 ceased for more than 20 years, resulting in lower exchanges between liquid and solid  
6 phases due to physically- and microbially-enhanced immobilisation of PAHs and heavy  
7 metals during ageing of the soil matrix (Sayer et al., 1999; Eom et al., 2007; Zhao et al.,  
8 2009). This may explain the low level of liquid-phase ecotoxicity observed in these soils,  
9 when compared to solid phases, and reinforces the usefulness of an ecotoxicological  
10 approach incorporating the whole soil (Vasseur et al., 2008).

11 We showed that (i) among aquatic-phase (indirect) toxicity bio-assays the  
12 Microtox<sup>®</sup> test (inhibition of bioluminescence of *V. fischeri*) and the algal  
13 (*Pseudokirchneriella*) growth test were more sensitive than the *Daphnia* mobility and the  
14 rotifer (*Brachionus*) growth tests, (ii) among solid-phase (direct) toxicity bio-assays the  
15 inhibition of lettuce (*Lactuca*) germination, the springtail (*Folsomia*) population growth and  
16 avoidance tests were more sensitive than earthworm (*Eisenia*) mortality and lettuce  
17 growth inhibition tests. All these bio-assays exhibited ecoscores higher than 40 (Table 9).  
18 To these ecotoxicity tests should be added the micronucleus test, which detected with a  
19 high ecoscore (80) the genotoxicity of the aqueous phase of Soil 3 (Table 9). That  
20 genotoxicity was detected by the micronucleus test in the absence of S9 activator  
21 indicated direct genotoxicity. This may allow us to suggest a reduced test battery of six  
22 tests (Table 9) including two rapid bio-assays (Microtox<sup>®</sup> and springtail avoidance, a few  
23 minutes each) and three bio-assays of a longer duration (algal growth, lettuce germination  
24 and springtail reproduction, a few days to a few weeks each). Our method of selection,  
25 based on ecoscores, differed from that of Pandard et al. (2006), who used a multivariate  
26 analytical method, Principal Components Analysis followed by cluster analysis of a data  
27 matrix crossing a variety of soils and end-points: in their evaluation procedure, which did

1 not include springtail tests, the classification of soils by a reduced battery of tests was  
2 compared to that obtained with the complete set of bioassays. Both methods selected  
3 Microtox<sup>®</sup> and lettuce germination for the restricted battery, but they differed mainly in the  
4 inclusion of springtail avoidance and reproduction tests in our complete battery: these  
5 tests proved to be particularly sensitive and were kept in our restricted battery. Compared  
6 to multivariate analyses, the use of ecoscores, each endpoint being evaluated separately  
7 from the others (Lors et al., 2010a) allows addition or deletion of a test (or of a sample)  
8 without interfering with the evaluation of other endpoints (or samples).

9

## 10 **5. Conclusion**

11

12 In the present study, environmental toxicity methods were compared in a restricted  
13 array of four coke factory soils contaminated mainly with PAHs at varying concentrations  
14 (all above expected risk) and to a lesser extent with heavy metals and cyanides. Toxicity  
15 was evaluated both on the whole soil and on the soil extract. Solid-phase bio-assays were  
16 based on the germination and growth of the lettuce *Lactuca sativa*, the survival of the  
17 earthworm *Eisenia fetida* and the avoidance behaviour and the reproduction of the  
18 springtail *Folsomia candida*. The toxicity of water extracts of soils was determined by  
19 acute Microtox<sup>®</sup> and *Daphnia magna* tests, chronic *Pseudokirchneriella subcapitata* and  
20 *Brachionus calyciflorus* tests and genotoxic micronucleus test on the mouse lymphoma  
21 L5178Y.

22 On the basis of the ecoscore method of evaluation of test performance, we  
23 showed that liquid-phase bio-assays allowed classing the four contaminated soils in the  
24 same order than soil-phase bio-assays, although at a somewhat lower level of sensitivity  
25 ( $\times 0.8$ ).

