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# Environmental hazard assessment by the Ecoscore system to discriminate PAH-polluted soils

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**Abstract** A bioassay battery-integrated index was applied to different soils sampled from a former coke factory, with the aim to evaluate the discriminating capacity of the Ecoscore system (ES) to assess the environmental hazard of PAH-polluted soils. Two soils from a former coke factory, polluted with polycyclic aromatic hydrocarbons (PAHs), were evaluated for their ecotoxicity to terrestrial and aquatic organisms and their genotoxicity. These soils have been already presented in a previous paper but data have been reanalyzed for the present article in an endeavor to standardize the ES. One soil was sampled in the untreated site and the second underwent a windrow treatment. While these soils had a similar total concentrations of US-EPA 16PAHs (around 3000 mg kg$^{-1}$), different ecoscores were obtained when subjected to a set of solid- and liquid-phase bioassays measuring acute, chronic, and genotoxic effects. The total PAH content of the soil is not a pertinent parameter to assess soil pollution hazards contrary to the ES. ES is a robust method to classify soils according to their toxicity level. Four levels of toxicity have been defined: no (ecoscore = 0), weak (0 < ecoscore ≤33), moderate (33 < ecoscore ≤67), and strong toxicity (67 < ecoscore ≤ 100). The combination of chemical and toxicological data highlights the relationship between three-ring PAHs and acute ecotoxicity. Conversely, chronic effects of water extracts on algal growth could be explained by high molecular weight PAHs, such as five- and six-ring PAHs.

**Keywords** PAHs · Contaminated soils · Ecotoxicity · Solid bioassays · Liquid bioassays · Bioavailability · Ecoscores

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## Introduction

Industrial activities led to the discharge of a wide range of hazardous chemicals in soils. These pollutants include mostly hydrocarbon aromatic hydrocarbons (PAHs) and heavy metals. PAHs are a group of persistent hydrophobic organic pollutants which contains two or more fused aromatic rings (Cerniglia 1992). PAHs are mainly generated from the incomplete combustion and pyrolysis of organic materials (wood, coal, oil, petrol, and plastics), coal liquefaction and gasification, creosote production, petroleum refining, and other high-temperature industrial processes (Cerniglia 1992; Bispo et al. 1999). PAHs are a major environmental and health concern because of their potentially toxic, mutagenic, and carcinogenic properties (White and Claxton 2004). The US Environmental Protection Agency (US-EPA) has established that 7 compounds among 16 PAHs are potentially carcinogenic to humans (http://www.epa.gov/region5/cleanup/indianaharbor/pdfs/supplementalriskassessmentchapt6.pdf).

PAHs can adversely affect not only human health but also terrestrial and aquatic ecosystems. Hazard risk assessments of polluted soils are usually performed by chemical analysis to determine concentrations of target compounds. However, chemical analysis does not allow to identify all the compounds but only to quantify those which are analyzed. Moreover, it does not provide information on the bioavailability of

pollutants, neither on synergic or antagonistic phenomena between pollutants, nor on their effect on living organisms (Juvonen et al. 2000). In the case of PAHs that are hydrophobic organic pollutants, they may be adsorbed onto soil matrices and thus be less bioavailable (Semple et al. 2003). Soil properties, such as organic matter content and ageing, also play an important role in the bioavailability of PAHs (Chung and Alexander 2002; Riding et al. 2013). These parameters can significantly affect their fate in ecosystems and their impact on species and populations (Peijnenburg et al. 2002). Thus, chemical analysis needs to be complemented with an ecotoxicological approach allowing integrating the effects of all bioavailable contaminants (Fernandez et al. 2005; Leitgib et al. 2007). As a species sensitive to all environmental contaminants does not exist, a battery of bioassays involving organisms at different levels of biological organization and accounting for acute/chronic ecotoxicity and genotoxicity is recommended to evaluate environmental hazards of contaminated soils (Rila and Eisentraeger 2003; Fernandez et al. 2005).

Different approaches were performed to assess soil ecotoxicity: bioassays on whole soil using bacteria, earthworms, collembolans, and plants (Lors et al. 2010); liquid bioassays applied on both water and organic extracts (Bispo et al. 1999); and combinations of both bioassays on whole soil and on soil water extracts (Mendonça and Picado 2002; Eom et al. 2007). Direct toxic effects on terrestrial organisms may reflect the ecotoxicological potential of contaminated soils (Fent 2003; Lors et al. 2010), whereas soil organic extracts may lead to an overestimation of the real bioavailability of organic pollutants (Alexander 1991). A combined approach using terrestrial and aquatic bioassays gave satisfactory results from the viewpoint of ecological relevance (Lors et al. 2011).

Different bioassay battery-integrated indexes were reported to characterize the ecotoxicological risk of contaminated sites, particularly contaminated sediments: a test battery-integrated index (TBI) is reported in the handbook of ISPRA (2011) and a toxicity classification system (TCS) in Persoone et al. (2003). These indexes were used to integrate the results of several tested bioassays. TCS is based on two values: a ranking in five acute hazard classes (no, slight, acute, high acute, very high acute hazards) and a weight score for each hazard class (0 to 3). TBI allows ranking five levels of ecotoxicological risk (not significant, low, medium, high, very high), which differ from those of TCS for the first three classes. The quality of sediments of Taranto seas (Mar Grande and Mar Piccolo) was evaluated by these two integrated systems from a battery of five test species representing different trophic levels (Prato et al. 2015). The results obtained showed a similarity between TCS and TBI for the high levels of ecotoxicological risk. Nevertheless, for the lower levels of toxicity, TCS gave a more severe assessment of risk, classifying sediment samples as high acute toxic whereas TBI indicated a low toxicity.In the present study, the Ecoscore system (ES; Lors et al. 2010) was applied to contaminated soils providing from historical industrial sites.

