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► **To cite this version:**

Christine Lors, Jean-François Ponge, Denis Damidot. Microscopy in addition to chemical analyses and ecotoxicological assays for the environmental hazard assessment of coal tar-polluted soils. *Environmental Science and Pollution Research*, 2018, 25 (3), pp.2594-2602. 10.1007/s11356-017-0693-8 . hal-01691330

**HAL Id: hal-01691330**

**<https://hal.science/hal-01691330v1>**

Submitted on 25 Jan 2018

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1 **Microscopy in addition to chemical analyses and ecotoxicological assays**  
2 **for the environmental hazard assessment of coal tar polluted soils**

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9

10 **Abstract** Chemical analysis of soils contaminated with coal tar indicated that most organic  
11 compounds, and particularly PAHs, were contained in coarser particles (> 200 µm). Microscopic  
12 observations of this fraction, carried out on polished sections, reported the presence of organic  
13 particles in addition to mineral particles. Some organic particles had a very low porosity and their  
14 microstructure did not evolve during biotreatment. Alternatively, other organic particles had a large  
15 porosity composed of an interconnected pore network that was open to coal tar surface and thus in  
16 contact with soil water. Interconnected porosity seemed to increase during biotreatment in relation  
17 to a decrease in the amount of organic compounds. The amount of open porosity in contact with  
18 soil water was expected to increase the desorption rate of PAHs. Consequently, the environmental  
19 hazard could depend on the amount of open porosity in addition to chemical properties of organic  
20 particles, such as their concentration in PAHs. Thus, microscopy can be complementary to  
21 chemical analysis and ecotoxicological assays to assess the best strategy for remediation but also to  
22 follow the advancement of a biotreatment.

23

24 **Keywords** Coal tar; PAHs; Environmental hazard assessment; Ecotoxicity; Pore network;  
25 Microscopy

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## 27 **Introduction**

28 Industrial activities have led to the discharge of a wide range of hazardous chemical in soils, such  
29 as coal tar that contains Non-Aqueous Phase Liquids (NAPLs). The composition of coal tar varies  
30 widely depending on its origin (Brown et al. 2006). Nevertheless, coal tars are always composed of  
31 hundreds of organic compounds but also of some inorganic constituents. Main organic compounds  
32 are polynuclear aromatic hydrocarbons (PAHs), phenolic compounds, such as cresols, BTEX as  
33 well as aliphatic and polar hydrocarbons (EPRI 1993). The microstructure of coal tars and their  
34 cokes is complex and also varies markedly according to processing conditions. Some more or less  
35 anisotropic textures are found along with variable amounts of porosity (Panaitescu and Predeanu  
36 2007; Picon-Hernandez et al. 2008).

37 Most organic substances contained in coal tars are potentially toxic, mutagenic and  
38 carcinogenic (White and Claxton 2004). Thus, once soils have been polluted by coal tar, it is  
39 important to assess their environmental risk and try to suppress or at least to mitigate it. Some  
40 remediation techniques are applied, such as bioremediation that is often used to cure PAH-  
41 contaminated soils (Johnsen et al. 2005). Finally, the efficiency of a treatment also requires the  
42 evaluation of post-treatment environmental risk.

43 Microbial degradation, one of the most important processes of PAH destruction in soils,  
44 has been extensively studied. The transfer of PAHs from coal tar to soil water is the first critical  
45 step before PAHs can be metabolized (Mahjoub et al. 2000; Nambi and Powers 2000; Semple et al.  
46 2003; Benhabib et al. 2010). Thus, PAH availability to microorganisms depends primarily on PAH  
47 transfer rate that is controlled by several mechanisms, such as (i) the diffusion of PAHs from coal  
48 tar to soil water, (ii) the solubility of PAHs that decreases with increasing number of rings, (iii) the  
49 contact surface of coal tar in soil water, and (iv) soil properties. The surface of coal tar particles in  
50 contact with soil water depends on their shape, size and distribution in the different soil fractions  
51 (Chung and Alexander 2002; Vulava et al. 2007; Al-Raoush 2014). However, despite of the  
52 importance of coal tar microstructure in the bioremediation process, microscopic observations of  
53 coal tar in polluted soils are scarce (Ghosh et al. 2000). A precise description of polluted soils is  
54 also infrequent despite its usefulness to better understand the physicochemical processes involved.  
55 For example, PAH availability to microorganisms can be reduced if PAHs are adsorbed to some  
56 compounds of the soil, such as organic matter or clay particles, which protect them from  
57 degradation (Vulava et al. 2012). Consequently, higher microbial degradation rates occur in soils  
58 with lower amounts of soil organic matter and clay (Thiele-Bruhn and Brümmer 2004; Rhodes et  
59 al. 2008).

60 Considering the complexity of the mechanisms involved, environmental risk is thus often

61 assessed by a global approach thanks to ecotoxicological bioassays. These bioassays indirectly  
62 assess the bioavailability of PAHs in polluted soils (Peijnenburg et al. 2002; Eom et al. 2007;  
63 Riding et al. 2013). However, bioassays are not compound-specific and the availability of  
64 pollutants is also specifically linked to soil properties (Riding et al. 2013). PAH bioavailability is  
65 thus related to the fraction actually available for the organisms used in the bioassay.

66 The present work aimed at further assessing the environmental hazard of three soils  
67 polluted by coal tar that was previously estimated by an ecoscore calculated from a complete  
68 battery of acute and chronic toxicity bioassays (Lors et al. 2010, 2011). In these previous studies,  
69 ecotoxicity assessed by ecoscores was not connected with the total concentration of PAHs. A more  
70 precise study of these three soils was thus performed to bring further explanations for the reported  
71 differences. First, a chemical analysis was performed on several fractions of each soil to assess how  
72 coal tar particles and PAHs were distributed. Second, a special attention was paid to the  
73 observation of the microstructure of coal tar particles by several microscopic techniques. Finally,  
74 the impact of both PAHs concentration in coal tar particles and their microstructure was discussed  
75 with respect to the desorption rate of PAHs, and consequently to environmental hazard assessment.

76

## 77 **Material and methods**

### 78 **Soil samples**

79 Experiments were carried out on three coal tar-contaminated sandy soils sampled from two  
80 historical industrial sites located in the North of France, in which the distillation of coal tar was the  
81 main activity. Properties of these soils were detailed in previous papers (Lors et al. 2010, 2011).  
82 Briefly, a soil was collected in one of the two sites, before (soil S3) after 6-month windrow  
83 biotreatment (soil S3T), while soil S2 was collected in another site after 18-month windrow  
84 biotreatment.

85

### 86 **Chemical and ecotoxicological analyses**

87 The three sandy soils were separated into six fractions: < 2, 2-20, 20-50, 50-200, 200-2000, and  
88 > 2000  $\mu\text{m}$ . The first three fractions were obtained by dry sieving using sieves between 50 and  
89 200  $\mu\text{m}$ . The smaller fractions (< 50  $\mu\text{m}$ ) were obtained by moist way, at first by sieving soil under  
90 water through a sieve of 20  $\mu\text{m}$ . The fraction retrieved on the sieve, corresponding to the fraction  
91 20-50  $\mu\text{m}$ , was dried to 30°C. The filtrate suspension, corresponding to the fraction < 20  $\mu\text{m}$ , was

92 centrifuged to 1000 rpm during 3.5 min, enabling us to separate fractions 2-20  $\mu\text{m}$  and  $< 2 \mu\text{m}$ . The  
93 different fractions were dried at 30°C, then analysed to measure the concentration of the 16 PAHs  
94 of the US-EPA list according to ISO 13877 (ISO 1998) and the total organic carbon (TOC)  
95 concentration according to ISO 10694 (ISO 1995). Additionally, the mineral composition of the  
96 soils was studied by X-ray diffraction.