1           In addition, both solid- and liquid-phase bio-assays were strongly correlated with  
2 concentration of 3-ring PAHs. According to our results, and within the limits of our  
3 restricted study which did not include a variety of pollution sources, a restricted battery of  
4 six bio-assays can be proposed, including both solid and liquid phases and addressing  
5 acute as well as chronic toxicity and genotoxicity: two rapid bio-assays (Microtox<sup>®</sup> and  
6 springtail avoidance, a few minutes each), a one-day bio-assay (micronucleus test) and  
7 three bio-assays of a longer duration (algal growth, lettuce germination and springtail  
8 reproduction, a few days to a few weeks each). This battery of bio-assays could be  
9 advised for a cost-effective assessment of contaminated/remediated soils.

10

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12

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17 genotoxicological analyses.

18

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2

1 **Figure captions**

2

3 **Fig. 1.** Distribution of PAHs in soil water extracts.

4

5 **Fig. 2.** Correlation between ecoscores calculated for liquid-phase (abscissa) and solid-  
6 phase (ordinate) bio-assays. \*\* = significant at 0.01 level. 1, 2 or 3T = Soil or Water  
7 extract 1, 2 or 3T according to solid- or liquid-phases, respectively.

8

1 **Table 1.** Characteristics of bioassays used to assess the toxicity of the studied soils and  
 2 water extracts

<b>Organisms</b>	<b>Contact</b>	<b>Test duration</b>	<b>Endpoint</b>	<b>Type of toxicity</b>	<b>Test procedure</b>
Plants <i>Lactuca sativa</i>	Direct	14 d	Germination and growth inhibition	Acute	ISO (2005)
Earthworms <i>Eisenia fetida</i>	Direct	14 d	Survival	Acute	ISO (1993)
Collembola <i>Folsomia candida</i>	Direct	20 min	Avoidance	Acute	Not standardized
Collembola <i>Folsomia candida</i>	Direct	40 d	Reproduction	Chronic	ISO (1999)
Bacteria <i>Vibrio fischeri</i> (Microtox®)	Water	30 min	Luminescence inhibition	Acute	ISO (1998)
Micro-crustaceans <i>Daphnia magna</i>	Water	48 h	Immobilization	Acute	ISO (1996)
Algae <i>Pseudokirchneriella subcapitata</i>	Water	72 h	Growth inhibition	Chronic	ISO (2004)
Rotifers <i>Brachionus calyciflorus</i>	Water	48 h	Growth inhibition	Chronic	ISO (2008)
Animals Mouse lymphoma cells	Water	24 h	Chromosomic change	Genotoxicity	Not standardized

3

4

1 **Table 2.** Concentrations of 16 PAHs, cyanides and heavy metals in tested soils  
 2 (expressed in mg kg<sup>-1</sup> dry soil ± SE), compared with the geochemical background, i.e.  
 3 concentrations measured over a wide range of unpolluted agricultural and forest soils  
 4 (Sterckeman et al., 2002) and with Predicted No Effect Concentrations (PNEC) on various  
 5 terrestrial organisms, according to CTPHT (2008) for PAH and Smolders et al. (2009) for  
 6 trace metals