The first goal of this work is to demonstrate through two historical contaminated soils presenting a similar total concentration of PAHs that chemical analyses are not sufficient to evaluate the hazard of polluted soils and these analyses have to be complemented by ecotoxicological bioassays.

The second goal is to confirm the robustness of a method previously defined by Lors et al. (2011), in order to evaluate the environmental risk through the calculation of an ecoscore. It was thus applied in the present article to two polluted soils, in order to check its robustness. Additionally, the combination of chemical and toxicological data was used to bring some new insights on the ecotoxicity of different types of PAHs.

Data on the factory soils used for the present study have been already presented in Lors et al. (2011), but they have been reanalyzed for the present article, in order to standardize the ES for more user-friendly comparisons between soils. In order to allow a better appraisal of the interest of the ES method to ESPR readers and to facilitate comparison with similar studies, data obtained on the soils under study, as well as the methods we used to analyze them from a chemical and ecotoxicological point of view, will be presented in the foregoing chapters.

## Materials and methods

As mentioned above, analytical and ecotoxicological data have been already presented in Lors et al. (2011). They have been briefly summarized below.

### Soil samples

Experiments were carried out on two contaminated soils, named soil A and soil B, sampled from two historical industrial sites located in the north of France. The distillation of coal tar was the main activity on these sites, taking place from 1923 to 1987 and 1925 to 1973, respectively. Soil A was sampled in the untreated industrial site whereas soil B underwent a windrow treatment for 18 months from October 1995 to June 1997. Despite bioremediation, this soil was still polluted with PAHs, with a total concentration still around that of soil A.

Unpolluted soils were sampled in the two studied sites in uncontaminated areas. Chemical characteristics of these control soils, named control A and control B, are reported in Table 1. These soils were used as controls in the avoidance test and as a dilution matrix in solid-phase bioassays. The ecotoxicological characterization of control soils did not show any ecotoxicity. The procedure used for soil sampling has been described by Lors et al. (2010).

**Table 1** Concentrations of PAHs in soils A and B and in their control soils (expressed in mg $kg^{-1}$ dry soil ± SE) and concentrations of total organic carbon, total organic nitrogen, and total phosphorus in soils A and B (expressed in mg $kg^{-1}$ dry soil ± SE)

| | No. of rings | Soil A | Soil B | Control A | Control B |
|---|---|---|---|---|---|
| Naphthalene | 2 | 594.2 ± 13.8 | 150.9 ± 10.6 | 0.13 ± 0.01 | 0.41 ± 0.01 |
| Acenaphthylene | 3 | 3.1 ± 0.1 | 23.5 ± 1.1 | 0.04 ± 0.001 | 0.01 ± 0.00 |
| Acenaphthene | 3 | 217.4 ± 1.2 | 2.0 ± 0.1 | 0.10 ± 0.11 | 0.01 ± 0.00 |
| Fluorene | 3 | 226.8 ± 2.8 | 83.1 ± 3.7 | 0.02 ± 0.001 | 0.06 ± 0.00 |
| Phenanthrene | 3 | 629.3 ± 4.2 | 308.2 ± 17.7 | 0.08 ± 0.01 | 1.01 ± 0.03 |
| Anthracene | 3 | 202.5 ± 31.7 | 206.7 ± 7.0 | 0.01 ± 0.00 | 0.14 ± 0.01 |
| Fluoranthene | 4 | 414.3 ± 1.2 | 625.2 ± 30.7 | 0.16 ± 0.03 | 2.01 ± 0.05 |
| Pyrene | 4 | 233.4 ± 0.4 | 299.4 ± 10.9 | 0.13 ± 0.01 | 0.55 ± 0.04 |
| Benzo[a]anthracene | 4 | 85.7 ± 0.9 | 391.9 ± 13.1 | 0.08 ± 0.00 | 0.37 ± 0.03 |
| Chrysene | 4 | 75.4 ± 0.9 | 410.4 ± 8.1 | 0.08 ± 0.01 | 0.70 ± 0.02 |
| Benzo[b]anthracene | 5 | 56.2 ± 0.3 | 210.8 ± 5.8 | 0.02 ± 0.001 | 1.13 ± 0.25 |
| Benzo[k]fluoranthene | 5 | 25.8 ± 0.3 | 161.9 ± 2.6 | 0.02 ± 0.001 | 0.25 ± 0.00 |
| Benzo[a]pyrene | 5 | 60.4 ± 6.7 | 364.1 ± 2.9 | 0.03 ± 0.001 | 0.92 ± 0.17 |
| Dibenzo[ah]anthracene | 5 | 6.9 ± 0.2 | 59.3 ± 0.3 | 0.02 ± 0.001 | 0.09 ± 0.00 |
| Benzo[ghi]perylene | 6 | 32.5 ± 1.0 | 196.1 ± 6.5 | 0.10 ± 0.05 | 0.47 ± 0.01 |
| Indeno[123-*cd*]pyrene | 6 | 30.8 ± 0.3 | 193.7 ± 1.6 | 0.10 ± 0.05 | 0.42 ± 0.02 |
| 2-Ring PAH | | 594.2 ± 8.0 | 150.9 ± 6.1 | 0.13 ± 0.01 | 0.41 ± 0.01 |
| 3-Ring PAHs | | 1279.2 ± 20.3 | 623.5 ± 17.1 | 0.26 ± 0.12 | 1.23 ± 0.05 |
| 4-Ring PAHs | | 808.9 ± 1.0 | 1726.9 ± 36.2 | 0.44 ± 0.04 | 3.63 ± 0.14 |
| 5-Ring PAHs | | 149.2 ± 3.7 | 796.1 ± 6.7 | 0.09 ± 0.003 | 2.40 ± 0.43 |
| 6-Ring PAHs | | 63.2 ± 0.4 | 389.8 ± 4.6 | 0.19 ± 0.10 | 0.88 ± 0.02 |
| ∑ 16 PAHs | | 2894.8 ± 38.1 | 3687.2 ± 48.8 | 1.10 ± 0.70 | 8.56 ± 0.65 |
| Total organic carbon | | 90,000 | 442,000 | – | – |
| Total organic nitrogen | | 1700 | 5600 | – | – |
| Total phosphorus | | 620 | 1900 | – | – |