97 Ecotoxicity was evaluated with the help of ecoscores, based on a restricted array of  
98 biological endpoints. The calculation of ecoscores has been detailed in Lors et al. (2010, 2011,  
99 2017). The set of bioassays was selected by Lors et al. (2011) from nine bioassays tested on the  
100 basis of their best sensitivity to PAH pollution. This set of bioassays included both solid and liquid  
101 phases and addressed acute and chronic toxicities and genotoxicity: two rapid bioassays (Microtox<sup>®</sup>  
102 and springtail avoidance), a micronucleus test and three bioassays of a longer duration (algal  
103 growth, lettuce germination and springtail reproduction).

104

#### 105 **Microscopic observations of soil samples**

106 The soil fraction larger than 200  $\mu\text{m}$  was prepared, in order to be observed by scanning electron  
107 microscopy and by optical microscopy. The soil fraction was impregnated under vacuum with  
108 epoxy resin in circular moulds (diameter: 2 cm). After hardening of the epoxy resin, the surface  
109 was pre-polished under water using several diamond-covered polishing discs having a decreasing  
110 particle size: 151, 75, 46 and 16  $\mu\text{m}$ . To obtain a flat surface prior to fine polishing steps, samples  
111 were polished using a silicon carbide grinding wheel (particle size: 8  $\mu\text{m}$ ). The fine polishing steps  
112 were performed using diamond pastes of decreasing particle size (9, 6, 3, 1,  $\frac{1}{2}$ , and  $\frac{1}{4} \mu\text{m}$ ).  
113 Samples were gold coated before observation under a scanning electron microscope (Hitachi S-  
114 4300SE/N) operating in backscattered electron (BSE) mode (20 KeV and 2 KA). Elemental  
115 chemical analysis was performed with a Thermoscientific Ultradry EDX detector running at 15 kV.  
116 Complementary experiments carried out with an optical microscope (Leica DMRXP) under  
117 reflected light were performed directly on uncoated samples. Image analysis of BSE images was  
118 performed using a specific procedure adapted from the statistical analysis of BSE images  
119 performed on hydrated cement paste (Hu and Ma. 2016). First, one hundred coal tar particles were  
120 cut manually using Adobe Photoshop<sup>®</sup> from several BSE images (3968 x 2232 pixels) taken at a  
121 magnification of 100 x. Then, each digital coal tar particle was pasted in a separate rectangular  
122 image having a white background colour. The total surface of coal tar particles was estimated by  
123 counting the number of pixels having a level of grey different than white, using a MATLAB<sup>®</sup>  
124 procedure. Then, coal tar images were segmented based on grey levels of pixels associated to the  
125 organic compounds of coal tar. Indeed, the contrast organic phases, pore-intruding resin, empty

126 pores and mineral phases contained in the pores of coal tar particles was sufficient to easily depict  
127 organic compounds. The content of organic compounds was determined by dividing the pixels of  
128 segmented images by the total pixels of coal tar particles. Finally, the porosity of coal tar grains  
129 was equal to the surface fraction that was not attributed to organic compounds. As 100 coal tar  
130 particles were treated by image analysis for each soil, the surface fractions of coal tar particle  
131 components were equal to their volume fraction (Igarashi et al., 2004).

132

## 133 **Results**

### 134 **Chemical analysis of soils as a function of soil fractions**

135 The biotreatment decreased PAH and total organic carbon (TOC), resulting in decreased  
136 ecotoxicity, as expected (Fig. 1). However, soil S2 was much less ecotoxic than soil S3 despite its  
137 higher amount of PAHs (Lors et al. 2017).

138 For all three soils, the fraction larger than 200  $\mu\text{m}$  ( $> 200 \mu\text{m}$ ) represents more than 70% of  
139 the soil mass (Table 1). From a mineralogical point of view, all soils are mostly composed of  
140 quartz with some feldspar as minor phase and traces of clay in accordance with the sandy nature of  
141 the soils. The windrow treatment (S3T versus S3) did not alter the distribution of the different  
142 mineral fractions. In all three soils the fraction 200-2000  $\mu\text{m}$  contains the majority of total soil  
143 organic carbon (TOC) and PAHs (Tables 2-4).

144 The advancement of the microbial degradation of PAHs contained in coal tar particles can  
145 be estimated by considering the relative amount of most soluble (better bioavailable, with a lower  
146 number of rings) over less soluble PAHs (Bamforth and Singleton 2005). This parameter, noted as  
147 2-4/5-6 ratio, was calculated by dividing the concentrations of 2-4-ring PAHs by the concentrations  
148 of 5-6-ring PAHs. This ratio is thus expected to decrease with the advancement of degradation. The  
149 concentration of 5-6-ring PAHs is high relatively to 2-4-ring PAHs in all fractions of soil S2 (2-  
150 4/5-6 ratio ranging from 0.83 to 1.55), in agreement with its previous windrow treatment (Table 2),  
151 although a comparison with this soil previous to 18-month biotreatment is not possible. In soil S3;  
152 the 2-4/5-6 ratio is much higher than in soil S2 and decreases from 9.8 to 4.4 from the coarsest to  
153 the finest fraction, with an average value of 8.4 (Table 3).

154 Given that all three soils are strongly polluted with coal tar (Lors et al. 2010, 2011), tar  
155 concentration can be approximated from the total organic content of the soil, other sources of  
156 organic carbon being negligible compared to coal tar. The PAH concentration of coal tar particles  
157 was thus estimated by the PAH concentration of the soil divided by its total organic content (noted

158 thereafter PAHs/TOC). This ratio is low for all fractions, with an average value of 0.83% indicating  
159 a low PAH concentration in the remaining coal tar particles. Indeed, as the amounts of clay and  
160 organic matter are very low in soil S2, most of the PAHs remaining after biotreatment are  
161 entrapped in coal tar particles. The value of PAHs/TOC is higher in soil S3 than in soil S2, with an  
162 average value of 3.2% (Tables 2 and 3). Thus, despite similar total PAH concentrations in these  
163 soils, PAHs are more concentrated in the coal tar of soil S3. Additionally, soil S3 is also richer in 3-  
164 and 4-ring PAHs and poorer in 2-, 5- and 6- ring PAHs.