	Soil 1	Soil 2	Soil 3	Soil 3T	Geochemical background	PNEC
<b>Naphthalene</b>	19.2 ± 0.7	150.9 ± 10.6	594.2 ± 13.8	11.1 ± 4.9		1.0
<b>Acenaphthylene</b>	1.9 ± 0.04	23.5 ± 1.1	3.1 ± 0.1	0.2 ± 0.4		0.29
<b>Acenaphthene</b>	1.9 ± 0.04	2 ± 0.1	217.4 ± 1.2	7.4 ± 0.6		0.038
<b>Fluorene</b>	19.3 ± 0.3	83.1 ± 3.7	226.8 ± 2.8	4.5 ± 4.2		1.0
<b>Phenanthrene</b>	119.4 ± 17.7	308.2 ± 17.7	629.3 ± 4.2	3.8 ± 0.8		1.8
<b>Anthracene</b>	183.2 ± 8.3	206.7 ± 7	202.5 ± 31.7	19.1 ± 5.9		0.13
<b>Fluoranthene</b>	130.8 ± 22.5	625.2 ± 30.7	414.3 ± 1.2	55.1 ± 11.9		1.5
<b>Pyrene</b>	54.5 ± 9.9	299.4 ± 10.9	233.4 ± 0.4	50.6 ± 9.9		1.0
<b>Benzo[a]anthracene</b>	48.4 ± 9.2	391.9 ± 13.1	85.7 ± 0.9	21.2 ± 2.9		0.079
<b>Chrysene</b>	47.7 ± 8.2	410.4 ± 8.1	75.4 ± 0.9	19.0 ± 2.7		0.55
<b>Benzo[b]anthracene</b>	2.6 ± 1	210.8 ± 5.8	56.2 ± 0.3	42.9 ± 5.4		0.27
<b>Benzo[k]fluoranthene</b>	2.8 ± 0.8	161.9 ± 2.6	25.8 ± 0.3	15.7 ± 1.7		0.27
<b>Benzo[a]pyrene</b>	38.2 ± 2.6	364.1 ± 2.9	60.4 ± 6.7	37.1 ± 4.8		0.053
<b>Dibenzo[ah]anthracene</b>	4.9 ± 0.5	59.3 ± 0.3	6.9 ± 0.2	1.4 ± 0.1		0.054
<b>Benzo[ghi]perylene</b>	15.1 ± 1.5	196.1 ± 6.5	32.5 ± 1	21.4 ± 4.2		0.17
<b>2-ring PAH</b>	19.2 ± 0.4	150.9 ± 6.1	594.2 ± 8	11.1 ± 1		
<b>3-ring PAHs</b>	325.7 ± 15.1	623.5 ± 17.1	1279.2 ± 20.3	35 ± 5.7		
<b>4-ring PAHs</b>	281.3 ± 28.8	1726.9 ± 36.2	808.9 ± 1	145.9 ± 15.8		
<b>5-ring PAHs</b>	48.6 ± 2.8	796.1 ± 6.7	149.2 ± 3.7	97.2 ± 6.9		
<b>6-ring PAHs</b>	26.3 ± 1.4	389.8 ± 4.6	63.2 ± 0.4	56.2 ± 5.4		
<b>Σ 16 PAHs</b>	701.2 ± 48.6	3687.2 ± 48.8	2894.8 ± 38.1	345.4 ± 23.4		
<b>CN<sup>-</sup></b>	6.6	67	0.8	0.5		
<b>As</b>	21.7 ± 0.9	33.4 ± 1.5	6.5 ± 0.3	6.5 ± 0.5	8.9 ± 0.7	
<b>Cd</b>	< 4	5.2 ± 0.01	< 4	< 4	0.4 ± 0.02	1.1
<b>Co</b>	8.0 ± 0.06	48.9 ± 0.8	9.6 ± 0.1	8.9 ± 0.06	9.3 ± 0.5	
<b>Cr</b>	31.9 ± 0.4	52.2 ± 2.3	32.9 ± 0.2	31.3 ± 0.5	48.8 ± 1.6	
<b>Cu</b>	47.8 ± 2.2	126.4 ± 0.2	19.3 ± 0.2	21.0 ± 0.3	16.7 ± 1	33.8
<b>Ni</b>	21.1 ± 0.2	46.9 ± 1.1	19.8 ± 0.1	26.8 ± 1.9	24.7 ± 3.3	13.8
<b>Pb</b>	35.1 ± 2.2	345.7 ± 4	23.9 ± 0.5	25.9 ± 2.1	38.4 ± 3.2	167.9
<b>Zn</b>	75.1 ± 6.9	514.0 ± 6.1	92.7 ± 0.5	104 ± 3.3	73.7 ± 3.6	81.9

7  
8

1 **Table 3.** Concentration ( $\mu\text{g l}^{-1}$ ) of PAHs in water extracts of studied soils (mean  $\pm$  SE)  
 2 compared with Predicted No Effect Concentrations (PNEC) on various fresh water  
 3 organisms, according to CTPHT (2008)