Values are means of three replicate measures. Data from Lors et al. (2011)

## Analytical data

Soil water extraction was carried out according to ISO 21268–2 (2007). PAH-releasing capacity was expressed by the ratio between PAH concentration in water extract (per unit mass of soil for water extraction) and PAH concentration in soil.

Soil $pH_{water}$ was determined using a Consort C83 pH-meter fitted with a glass electrode corrected for temperature. Total organic carbon concentration was obtained from total carbon and inorganic carbon contents, determined with a TOC-5000A Shimatzu® analyzer, according to ISO 10694 (1995).

Concentrations of the 16 PAHs of the US-EPA list (Verschueren 2001) were dosed in soil and water extracts according to ISO 13877 (1998). However, PAH concentration in water extracts did not include acenaphthylene. The extraction of PAHs from soil samples was carried out with the solvent extractor system Dionex® ASE 200 (Dionex Corporation®, Sunnyvale, CA). Concentrations of the 16 PAHs were dosed in the extracts by high performance liquid chromatography (Waters® HPLC 2690, Milford, MS), coupled to a UV photodiode array detector (Waters® 996). The ratio between PAH concentration in water extract and PAH concentration in soil allowed determining the PAH water extraction capacity of the studied soils. All chemical analyses were done in triplicate.

The particle size distribution of soils was determined by separating soil samples into six fractions: > 2000, 2000–200, 200–50, 50–20, 20–2, and < 2 µm. The first three fractions were obtained by dry sieving using sieves between 50 and 200 µm. The smaller fractions (< 50 µm) were obtained by moist procedure, at first by sieving the soil under water through a 20-µm sieve. The fraction retrieved on the sieve, corresponding to the fraction 20–50 µm, was dried to 20 °C. The suspension, corresponding to the fraction < 20 µm, was centrifuged to 1000 rpm for 3.5 min. The recovered fraction was dried at 30 °C.

## Ecotoxicological data

Bioassays were performed to assess the direct toxicity of soils and soil water extracts to terrestrial and aquatic organisms, respectively. The set of bioassays included acute, chronic, and genotoxicity effects, using organisms representative of a variety of trophic levels.

Toxicity endpoints were the responses of test organisms to soils or water extracts in test media (%, *w*/*w*). Results were calculated as concentrations producing no significant effect (NOEC), percent inhibition at the highest concentration of the tested sample or as concentrations decreasing the measured endpoint by 10, 20, and 50% [$E(L)C_{10}$, $E(L)C_{20}$, and $E(L)C_{50}$, respectively] compared to controls. $E(L)C_x$ values were calculated following adjustment of data to a log-probit logistic model (Litchfield and Wilcoxon 1949).

The toxicity of soils was evaluated by Lors et al. (2011) from nine bioassays tested on the basis of their best sensitivity to PAH pollution. This set of bioassays included both solid and liquid phases and addressed acute and chronic toxicities and genotoxicity: two rapid bioassays (Microtox® and springtail avoidance), a micronucleus test and three bioassays of a longer duration (algal growth, lettuce germination, and springtail reproduction).

Bioassays applied directly on the soil included an acute phytotoxicity bioassay on *Lactuca sativa* (ISO 11269–2 2005). A chronic toxicity bioassay based on springtail (*Folsomia candida*) reproduction was conducted according to ISO 11267 (1999) modified by Martínez Aldaya et al. (2006). An avoidance test was conducted on *Folsomia candida* according to Martínez Aldaya et al. (2006) and Lors et al. (2006). Terrestrial toxicity bioassays were performed by using control A and control B as a dilution matrix for soil A and soil B, respectively. The pH of both soils was around 8 and thus was compatible with requirements of test organisms.

The toxicity of water extracts to aquatic organisms was assessed through both acute and chronic effects. An acute ecotoxicity test was performed by measuring the inhibition of bioluminescence of the bacterium *Vibrio fischeri* according to ISO 11348–3 (1998). Chronic ecotoxicity was determined on the growth of the freshwater alga *Pseudokirchneriella subcapitata* according to ISO 8692 (2004). Each concentration was tested in six replicate microplates. The % inhibition of the population growth was determined for each concentration by comparison with the control.