165 As expected, soil S3T that was subjected to a windrow-treatment has a lower PAH  
166 concentration than soil S3 whatever the fraction considered (Table 4). The windrow treatment did  
167 not modify the distribution of PAHs between the different soil fractions, most PAHs being still  
168 contained in the 200-2000  $\mu\text{m}$  fraction (82%). The 2-4/5-6 ratios range from 1.4 to 4.0 according to  
169 fraction, with an average value of 2.6 compared to 8.4 for the untreated soil. The reported values  
170 indicate that 2- to 4-ring PAHs were more degraded than 5- to 6-ring PAHs during the  
171 biotreatment. This can be explained by the higher bioavailability of 2- to 4-ring PAHs in  
172 connection to their higher solubility (Bamforth and Singleton 2005). Nevertheless, despite of 6-  
173 month windrow treatment, soil S3T has a higher 2-4/5-6 ratio than soil S2 even after 18-month  
174 biotreatment, suggesting (in the absence of knowledge of initial values) that soil S2 is probably at a  
175 more advanced stage of bioremediation than soil S3T. However, despite that bioremediation is  
176 more advanced in soil S2, soil S3T has a value for PAHs/TOC lower than soil S2 (0.62% compared  
177 to 0.83%) and its total amount of PAHs is also lower than soil S2 that contains a greater amount of  
178 coal tar estimated by TOC. This suggests that soil S2 had been more heavily polluted than soil S3  
179 before biotreatment. Additionally, the efficiency of the biotreatment applied to soil S3 can be  
180 assessed by a strong drop in PAHs/TOC value, from 3.2% to 0.6%. Some of the organic  
181 compounds contained in coal tar were thus degraded more slowly than PAHs or even not degraded  
182 at all.

183

#### 184 **Microscopic observations**

185 The observation of the soil fractions above 200  $\mu\text{m}$  was performed on polished sections instead of  
186 soil particles directly deposited on a sample holder. This method allows observing the internal  
187 microstructure of the particles as cross sections are made during polishing. Moreover, chemical  
188 analyses performed by EDS under SEM are more accurate on the flat surfaces of polished sections.

189 Microscopic observations by SEM of coarse grains of soil S2 indicate that most of them  
190 are aggregates of small particles showing a large array of sizes, generally from 1 to 500  $\mu\text{m}$ , with

191 different levels of grey (Fig. 2a). These levels of grey are linked to the mean atomic weight of the  
192 compounds contained in the particles, darker levels corresponding to lower mean atomic weights.  
193 Thus, organic compounds (with a skeleton made of carbon,  $A_r = 12.01$ ) are dark grey while  
194 mineral compounds (with a skeleton made of silicon in the present case,  $A_r = 28.09$ ) are light grey.  
195 The resin, used to impregnate the soil before sectioning it, appears dark grey like organic  
196 compounds contained in soil particles.

197 Some organic compounds of soil S2 have angular shapes and are very homogeneous,  
198 without visible internal porosity. These angular shapes can be linked to the break-up of larger  
199 fragile particles, suggesting that these organic compounds are solid. Other organic particles have a  
200 smooth rounded shape and are seemingly composed of high-viscosity organic compounds liquid.  
201 Polished sections reveal the presence of a pore network that was not intruded by resin during  
202 preparation. Pores have a flat shape and a size most often smaller than  $10\ \mu\text{m}$  in diameter (Fig. 2b,  
203 enlargement of Fig. 2a). These two major types of organic particles cannot be differentiated by  
204 their chemical composition (EDS analysis): most carbon is found along with some oxygen and  
205 traces of sulphur, as frequently observed in coal tar or coke particles (Picon-Hernandez et al. 2008).

206 Soil S3 also presents some large grains made of smaller organic and mineral particles (Fig.  
207 2c). Contrary to soil S2, most coal tar particles are lens shaped, in agreement with viscous organic  
208 compounds contained in quartziferous sandy soils (Vulava et al. 2007). Smaller soil grains are  
209 often mostly comprised of a unique coal tar particle finely covered with very small mineral  
210 particles glued to its surface (Fig. 2d). Almost all coal tar particles display a large porosity. Pore  
211 networks are most often filled by the resin intruded during the preparation. EDS chemical analysis  
212 was used to differentiate coal tar from resin, both showing almost identical grey levels: the resin  
213 contains chloride whereas coal tar contains sulphur. The presence of resin in the pores indicates  
214 that most pores are connected with the surface of coal tar particles. Some pores opening to the  
215 surface have been reported previously from the observation of the surface of coal tar or coke  
216 particles, the amount of pores varying markedly depending on origin and processing of the  
217 constituents (Panaitescu and Predeanu 2007; Picon-Hernandez et al. 2008).

218 At least two distinct organic compounds are associated with distinct porosities: one organic  
219 compound contains large pores (soil S3) and the other one is denser and displays a porosity looking  
220 like that of soil S2 (Fig. 2d). Some particles, called cenospheres, that can be almost entirely porous,  
221 were also observed by other authors (Panaitescu and Predeanu 2007; Ghosh et al. 2000).

222 In summary, the microstructure of coal tar is very heterogeneous with some anisotropy in  
223 agreement with previous observations by Panaitescu and Predeanu (2007). However, the present  
224 work demonstrates for the first time that the pores open to the surface are connected and thus that



225 pore water can be in contact with the internal porosity of coal tar particles. Observations in optical  
226 microscopy demonstrate even more easily the presence of a connected pore network (Fig. 2e).  
227 Here, the differentiation between coal tar and resin is obvious: organic compounds of coal tar  
228 appear in white with some mosaic patterns in relation to coal tar anisotropy while resin appears  
229 homogeneous with a medium grey level. The connected internal pore network of coal tar particles  
230 is thus well defined by grey-level differences between resin and coal tar organic compounds.  
231 Unconnected pores in which resin cannot penetrate are also well defined, appearing black. Optical  
232 microscopy, simpler and less expensive than SEM, can give satisfactory results when estimating  
233 the amount of porosity within coal tar particles. However, mineral compounds are not so well  
234 discriminated compared to SEM observations with a better contrast due to differences in mean  
235 atomic number. In addition, magnification is rapidly limited for optical microscopy compared to  
236 SEM.

237 SEM observations on soil S3T reveal, in agreement with the distribution of soil fractions  
238 (Table 1), that the biotreatment did not modify the initial size of coal tar particles. Like soil S3, soil  
239 S3T shows large coal tar particles engulfed in a matrix of small mineral grains (Fig. 2f). However,  
240 the volume of the internal pore network of coal tar particles was markedly increased by the  
241 biotreatment (Fig. 2g). In order to further assess the connection of the internal pore system of coal  
242 tar particles with soil water, polished sections of soil S3T were prepared with less pressure applied  
243 to the resin when embedding the soil, the internal pore system of coal tar particles being not always  
244 intruded by the resin. With this specific setup, it was possible to observe mineral particles in  
245 contact with pores open at the coal tar surface. This is an additional evidence of the presence of an  
246 open connected pore network that had developed over the whole volume of coal tar particles during  
247 biotreatment.

248 Image analysis indicated that coal tar particles of soil S2 have a small pore volume fraction  
249 of  $6 \pm 1\%$  while pore volume fractions of  $47 \pm 3\%$  and  $65 \pm 4\%$  are found for soils S3 and S3T,  
250 respectively.

251

## 252 **Discussion**

253 Microscopic observations of coal tar particles brought some helpful additional data to previous  
254 ecotoxicological assays and chemical analyses (Lors et al. 2010, 2011), more especially by  
255 revealing the presence of a connected network of pores open to the surface that depended on the  
256 type of organic compounds contained in coal tar particles. When an open porosity was found, the  
257 coal tar surface potentially in contact with soil water was expected to increase markedly.