	<b>Water extract 1</b>	<b>Water extract 2</b>	<b>Water extract 3</b>	<b>Water extract 3T</b>	<b>PNEC</b>
<b>Naphthalene</b>	0.09 $\pm$ 0.02	0.62 $\pm$ 0.18	0.20 $\pm$ 0.02	0.28 $\pm$ 0.02	2.0
<b>Acenaphthene</b>	0.09 $\pm$ 0.01	0.62 $\pm$ 0.07	154.1 $\pm$ 1.5	1.03 $\pm$ 0.03	3.8
<b>Fluorene</b>	0.11 $\pm$ 0.02	0.72 $\pm$ 0.14	198.7 $\pm$ 3.7	0.82 $\pm$ 0.03	2.5
<b>Phenanthrene</b>	0.19 $\pm$ 0.02	1.37 $\pm$ 0.2	151.4 $\pm$ 2.8	1.63 $\pm$ 0.1	1.3
<b>Anthracene</b>	0.66 $\pm$ 0.04	1.34 $\pm$ 0.3	26.91 $\pm$ 0.36	1.20 $\pm$ 0.1	0.1
<b>Fluoranthene</b>	0.14 $\pm$ 0.02	4.05 $\pm$ 0.72	51.2 $\pm$ 0.9	20.7 $\pm$ 0.6	0.01
<b>Pyrene</b>	1.18 $\pm$ 0.1	3.11 $\pm$ 0.58	28.9 $\pm$ 0.5	23.8 $\pm$ 0.9	0.023
<b>Benzo(a)anthracene</b>	0.08 $\pm$ 0.03	3.11 $\pm$ 0.56	7.97 $\pm$ 0.14	7.86 $\pm$ 0.26	0.012
<b>Chrysene</b>	0.13 $\pm$ 0.03	3.43 $\pm$ 0.55	8.78 $\pm$ 0.12	3.98 $\pm$ 0.13	0.07
<b>Benzo(b)fluoranthene</b>	0.31 $\pm$ 0.05	8.42 $\pm$ 1.44	3.85 $\pm$ 0.05	10.57 $\pm$ 0.4	0.017
<b>Benzo(k)fluoranthene</b>	0.13 $\pm$ 0.02	2.71 $\pm$ 0.46	2.30 $\pm$ 0.03	5.48 $\pm$ 0.21	0.017
<b>Benzo(a)pyrene</b>	0.33 $\pm$ 0.05	6.86 $\pm$ 1.37	4.41 $\pm$ 0.02	10.79 $\pm$ 0.43	0.022
<b>Dibenzo(a,h)anthracene</b>	0.14 $\pm$ 0.01	3.43 $\pm$ 0.56	0.72 $\pm$ 0.01	1.22 $\pm$ 0.05	0.0014
<b>Benzo(g,h,i)perylene</b>	0.18 $\pm$ 0.02	8.42 $\pm$ 1.70	3.00 $\pm$ 0.03	6.39 $\pm$ 0.27	0.0082
<b>Indeno(1,2,3)pyrene</b>	0.21 $\pm$ 0.02	9.04 $\pm$ 1.77	2.30 $\pm$ 0.02	4.06 $\pm$ 0.17	0.0027
<b>2-ring PAHs</b>	0.09 $\pm$ 0.02	0.62 $\pm$ 0.18	0.20 $\pm$ 0.02	0.28 $\pm$ 0.02	
<b>3-ring PAHs</b>	1.05 $\pm$ 0.09	4.05 $\pm$ 0.72	531.1 $\pm$ 8.3	4.68 $\pm$ 0.27	
<b>4-ring PAHs</b>	1.53 $\pm$ 0.19	13.7 $\pm$ 2.4	96.9 $\pm$ 1.7	56.3 $\pm$ 1.9	
<b>5-ring PAHs</b>	0.91 $\pm$ 0.13	21.4 $\pm$ 3.8	11.3 $\pm$ 0.1	28.06 $\pm$ 1.09	
<b>6-ring PAHs</b>	0.39 $\pm$ 0.03	17.5 $\pm$ 3.5	5.30 $\pm$ 0.05	10.45 $\pm$ 0.44	
<b><math>\Sigma</math>16-PAHs</b>	3.97 $\pm$ 0.46	57.3 $\pm$ 11	645 $\pm$ 12	99.7 $\pm$ 3.6	

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1 **Table 4.** PAH water extraction capacity in water extracts of studied soils expressed by the  
 2 ratio PAH concentration in water extract/soil ( $\text{mg l}^{-1}/\text{mg kg}^{-1}$ )