The genotoxicity of water extracts was evaluated with the in vitro micronucleus assay applied on mouse lymphoma cells L5178Y according to the procedure described by Nesslany and Marzin (1999). The criteria for determining a genotoxic effect were a concentration-related increase in the number of micronucleated cells and a statistically significant increase over the spontaneous level in at least one treatment schedule.

### Calculation of ecoscores

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

From five ecotoxicological parameters, $E(L)C_{50}$, $E(L)C_{20}$, $E(L)C_{10}$, NOEC, and % inhibition, scores were calculated by assigning to each endpoint value a score between 0 and 100 as a function of its intensity. It was noticed that the scale of the scores was modified compared to that defined by Lors et al. (2010) ($0 < \text{score} < 3$), in order to normalize to 100 the maximum effect.

For $E(L)C_{50}$, $E(L)C_{20}$, $E(L)C_{10}$, and NOEC, the following scale ($x$ = endpoint value) was used:

- 0 = No effect ($x > 100$)
- 33 = Weak effect ($50 < x \leq 100$)
- 67 = Medium effect ($20 < x \leq 50$)
- 100 = Strong effect ($x \leq 20$)

For % inhibition, the following scale was used:

- 0 = No effect ($x \leq 5$)
- 33 = Weak effect ($5 < x \leq 20$)
- 67 = Medium effect ($20 < x \leq 60$)
- 100 = Strong effect ($x > 60$)

For each bioassay, the five scores were summed up and divided by the number of endpoints, in order to calculate a bioassay score. Bioassay score values allowed evaluating the sensitivity of the different bioassays.

An ecoscore was calculated for each soil by averaging the values of the different bioassay scores. The following scale was used to define the environmental risk of PAH-polluted soils and classify soils in function of the intensity of toxicity:

- No toxicity (ecoscore = 0)
- Weak toxicity ($0 < \text{ecoscore} \leq 33$)
- Moderate toxicity ($33 < \text{ecoscore} \leq 67$)
- Strong toxicity ($67 < \text{ecoscore} \leq 100$)

## Results and discussion

### Chemical and toxicological characteristics of the soils

The particle size distribution of the studied soils is reported on Fig. 1. Soil B was composed almost exclusively of particles between 2000 and 50 μm (79%), corresponding to sand. Within this class, the fraction 2000–200 μm (coarse sand) was dominant (56%) compared with fine sand (23%). Silt and clay fractions were present in very small proportion, 3.3

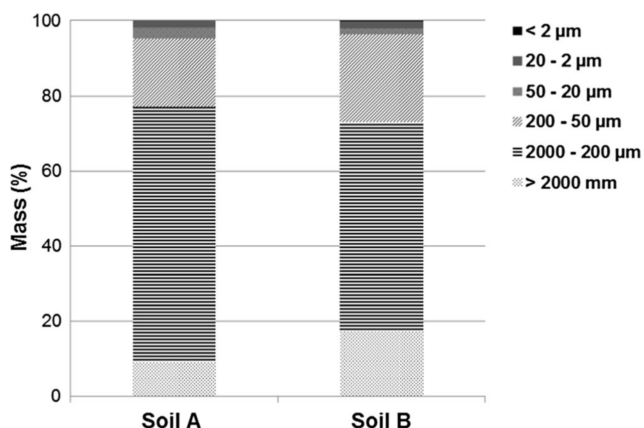


**Fig. 1** Distribution of soil textural fractions (in mass %) for each studied soil

and 0.2%, respectively. The particle size distribution of soil A was comparable to that of soil B: the sand fraction was dominant (86%), with a similar dominance of coarse sand (68%). Finer textural classes were distributed with proportions almost similar to those of soil B: 4.4 and 0.3% for silt and clay fractions, respectively.

Soil A was mainly contaminated with organic compounds. This soil was heavily polluted with PAHs, with a global content of the 16PAHs of the US-EPA list around 3 g kg$^{-1}$ dry soil, mainly represented by two-, three-, and four-ring compounds (Table 1). Three-ring PAHs were the most represented (44% of $\sum$ 16PAHs—1279.2 mg kg$^{-1}$ dry soil), followed by two- and four-ring compounds (28 and 20%, 594.2 and 808.9 mg kg$^{-1}$ dry soil, respectively) (Fig. 2). Five- and six-ring PAHs were hardly present in this soil (5 and 2%, 149.2 and 63.2 mg kg$^{-1}$ dry soil, respectively).

Soil B was highly polluted with PAHs, to a level similar to soil A ($\sum$ 16PAHs = 3687.2 mg kg$^{-1}$) (Table 1). However, the PAH distribution pattern was different from soil A. Four-ring PAHs were the most represented (50% of $\sum$ 16PAHs—1726.9 mg kg$^{-1}$ dry soil) followed by five- and six-rings PAHs (22 and 11%—796.1 and 389.8 mg kg$^{-1}$ dry soil) (Fig. 2). Contrary to soil A, three-ring PAH content was lower, 623.5 against 1279.2 mg kg$^{-1}$ dry soil for soil A. This difference of distribution is probably the consequence of the partial biodegradation of PAHs during biotreatment that was more efficient to decrease two- and three-ring PAHs, as expected (Lors et al. 2010). Thus, the concentrations of four-, five-, and six-ring PAHs were higher for soil B contrary to two- and three-ring PAHs.