258 Additionally, the presence of an open porosity can be beneficial to speed up the rate of  
259 biodegradation of coal tar particles. Indeed, the biodegradation of coal tar particles increased the  
260 amount of porosity indicating that biodegradation occurred on the whole available surface of coal  
261 tar and thus even in the internal porosity of coal tar particles. The relative increase of porosity  
262 between soils S3 and S3T due to biotreatment was 1.38. This increase can be related to the  
263 microbial consumption of organic compounds ( $33\ 000\ \text{mg kg}^{-1}$ ) contained in coal tar particles. As  
264 the volume of coal tar particles remained constant, the proportion of consumption of coal tar can be  
265 related to the relative increase of porosity if the density of coal tar is known. Considering an  
266 average density of 1.3 for coal tar (Brown et al. 2006), the observed decrease of 36.6% of TOC  
267 would lead to increase the porosity of soil S3 by 1.28. This value compares well with 1.38 that  
268 corresponds to the relative increase of porosity between soils S3 and S3T.

269 The diffusion of pollutants and mostly PAHs from coal tar particles to soil water depends  
270 first on the gradient of PAH concentration between coal tar particles and soil water. Thus,  
271 ecoscores have been represented as a function of PAHs/TOC that gives an estimate of PAH  
272 concentration in coal tar particles rather than their concentration in bulk soil as presented in Fig 1.  
273 This estimate is expected to be pertinent for the three studied soils due to their low content in clay  
274 and organic matter, known for having high sorptive affinities to PAHs (Lamichlane et al. 2016).  
275 Ecoscores increase with PAHs/TOC: soil S2 now ranges between soils S3 and S3T (Fig. 3,  
276 compare with Fig. 1). Thus, PAH concentration in coal tar particles differentiates soil S2 from soil  
277 S3 despite a similar concentration of PAHs in both soils. Despite a strong hazard potential, soil S2  
278 gave a moderate ecotoxic response as its coal tar particles exhibited a small internal pore volume.  
279 This comparison of ecoscores and microscopic observations also confirms that analytical results of  
280 solvent extracts are not representative of environmental hazard of PAH-polluted soils (Semple et al.  
281 2003).

282 The amount of internal porosity of coal tar particles can also indicate the best strategy for  
283 remediation. Indeed, if this amount is low, other techniques than biodegradation may be preferred.  
284 The advancement of a biotreatment can also be surveyed by the increase of the internal porosity of  
285 coal tar particles in relation to the biodegradation of organic compounds. Moreover, the value of  
286 internal porosity can be used as a criterion to determine when the biotreatment can be ended.  
287 Despite the necessary statistical approach of microscopy, this technique is the only one that can  
288 assess quantitatively and qualitatively the porosity of coal tar particles. Other techniques, such as  
289 mercury intrusion porosimetry or adsorption isotherm measurements, are not able to differentiate  
290 the surface of organic compounds relative to that of mineral compounds contained in the soil.

291

## 292 **Conclusion**

293 As already known, the assessment of the environmental hazard of soils polluted by NAPLs and  
294 especially by coal tar is complex and a multi-disciplinary approach is thus required. The  
295 bioavailability of PAHs is linked to their rates of desorption from coal tar particles to the soil water  
296 that depends first on diffusion but also on additional mechanisms. The diffusion of PAHs to soil  
297 water can be correlated to the PAH concentration in coal tar particles. It can be estimated in the  
298 case of the reported sandy soils by the overall ratio between PAH concentration and the total  
299 organic content. Indeed, ecoscores increase almost linearly with PAH concentration in coal tar  
300 particles, although this relationship should be verified on a wider array of soils. The rate of release  
301 of PAHs is linked to additional parameters, such as the surface area of coal tar in contact with soil  
302 water. Microscopic observations of the coarser fraction ( $> 200 \mu\text{m}$ ) of two of the three studied soil  
303 samples showed an interconnected pore network opening to the surface of coal tar particles.  
304 Biotreatment increased the amount of porosity due to the microbial consumption of some organic  
305 compounds contained in coal tar particles. The presence of a pore network increases markedly the  
306 surface area of coal tar in contact with soil water and thus the potential release rate of pollutants.  
307 Thus, microscopy can be an additional method to chemical analyses and ecotoxicological assays to  
308 assess the best strategy for remediation but also to follow the efficiency of a biotreatment. Our  
309 study also confirmed that the analysis of PAHs transferred to the water fraction using water instead  
310 of solvent extraction could be advised in the perspective of environmental hazard assessment.

311

312 **Acknowledgements** The present study was performed with a financial support from the ADEME  
313 (Agence de l'Environnement et de la Maîtrise de l'Énergie, France), which is greatly  
314 acknowledged. We thank Total (France) and Charbonnages de France (France) to put industrial  
315 sites at our disposal.

316

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401 **Figure captions**

402 **Fig. 1.** Ecoscores as a function of total PAH concentration of soil. Ecoscores were calculated from  
403 a reduced battery of bioassays (Lors et al. 2011) that was normalized to 100%.

404 **Fig. 2.** Micrographs of studied soils in scanning electron microscopy. a) Soil S2 in scanning  
405 electron microscopy; b) Soil S2 in scanning electron microscopy, enlargement of the central  
406 organic particle of Figure 2a; c) Soil S3 in scanning electron microscopy; d) Soil S3 in scanning  
407 electron microscopy, other view; e) Soil S3 in optical microscopy; f) Soil S3T in scanning electron  
408 microscopy; g) Soil S3T in scanning electron microscopy, other view.

409 **Fig. 3.** Relation between ecoscores and ratios of PAH concentration over total organic  
410 concentration (PAHs/TOC) in the three studied soils. Ecoscores were calculated from a reduced  
411 battery of bioassays (Lors et al. 2011) that was normalized to 100% similarly to Figure 1.

412

401 **Figure captions**1  
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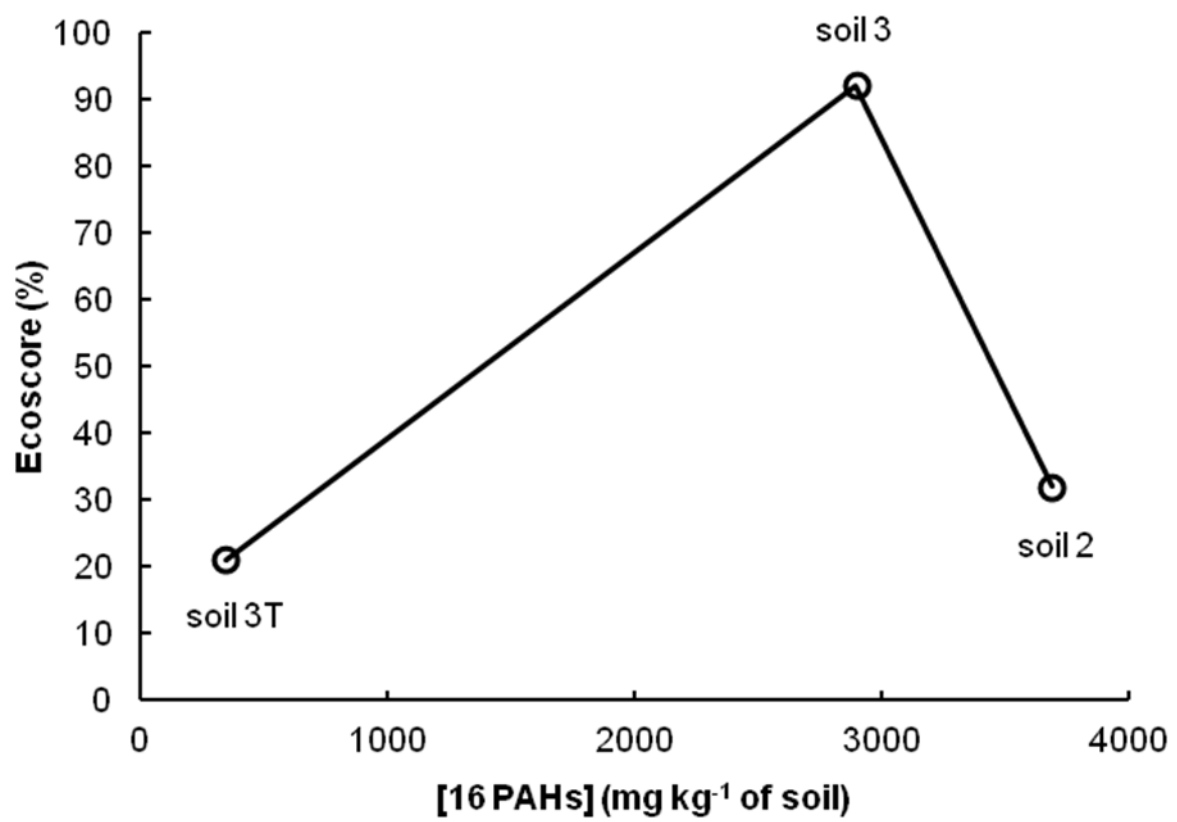