	<b>Water extract 1</b>	<b>Water extract 2</b>	<b>Water extract 3</b>	<b>Water extract 3T</b>
<b>2-ring PAHs</b>	$4.7 \cdot 10^{-6}$	$4.1 \cdot 10^{-6}$	$3.4 \cdot 10^{-7}$	$2.5 \cdot 10^{-5}$
<b>3-ring PAHs</b>	$3.2 \cdot 10^{-6}$	$6.5 \cdot 10^{-6}$	$4.2 \cdot 10^{-4}$	$1.3 \cdot 10^{-4}$
<b>4-ring PAHs</b>	$5.4 \cdot 10^{-6}$	$7.9 \cdot 10^{-6}$	$1.2 \cdot 10^{-4}$	$3.9 \cdot 10^{-4}$
<b>5-ring PAHs</b>	$1.9 \cdot 10^{-5}$	$2.7 \cdot 10^{-5}$	$7.6 \cdot 10^{-5}$	$2.9 \cdot 10^{-4}$
<b>6-ring PAHs</b>	$1.5 \cdot 10^{-5}$	$4.5 \cdot 10^{-5}$	$8.4 \cdot 10^{-5}$	$1.9 \cdot 10^{-4}$
<b><math>\Sigma</math> 16 PAHs</b>	$5.7 \cdot 10^{-6}$	$1.6 \cdot 10^{-5}$	$2.2 \cdot 10^{-4}$	$2.9 \cdot 10^{-4}$

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1 **Table 5.** Toxicity of Soil 1 according to the battery of solid and liquid bioassays

	<b>EC<sub>50</sub></b> (g.100 g <sup>-1</sup> )	<b>TU</b> 100/EC <sub>50</sub>	<b>EC<sub>20</sub></b> (g.100 g <sup>-1</sup> )	<b>EC<sub>10</sub></b> (g.100 g <sup>-1</sup> )	<b>NOEC</b> (g.100 g <sup>-1</sup> )	<b>Inhibition</b> (%)	<b>Ecoscore</b> (%)
<b><i>Lactuca</i> germination</b>	>100	<1	64.9 (48.9–86.2)	25.3 (13.5–47.5)	<35	20.4	47
<b><i>Lactuca</i> growth inhibition</b>	>100	<1	>100	87.4 (72.5->100)	100	0.7	13
<b><i>Eisenia</i> mortality</b>	NT	NT	NT	NT	NT	0	0
<b><i>Folsomia</i> population growth</b>	93.5 (75.3->100)	1.1	37.0 (30.0-45.7)	22.8 (17.2–30.3)	10	63.6	60
<b><i>Folsomia</i> avoidance</b>	27.1 (19.5–37.6)	3.7	2.9 (2.0–4.3)	0.9 (0.5–1.6)	<0.35	9.5	80
<b>Microtox<sup>®</sup> test</b>	>100	<1	52.7 (39.5-70.2)	23.1 (17.0-31.3)	<6.25	32.7	53
<b><i>Daphnia</i> mobility</b>	NT	NT	NT	NT	NT	0	0
<b>Algal growth</b>	92.5 (80.4->100)	1.1	55.5 (49.4-62.4)	42.5 (36.2-49.9)	25	47.4	53
<b>Rotifer growth</b>	NT	NT	NT	NT	NT	0	0
<b>Micronucleus test (-S9)</b>	NM	NM	NM	NM	NM	N/A	0

2 EC<sub>50</sub>, EC<sub>20</sub>, EC<sub>10</sub> = concentration causing 50%, 20%, 10% inhibition of germination rate,  
3 respectively (confidence interval at 0.05 risk level between brackets); TU = 100/EC<sub>50</sub>; NOEC = no  
4 observed effect concentration; NT = not toxic, observed response statistically indistinguishable from  
5 unpolluted soil sampled on the studied site; NM = not mutagenic; N/A = not applicable; inhibition  
6 (%) = relative decrease in response compared to control soil at the highest dose tested

7



1 **Table 6.** Toxicity of Soil 2 according to the battery of solid and liquid bioassays. Otherwise  
 2 as for Table 5