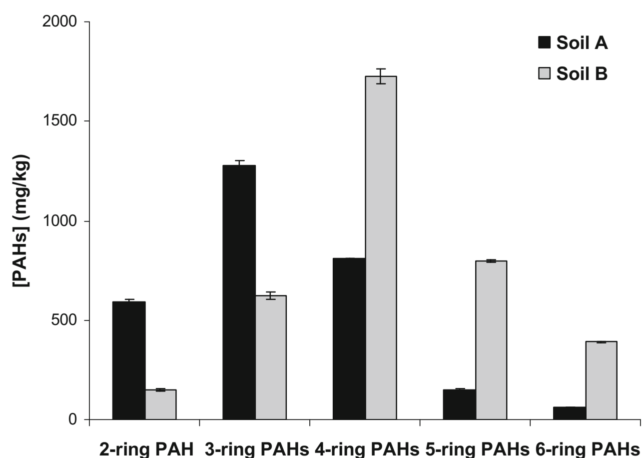


**Fig. 2** Concentrations of two- to six-ring PAHs in the two studied soils. Values are means of three replicate dosages, with SE as error bars

A higher amount of organic carbon was also detected in soil B (442 g kg$^{-1}$ dry soil) compared to soil A (90 g kg$^{-1}$ dry soil). This difference could be explained by the addition of compost during the windrow treatment of soil B. This can be confirmed by the high N and P amounts detected in this soil, amounting to 5600 and 1900 mg kg$^{-1}$, respectively (Table 1). As the dominant fraction of the two soils was coarse sand, it is possible that the organic part could be contained in this fraction.

Soil A induced a strong inhibition of lettuce germination. At the highest dose tested, lettuce germination was inhibited to about 70.9% (Table 2) and the bioassay score was to 93 (Table 4). Conversely, soil B showed a significantly lower phytotoxicity towards lettuce germination with an inhibition of 21.4% (Table 3) and a bioassay score of only 47 (Table 4).

The *Folsomia* population reproduction bioassay showed that soil A had a high chronic ecotoxicity, with an inhibition rate of 100% and a bioassay score of 100 (Tables 2 and 4) whereas soil B did not elicit any response by *Folsomia candida* populations (Table 3). For soil B, the behavioral test seemed to be more sensitive than ecotoxicity tests since it allowed to detect a significant repellence (bioassay score = 67, Table 4). Nevertheless, the repellence level was lower than that of soil A (bioassay score = 100, Table 4).

Soil A presented a strong ecotoxicity towards terrestrial organisms whereas the ecotoxicity of soil B was moderate. Ecoscores of soil A and soil B, obtained with this battery of bioassays, were 97.7 and 38.0, respectively (Table 4).

The fact that these two soils showed the same high level of organic pollution whereas their ecotoxicity assessed by solid bioassays was different demonstrates that the global content of PAHs is not the pertinent parameter to evaluate soil pollution hazards. Conversely, the distribution of PAHs gave a better picture of soil pollution and it can explain partly the ecotoxic responses obtained. In fact, the high ecotoxicity of soil A to terrestrial organisms is probably related to its high concentration in three-ring PAHs. Indeed, a positive and significant relationship between ecoscores pooled over the three solid bioassays and the concentration of three-ring PAHs was found by Lors et al. (2010). Soil A exhibited a high ecotoxicity on organisms tested (plants and Collembola), with bioassay scores of 93, 100, and 100 to *Lactuca* germination, *Folsomia* population growth, and *Folsomia* avoidance, respectively

**Table 2** Ecotoxicological characteristics of soil A according to a restricted battery of solid and liquid bioassays

| | $EC_{50}$ (g 100 $g^{-1}$) | TU 100/$EC_{50}$ | $EC_{20}$ (g 100 $g^{-1}$) | $EC_{10}$ (g 100 $g^{-1}$) | NOEC (g 100 $g^{-1}$) | Inhibition (%) |
|---|---|---|---|---|---|---|
| *Lactuca* germination | 21.3 (15.5–29.3) | 4.5 | 3.5 (1.9–6.5) | 1.4 (0.6–3.4) | < 5 | 70.9 |
| *Folsomia* population growth | 2.2 N/A | 45.5 | 2.1 N/A | 1.9 N/A | 1 | 100 |
| *Folsomia* avoidance | 0.8 (0.6–1) | 129 | 0.3 (0.2–0.4) | 0.042 (0.008–0.205) | < 0.35 | 100 |
| Microtox® test | 8.1 (6.4–10.1) | 12.4 | 1.5 (0.9–2.3) | 0.6 (0.3–1.1) | < 2.5 | 89.1 |
| Algal growth | 42.9 (40.4–45.6) | 2.3 | 28.0 (25.7–30.5) | 22.4 (20.0–25.0) | < 20 | 93.4 |
| Micronucleus test (−S9) | 16.1 (13.4–19.3) | 6.2 | 6.8 (5.0–9.4) | 4.4 (2.9–6.5) | < 12.5 | N/A |

Data from Lors et al. (2011)

*N/A* not applicable

(Table 4). These results confirmed those of Sverdrup et al. (2002) who showed that two-, three-, and four-ring PAHs significantly affected the survival and reproduction of Collembola and earthworms while PAHs with a high lipophily did not. The same results were obtained by Čvančarová et al. (2013) who indicated a better sensitivity of the earthworm *Eisenia fetida* to PAHs with three to four aromatic rings. The growth inhibition of this organism is linked to the accumulation of PAHs in earthworm tissues. Although soil A contains four-ring PAHs at a lesser concentration than three-ring PAHs, four-ring PAHs also contribute to the ecotoxicological effects measured.