Fig. 1

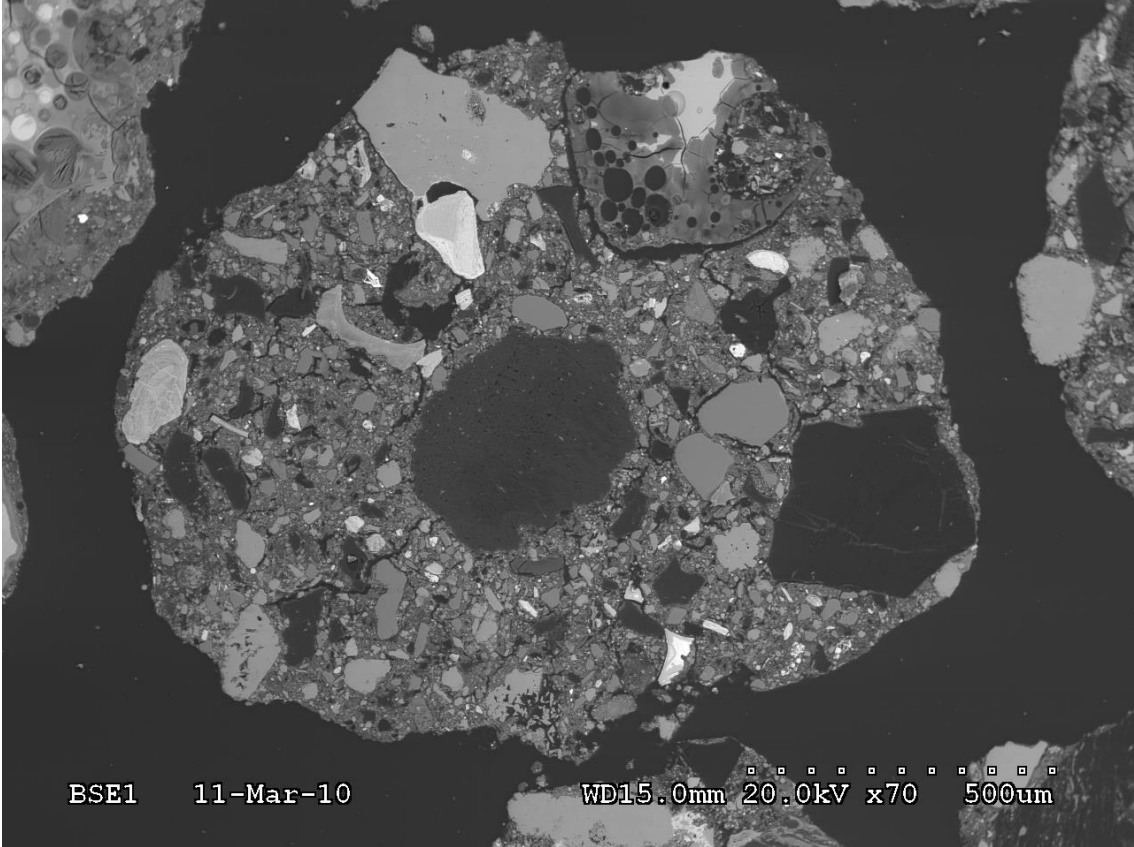


Fig. 2a

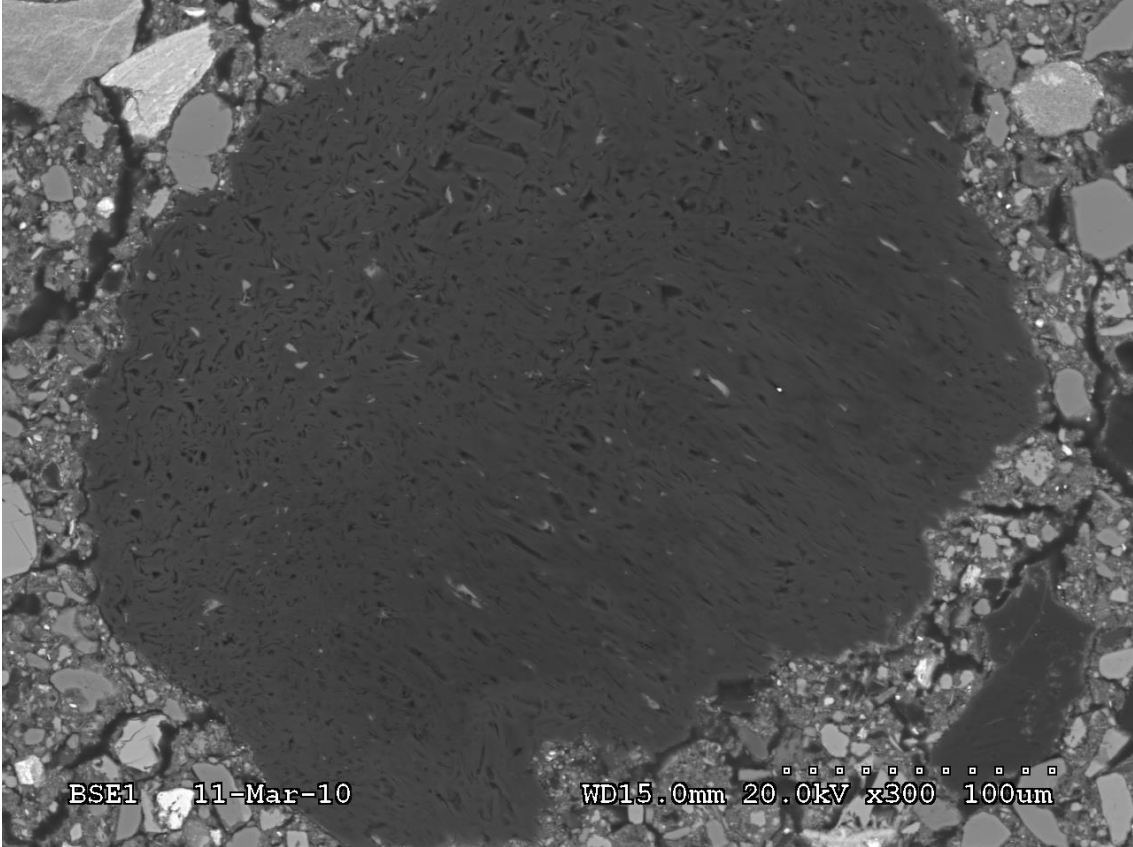


Fig. 2b