	<b>EC<sub>50</sub></b> (g.100 g <sup>-1</sup> )	<b>TU</b> 100/EC <sub>50</sub>	<b>EC<sub>20</sub></b> (g.100 g <sup>-1</sup> )	<b>EC<sub>10</sub></b> (g.100 g <sup>-1</sup> )	<b>NOEC</b> (g.100 g <sup>-1</sup> )	<b>Inhibition</b> (%)	<b>Ecoscore</b> (%)
<b><i>Lactuca</i> germination</b>	>100	<1	80.8 (62.9->100)	41.7 (29.9-58.1)	<35	21.4	47
<b><i>Lactuca</i> growth inhibition</b>	>100	< 1	>100	95.1 (83.5->100)	60	10	20
<b><i>Eisenia</i> mortality</b>	>100 (97.8->100)	<1	88.0 (83.0-93.2)	79.1 (72.2-86.7)	60	37.5	33
<b><i>Folsomia</i> population growth</b>	NT	NT	NT	NT	NT	0	0
<b><i>Folsomia</i> avoidance</b>	>100	<1	1.7 (0.6-5.1)	0.04 (0.004-0.403)	<0.35	12.5	67
<b>Microtox test</b>	NT	NT	NT	NT	NT	0	0
<b><i>Daphnia</i> mobility</b>	NT	NT	NT	NT	NT	0	0
<b>Algal growth</b>	80.5 (51.7->100)	1.2	8.4 (5.3-13.5)	2.6 (1.2-5.7)	<6.25	56.5	80
<b>Rotifer growth</b>	>100	<1	30.2 (18.6-49.1)	4.6 (1.7-12.4)	<6.25	32.1	67
<b>Micronucleus test (-S9)</b>	NM	NM	NM	NM	NM	N/A	0

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1 **Table 7.** Toxicity of Soil 3 according to the battery of solid and liquid bioassays. Otherwise  
 2 as for Table 5

	<b>EC<sub>50</sub></b> (g.100 g <sup>-1</sup> )	<b>TU</b> 100/EC <sub>50</sub>	<b>EC<sub>20</sub></b> (g.100 g <sup>-1</sup> )	<b>EC<sub>10</sub></b> (g.100 g <sup>-1</sup> )	<b>NOEC</b> (g.100 g <sup>-1</sup> )	<b>Inhibition</b> (%)	<b>Ecoscore</b> (%)
<b><i>Lactuca</i> germination</b>	21.3 (15.5–29.3)	4.5	3.5 (1.9–6.5)	1.4 (0.6–3.4)	<5	70.9	93
<b><i>Lactuca</i> growth inhibition</b>	16.6 (13.1–21.0)	5.8	2.9 (1.7–4.8)	1.2 (0.6–2.4)	<5	79.3	93
<b><i>Eisenia</i> mortality</b>	0.60 (0.56–0.64)	167	0.51 (0.46–0.55)	0.46 (0.41–0.51)	0.4	100	100
<b><i>Folsomia</i> population growth</b>	2.2 N/A	45.5	2.1 N/A	1.9 N/A	1	100	100
<b><i>Folsomia</i> avoidance</b>	0.8 (0.6–1)	129	0.3 (0.2–0.4)	0.042 (0.008–0.205)	<0.35	100	100
<b>Microtox test</b>	8.1 (6.4–10.1)	12.4	1.5 (0.9–2.3)	0.6 (0.3–1.1)	<2.5	89.1	100
<b><i>Daphnia</i> mobility</b>	50.0 N/A	2.0	50.0 N/A	50.0 N/A	25	100	73
<b>Algal growth</b>	42.9 (40.4–45.6)	2.3	28.0 (25.7–30.5)	22.4 (20.0–25.0)	<20	93.4	80
<b>Rotifer growth</b>	77.2 (58.1–>100)	1.3	19.1 (15.1–24.1)	9.2 (6.5–13.0)	<6.25	57.7	80
<b>Micronucleus test (-S9)</b>	16.1 (13.4–19.3)	6.2	6.8 (5.0–9.4)	4.4 (2.9–6.5)	<12.5	N/A	80