Moreover, soil B exhibited a weak acute ecotoxicity towards lettuce and no chronic ecotoxicity towards Collembola. Comparatively to soil A, soil B contains lesser concentrations of three-ring PAHs but higher concentrations of four-ring PAHs. If we consider that three- and four-ring PAHs were responsible for the impact of PAHs on Collembola and earthworms (Eom et al. 2007; Sverdrup et al. 2002), the results might indicate that three- and four-ring PAHs in soil B were less bioavailable to the exposed organisms. Indeed, soil B had 50% less three-ring PAHs and these compounds were very weakly dissolved by water during extraction (concentration in water extract B equal to 4.1 μg $L^{-1}$) (Table 5). In the same way, although the content of four-ring PAHs was 50% higher, a weak fraction was dissolved in water after extraction (concentration in water extract B equal to 13.7 μg $L^{-1}$). As a consequence, these compounds should be sequestered in other insoluble organic compounds in this soil.

Although any effect was detected on *Folsomia candida* population growth, soil B was repellent to the same organism at a lower level than soil A (bioassay scores of 100 and 67 for soils A and B, respectively—Table 4), confirming the better sensitivity of behavioral tests (avoidance by *Folsomia*) compared to ecotoxicity tests.

## Chemical and toxicological characteristics of water extracts

The water extraction of soils gives an information on the soluble fraction and thus on the fraction directly accessible to organisms through diffusion. Aqueous leaching tests showed that

**Table 3** Ecotoxicological characteristics of soil B according to a restricted battery of solid and liquid bioassays

| | $EC_{50}$ (g 100 $g^{-1}$) | TU 100/$EC_{50}$ | $EC_{20}$ (g 100 $g^{-1}$) | $EC_{10}$ (g 100 $g^{-1}$) | NOEC (g 100 $g^{-1}$) | Inhibition (%) |
|---|---|---|---|---|---|---|
| *Lactuca* germination | > 100 | < 1 | 80.8 (62.9 to > 100) | 41.7 (29.9–58.1) | < 35 | 21.4 |
| *Folsomia* population growth | NT | NT | NT | NT | NT | 0 |
| *Folsomia* avoidance | > 100 | < 1 | 1.7 (0.6–5.1) | 0.04 (0.004–0.403) | < 0.35 | 12.5 |
| Microtox® test | NT | NT | NT | NT | NT | 0 |
| Algal growth | 80.5 (51.7 to > 100) | 1.2 | 8.4 (5.3–13.5) | 2.6 (1.2–5.7) | < 6.25 | 56.5 |
| Micronucleus test (−S9) | NM | NM | NM | NM | NM | N/A |

Data from Lors et al. (2011)

*NT* not toxic, *NM* not mutagenic, *N/A* not applicable

**Table 4** Ecoscores of the two studied soils calculated from the bioassay scores obtained with the battery of solid and liquid bioassays

| | Bioassays | Effects | Scores (%) | |
|---|---|---|---|---|
| | | | Soil A | Soil B |
| **Solid-phase bioassays** | *Lactuca* germination | Acute ecotoxicity | 93 | 47 |
| | *Folsomia* population growth | Chronic ecotoxicity | 100 | 0 |
| | *Folsomia* avoidance | Behavior | 100 | 67 |
| | **Solid-phase bioassay battery** | | **97.7** | **38.0** |
| **Liquid-phase bioassays** | *Vibrio fischeri* inhibition | Acute ecotoxicity | 100 | 0 |
| | *Pseudokirchneriella* growth | Chronic ecotoxicity | 80 | 80 |
| | Micronucleus test | Genotoxicity | 80 | 0 |
| | **Liquid-phase bioassay battery** | | **86.7** | **26.7** |
| **Solid- and liquid-phase bioassays** | **Bioassay battery** | | **92.2** | **32.3** |

The values of ecoscores noted in bold type correspond to the average of bioassay scores

soil A had a strong capacity to release PAHs. In fact, the ratio PAH concentration in water extract/soil was equal to $2.2 \times 10^{-3}$. Water extract A mainly contained three-ring PAHs (82% of ∑ 16PAHs—531.1 μg $L^{-1}$) (Table 5). Their high concentration in solution was linked to their amount in soil A (1279.2 mg $kg^{-1}$ dry soil) and the higher solubility of PAHs of lower molecular weight (Table 1). Naphthalene was present at a small concentration (0.2 μg $L^{-1}$) despite its high amount in soil A. This was probably due to its volatilization during water extraction, given its high vapor tensile strength (37 Pa).

The leaching rate of PAHs decreased with the number of rings (Fig. 3). PAH concentrations in water extracts were

**Table 5** Concentration of PAHs in water extracts A and B (expressed in μg $L^{-1}$) and water solubility of PAHs at 25 °C (expressed in μg $kg^{-1}$)