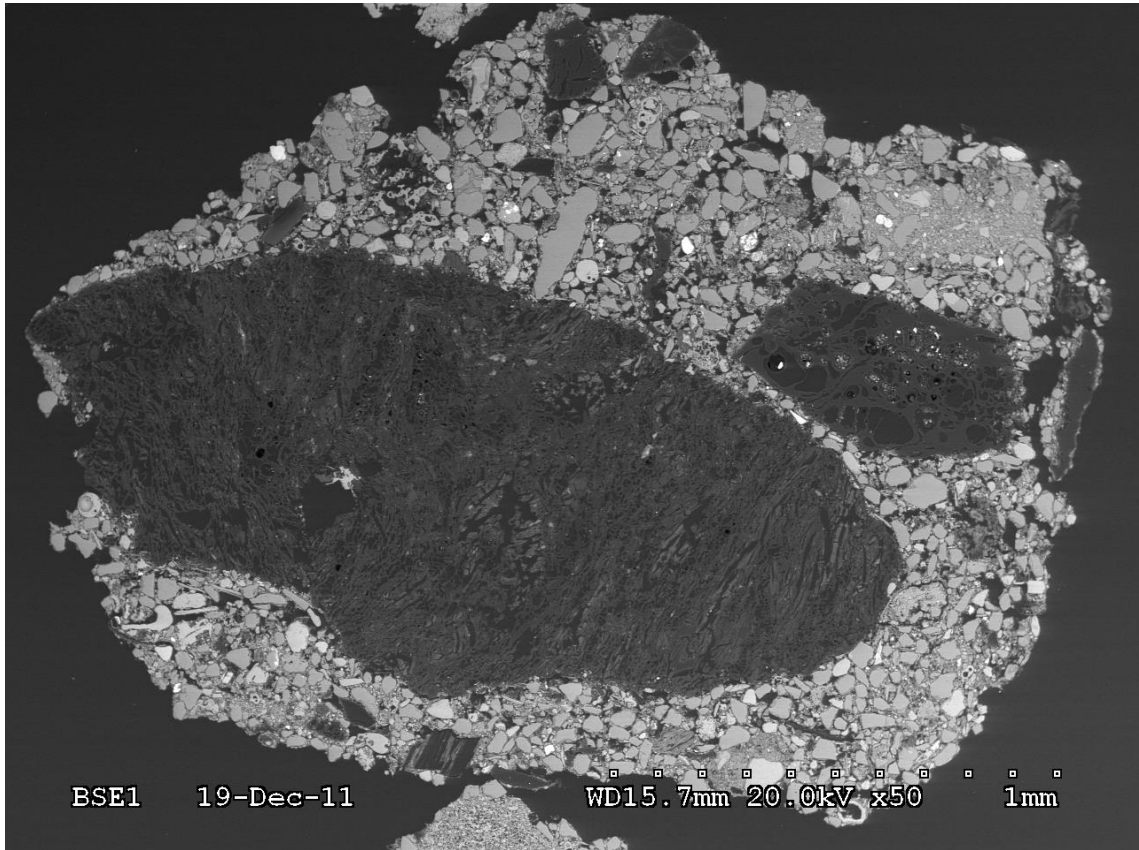


Fig. 2c

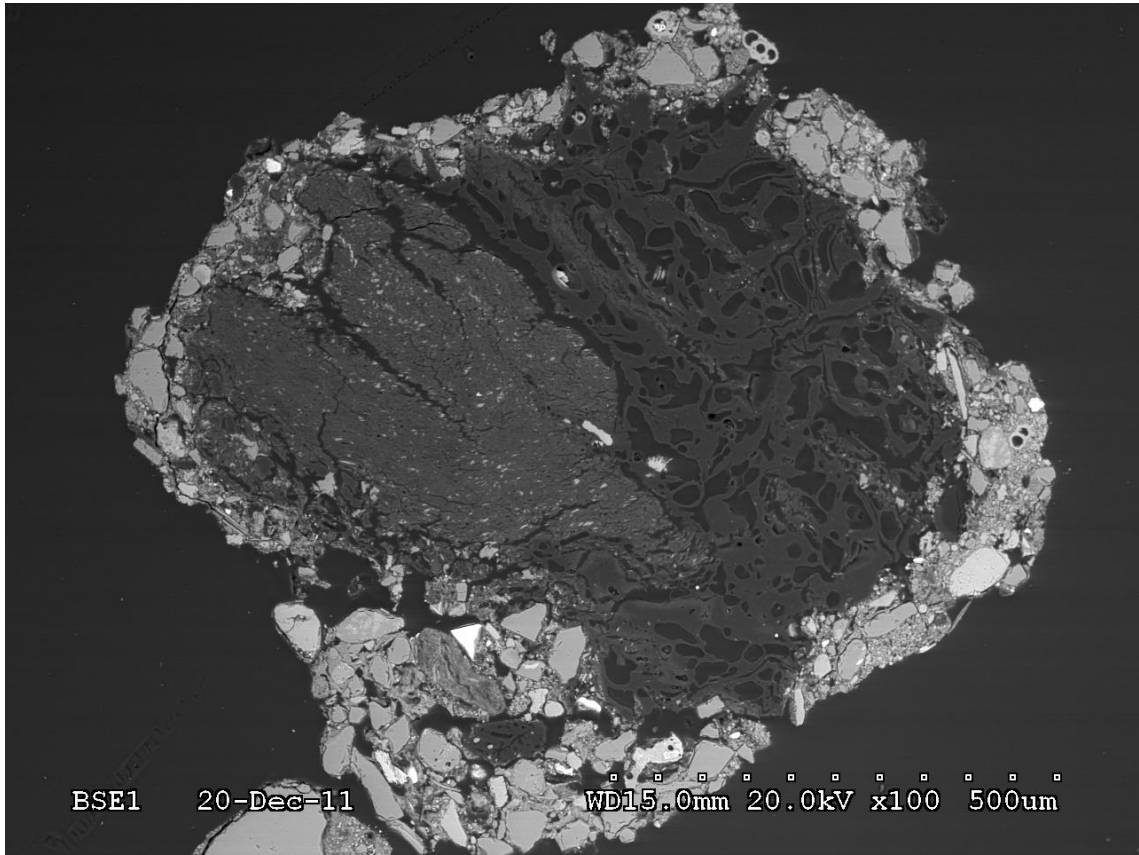


Fig. 2d