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- 1 **Table 8.** Toxicity of Soil 3T according to the battery of solid and liquid bioassays.
- 2 Otherwise as for Table 5

	<b>EC<sub>50</sub></b> (g.100 g <sup>-1</sup> )	<b>TU</b> 100/EC <sub>50</sub>	<b>EC<sub>20</sub></b> (g.100 g <sup>-1</sup> )	<b>EC<sub>10</sub></b> (g.100 g <sup>-1</sup> )	<b>NOEC</b> (g.100 g <sup>-1</sup> )	<b>Inhibition</b> (%)	<b>Ecoscore</b> (%)
<b><i>Lactuca</i> germination</b>	NT	NT	NT	NT	NT	0	0
<b><i>Lactuca</i> growth inhibition</b>	NT	NT	NT	NT	NT	0	0
<b><i>Eisenia</i> mortality</b>	NT	NT	NT	NT	NT	0	0
<b><i>Folsomia</i> population growth</b>	>100	<1	>100	5.2 (1.8–15.1)	<0.35	27	53
<b><i>Folsomia</i> avoidance</b>	NA	NA	NA	NA	NA	0	0
<b>Microtox test</b>	N/A	N/A	N/A	N/A	N/A	26.7	13
<b><i>Daphnia</i> mobility</b>	NT	NT	NT	NT	NT	0	0
<b>Algal growth</b>	>100	<1	39.1 (32.5-47.1)	21.1 (16.3-27.3)	<10	43.2	60
<b>Rotifer growth</b>	NT	NT	NT	NT	NT	0	0
<b>Micronucleus test (-S9)</b>	NT	NT	NT	NT	NT	N/A	0

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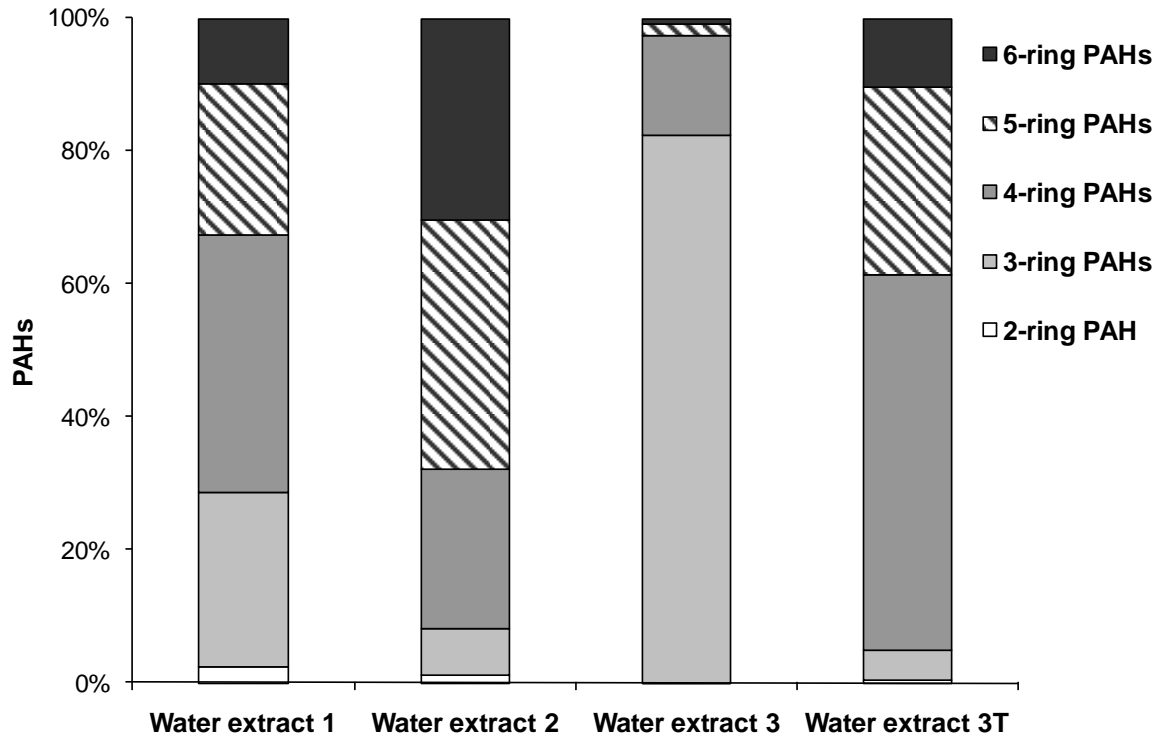
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1 **Table 9.** Ecoscores of the four studied soils obtained with five solid-phase bioassays and  
 2 five liquid-phase bioassays. Total ecoscores are average values of the ten tested  
 3 bioassays (rows) or of the four soils (columns).