| | No. of rings | Water extract A | Water extract B | Water solubility |
|---|---|---|---|---|
| Naphthalene | 2 | 0.20 ± 0.02 | 0.6 ± 0.2 | $3.2 \times 10^4$ |
| Acenaphthylene | 3 | – | – | $3.4 \times 10^3$ |
| Acenaphthene | 3 | 154.1 ± 1.5 | 0.6 ± 0.1 | $3.9 \times 10^3$ |
| Fluorene | 3 | 198.7 ± 3.7 | 0.7 ± 0.1 | $2.0 \times 10^3$ |
| Phenanthrene | 3 | 151.4 ± 2.8 | 1.4 ± 0.2 | $1.3 \times 10^3$ |
| Anthracene | 3 | 26.9 ± 0.4 | 1.3 ± 0.3 | $7.3 \times 10^1$ |
| Fluoranthene | 4 | 51.2 ± 0.9 | 4.1 ± 0.7 | $2.6 \times 10^2$ |
| Pyrene | 4 | 28.9 ± 0.5 | 3.1 ± 0.6 | $1.4 \times 10^2$ |
| Benzo[a]anthracene | 4 | 8.0 ± 0.1 | 3.1 ± 0.6 | $1.4 \times 10^1$ |
| Chrysene | 4 | 8.8 ± 0.1 | 3.4 ± 0.6 | $2.0 \times 10^0$ |
| Benzo[b]anthracene | 5 | 3.85 ± 0.05 | 8.4 ± 1.4 | $1.2 \times 10^0$ |
| Benzo[k]fluoranthene | 5 | 2.30 ± 0.03 | 2.7 ± 0.5 | $7.6 \times 10^{-1}$ |
| Benzo[a]pyrene | 5 | 4.41 ± 0.02 | 6.9 ± 1.4 | $3.8 \times 10^0$ |
| Dibenzo[ah]anthracene | 5 | 0.72 ± 0.01 | 3.4 ± 0.6 | *2.6 × 10*$^{-1}$ |
| Benzo[ghi]perylene | 6 | 3.00 ± 0.03 | 8.4 ± 1.7 | $6.2 \times 10^1$ |
| Indeno[123-*cd*]pyrene | 6 | 2.30 ± 0.04 | 9.0 ± 1.8 | $5.0 \times 10^{-1}$ |
| 2-Ring PAH | | 0.20 ± 0.02 | 0.6 ± 0.2 | – |
| 3-Ring PAHs | | 531.1 ± 8.3 | 4.1 ± 0.7 | – |
| 4-Ring PAHs | | 96.9 ± 1.7 | 13.7 ± 2.4 | – |
| 5-Ring PAHs | | 11.3 ± 0.1 | 21.4 ± 3.8 | – |
| 6-Ring PAHs | | 5.30 ± 0.05 | 17.5 ± 3.5 | – |
| ∑ 16 PAHs | | 644.8 ± 12.0 | 57.3 ± 11.2 | – |

Values are means of three replicate measures. Data from Lors et al. (2011)

correlated to the water solubility of these pollutants. More soluble PAHs, such as acenaphthene, fluorene, and phenanthrene, were found at the highest concentrations in water extract A (Table 5). Conversely, four-ring PAHs were present in a lesser proportion (15% of ∑ 16PAHs—96.9 μg $L^{-1}$), whereas five- and six- ring PAHs were not significantly represented in water extract A (1.8 and 0.8%—11.3 and 5.3 μg $L^{-1}$, respectively) (Table 5).

Soil B had a low capacity for PAH remobilization: this soil released ten times lower amounts of PAHs than soil A ($1.6 \times 10^{-4}$). Five- and six-ring PAHs were the most represented (respectively 37 and 30% of ∑ 16PAHs—21.4 and 17.5 μg $L^{-1}$) (Table 5). Among five-ring PAHs, benzo(*b*)anthracene and benzo(*a*)pyrene were the major compounds in water extract B. Water extract B also contained four-ring PAHs in a lesser proportion (23.9% of ∑ 16PAHs—13.7 μg $L^{-1}$). Three-ring PAHs were also hardly released, and thus, their concentrations were about 100 times less than water extract A. The windrow treatment applied to this soil was efficient because it removed low molecular weight PAHs, and remaining PAHs were the compounds most recalcitrant to microbial degradation. PAH degradation may also be limited by the slow desorption of PAHs from the soil matrix (Juhasz et al. 2005). In fact, soil B contains essentially four-, five-, and six-ring PAHs, which were characterized by a weak water solubility and a high lipophily.

Water extract A exhibited a strong acute ecotoxicity to *Vibrio fischeri* (bioassay score = 100) (Table 4). Conversely, water extract B did not show any acute ecotoxicity with the bioassay tested (bioassay score = 0) (Table 4). These results seemed to be linked to the three-ring PAH content of these water extracts: high amount in water extract A (∑ 3-ring PAHs = 531.1 μg $L^{-1}$) and small amount in water extract B (∑ 3-ring PAHs = 4.1 μg $L^{-1}$) (Table 5). This confirms the strong correlation between acute ecotoxicity and three-ring PAHs observed by Lors et al. (2011), which is linked to their higher water solubility compared to PAHs of higher molecular weight (Bispo et al. 1999).

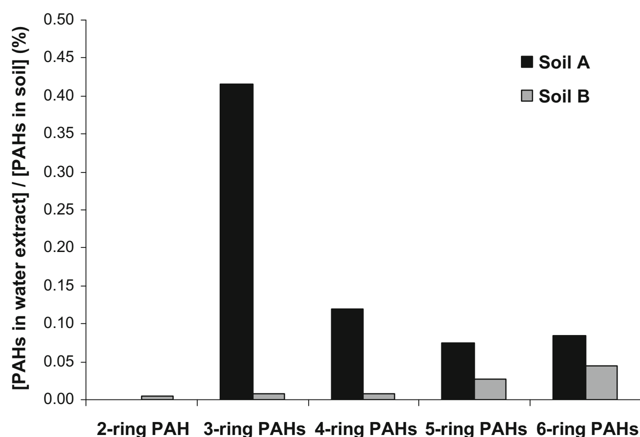


**Fig. 3** Ratio of PAH concentration in water extract to concentration in soil (expressed in %) for the two studied soils