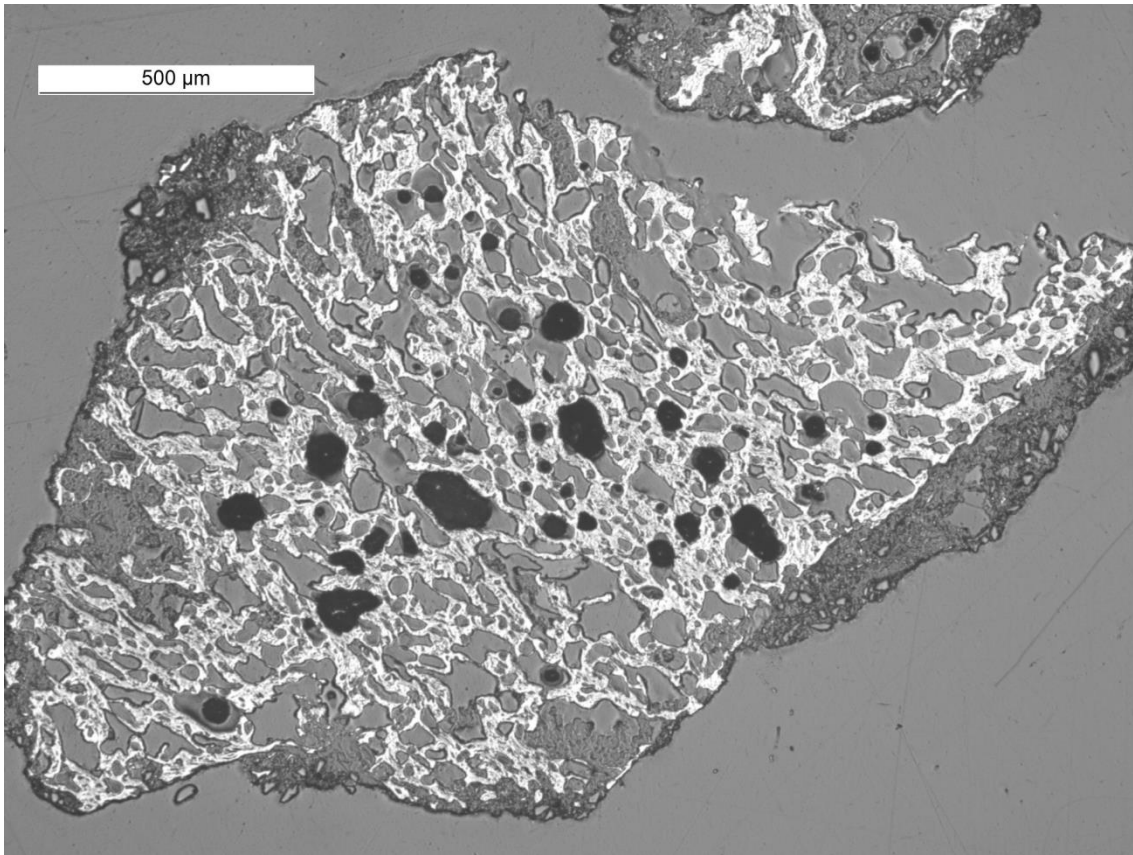
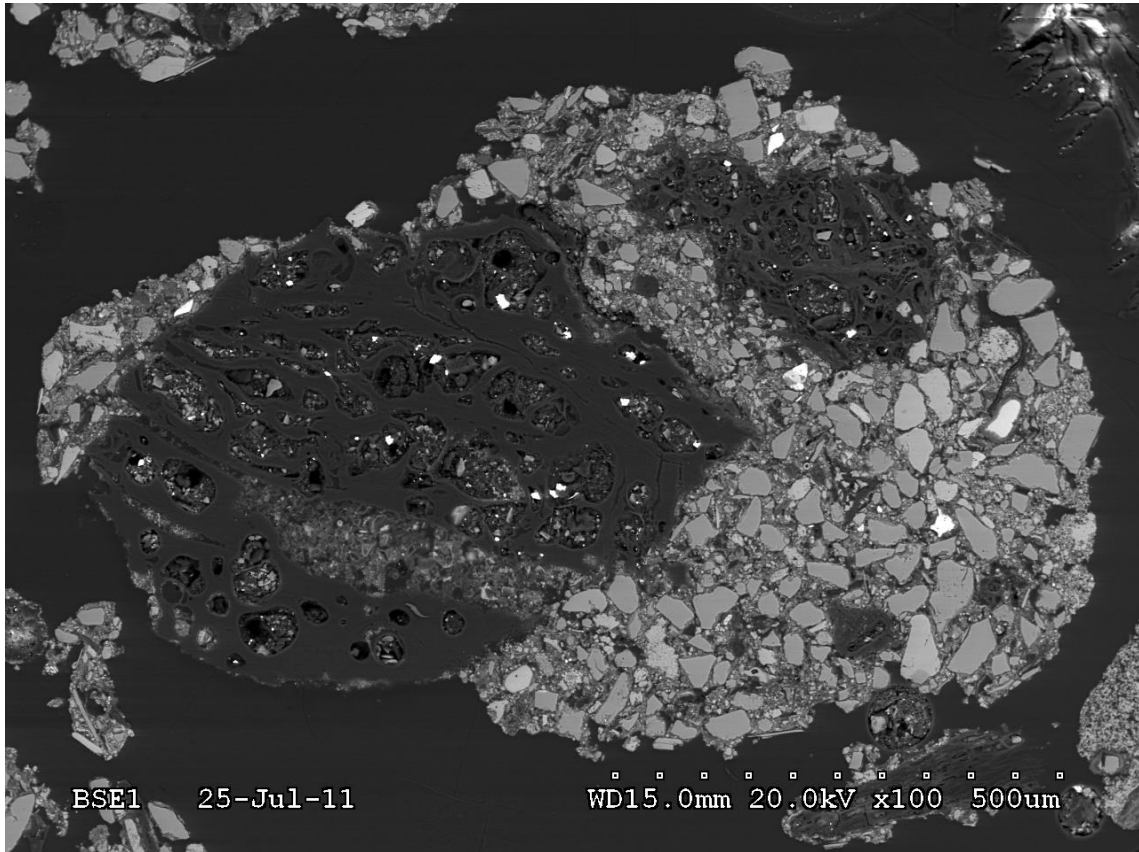
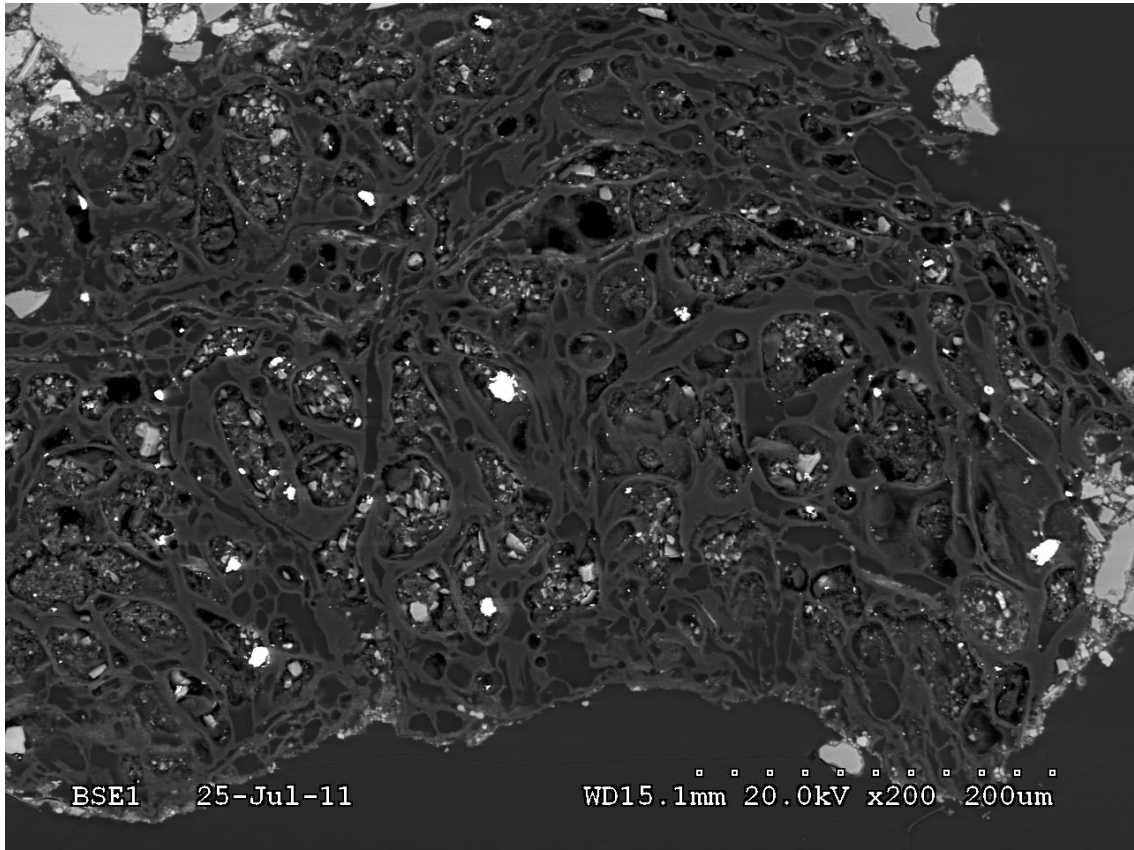