	<i>Lactuca</i> germination (*)	<i>Lactuca</i> Growth inhibition	<i>Eisenia</i> mortality	<i>Folsomia</i> Population growth (*)	<i>Folsomia</i> avoidance (*)	Microtox test (*)	<i>Daphnia</i> mobility	<i>Algal</i> growth (*)	<i>Brachionus</i> growth	Micro- Nucleus test (*)	Total	Total reduced battery (*)
Soil 1	47	13	0	60	80	53	0	53	0	0	31	59
Soil 2	47	20	33	0	67	0	0	80	67	0	31	39
Soil 3	93	93	100	100	100	100	73	80	80	80	82	95
Soil 3T	0	0	0	53	0	13	0	60	0	0	13	25
<b>Total</b>	47	32	33	53	62	42	18	68	37	20		

(\*) tests selected for the reduced test battery

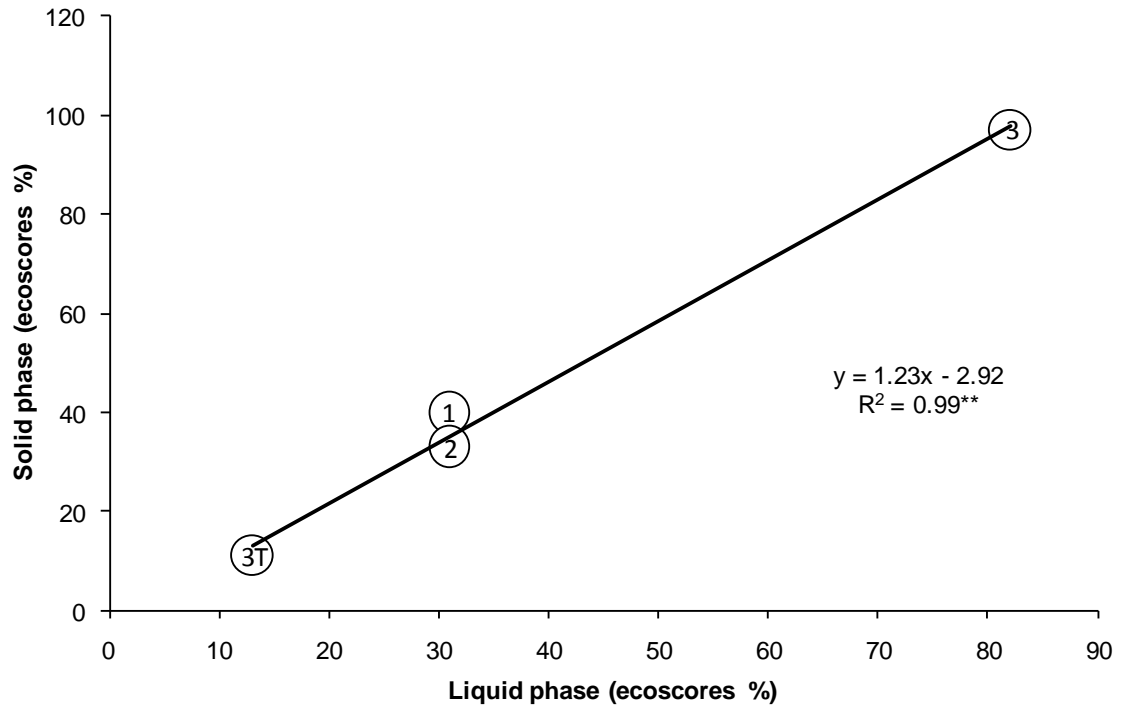
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2 Fig. 1

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2 Fig. 2

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