However, the effects of water extracts A and B on algal growth were comparable on the basis of ecoscores. Water extracts A and B showed a high chronic response towards *Pseudokirchneriella subcapitata* (bioassay scores of 80 for both water extracts) (Table 4). These results indicate that both soils had a potential impact to aquatic fauna in the long term and that ecotoxicological effects were due to five- and six-ring PAHs, although these were present in lower contents in both water extracts (Fig. 3). Nevertheless, the concentration in solution of five- and six-ring PAHs was above their hydrosolubility level (Table 5). The ratio between the concentration of each PAH in water extract and its water solubility was higher than 1 for all five- and six-ring PAHs to the exception of benzo[*g,h,i*]-perylene. This suggests that these high molecular weight PAHs were associated with other compounds, such as suspended organic matter or were in colloidal form, making them bioavailable to aquatic organisms. Although it had been biotreated, soil B contained a pool of PAHs that were potentially leachable. The battery must include bioassays taking into account different effects, acute, chronic toxicity, and behavioral endpoints, in order to prevent potential hazards for ecosystems in the long term (Prato et al. 2015).

The micronucleus test applied to mouse lymphoma cells showed a high genotoxicity only without S9 activator (bioassay score = 80) for water extract A, indicating the presence of directly genotoxic compounds in this water extract. Conversely, no any genotoxic effect was observed for water extract B. The fact that water extract A is genotoxic only without S9 activator suggests that its genotoxicity response is not due to PAHs but certainly to other compounds present in this water extract. In fact, PAHs are known to be activated into genotoxic metabolites by S9 rat liver enzymes (Otto et al. 1991; IARC 2010). The ecotoxicological approach gives a global response and takes into account all potentially toxic compounds and not only those that have been determined by chemical analysis.

### Comparison of ecoscores of soils and soil water extracts

The comparison of averaged ecoscores for solid- and liquid-phase bioassays showed that soil A was classified as strongly toxic by both types of bioassays. Ecoscores were to 97.7 and 86.7 for solid- and liquid-phase bioassays, respectively. Conversely, soil B, which was at the limit between weak and moderate effects, appeared weakly toxic by liquid phase bioassays (ecoscore = 26.7) and moderately toxic by solid-phase bioassays (ecoscore = 38.0). This was due to a lower sensitivity of liquid phase bioassays compared to that of solid-phase bioassays as shown by Lors et al. (2011), an observation already made on plant growth tests by Ferrari et al. (1999). Chemical and ecotoxicological analyses of water extracts allowed us to know which water-soluble fraction was directly accessible to organisms. The water extract was complementary to the bulk soil in the procedure of hazard

assessment. The weak toxicity of soil B compared to the strong toxicity of soil A was also confirmed by the calculation of ecoscores using both solid- and liquid-phase bioassays: ecoscores were 32.3 and 92.2, respectively (Table 4).

Our method of selection based on ecoscores was comparable to the TBI. However, TBI has an additional class, named "very high," and this level corresponds to the "high level" in the ES. Moreover, the high level in TBI corresponds to the medium level in ES. TBI thus overestimates the ecotoxicological risk in comparison with ES. In fact, Manzo et al. (2014) showed that TBI did not allow highlighting differences among the sites studied and showed a general high ecotoxicological risk. Nevertheless, while overestimating the ecotoxicological risk compared to ES, similar discriminating capacity could be obtained when using TBI ecotoxicological risk levels: according to TBI soils A and B are classed very highly and highly toxic, respectively.

## Conclusions

Two coke factory soils contaminated with similar total PAH concentration were characterized by chemical and ecotoxicological approaches applied to the whole soil and to the water extract. The battery of bioassays including solid and liquid bioassays took into account acute, chronic, and genotoxic effects. The chemical analysis of soil water extracts gave information on the soluble fraction corresponding to the pollutants directly accessible to organisms. Nevertheless, it was necessary to complete chemical analyses by ecotoxicity bioassays which proved to be more sensitive indicators of soil quality. The Ecoscore system (ES), based on ecoscores calculated from a battery of liquid and solid phase bioassays, proved to be a robust method which allowed us to evaluate the sensitivity of bioassays and to classify soils according to their toxicity level and particularly to differentiate the two studied soils despite their similar total concentration of PAHs. The total PAH content of soil was not the more pertinent parameter to assess the hazard of polluted soils, contrary to the distribution of PAHs: the combination of chemical and toxicological approaches highlighted the relationship between acute ecotoxicity and three-ring PAHs (most soluble compounds). In the same way, the chronic effect of both water extracts on algal growth can be explained by high molecular weight PAHs, such as five- and six-ring PAHs. A procedure involving the battery of bioassays proposed (two rapid bioassays, Microtox and springtail avoidance, a micronucleus test and three bioassays of a longer duration, algal growth, lettuce germination, and springtail reproduction) and the calculation of resulting ecoscores provides a discriminant assessment of soils contaminated by PAHs which could be implemented in bioremediation programs. It offers the advantage of allowing an easy comparison of various soils on the base of a wide array of acute and chronic solid- and liquid-phase toxicity tests, without resorting to complex data analyses. The proposed ES proved effective to discriminate between weak and strong toxicity hazard of the two studied soils, despite their similar total PAH concentration.

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