Fig. 2e



**Fig. 2f**



**Fig. 2g**



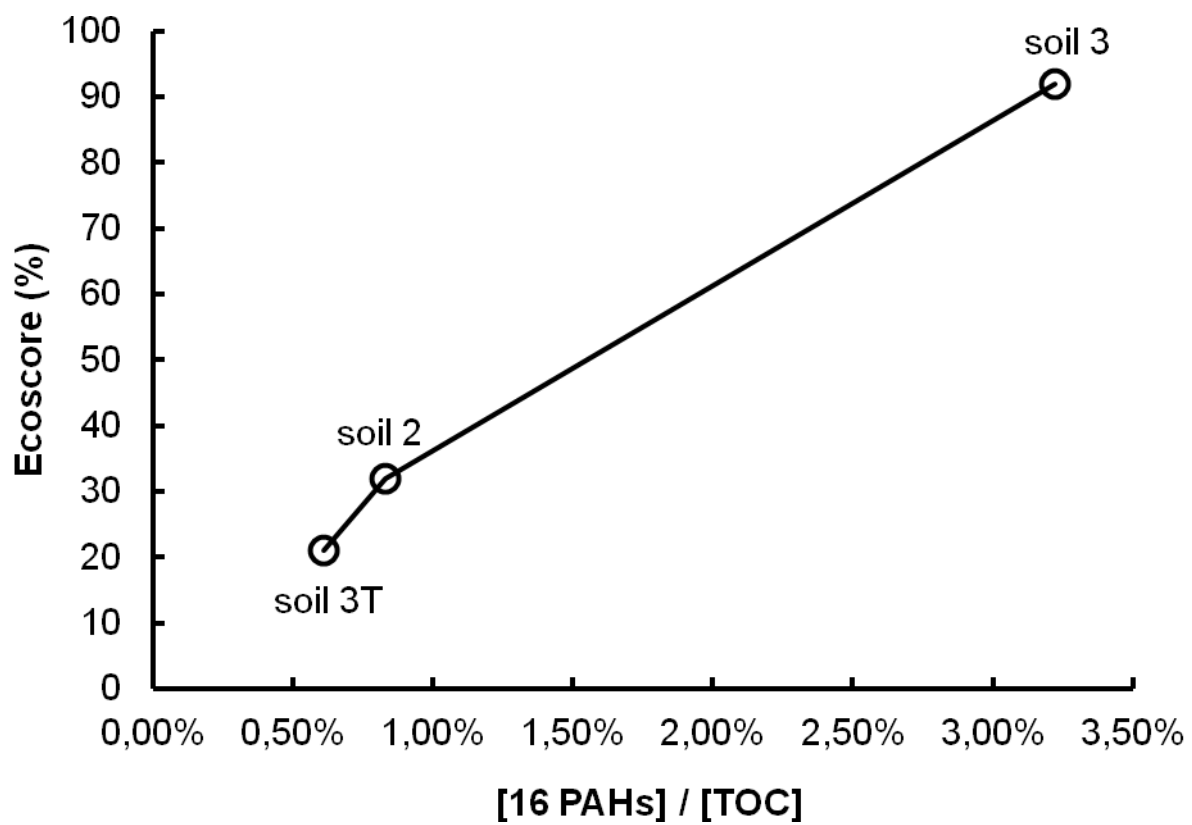


Fig. 3

**Table 1.** Distribution of soil particle size fractions (in mass %) in the three studied soils.

<b>Soil fractions</b>	<b>S2</b>	<b>S3</b>	<b>S3T</b>
<b>&gt; 2000 <math>\mu\text{m}</math></b>	17.19	9.14	9.12
<b>2000-200 <math>\mu\text{m}</math></b>	55.84	67.93	67.82
<b>200 - 50 <math>\mu\text{m}</math></b>	23.41	18.16	18.14
<b>50 - 20 <math>\mu\text{m}</math></b>	1.59	3.05	3.16
<b>20 - 2 <math>\mu\text{m}</math></b>	1.72	1.39	1.44
<b>&lt; 2 <math>\mu\text{m}</math></b>	0.25	0.32	0.33

**Table 2.** PAH and total organic carbon concentrations in each fraction of soil S2.

Concentration (mg kg <sup>-1</sup> of soil)	soil fraction (µm)						Soil = Σ fractions
	> 2000	2000-200	200-50	50-20	20-2.	< 2	
<b>2 ring PAHs</b>	41.6	247.5	109.2	6.2	14.0	1.9	420
<b>3 ring PAHs</b>	191.2	669.0	229.2	8.0	20.4	1.1	1 119
<b>4 ring PAHs</b>	28.3	223.3	97.1	4.2	10.7	0.1	364
<b>5 ring PAHs</b>	100.5	584.7	299.1	9.9	29.3	2.8	1 026
<b>6 ring PAHs</b>	68.4	430.2	228.2	7.3	22.3	1.6	758
<b>Total PAHs</b>	430	2 155	963	36	97	7	3 687
<b>distribution of PAHs</b>	11.66%	58.44%	26.11%	0.96%	2.62%	0.20%	100%
<b>TOC</b>	42 013	325 066	66 786	3 230	4 211	693	442 000
<b>2-4 / 5-6 ratio</b>	1.55	1.12	0.83	1.06	0.88	0.7	1.07
<b>PAHs / TOC (%)</b>	1.02	0.66	1.44	1.10	2.30	1.07	0.83

**Table 3.** PAH and total organic carbon concentrations in each fraction of soil S3.

concentration (mg kg <sup>-1</sup> of soil)	soil fraction (µm)						Soil = Σ fractions
	> 2000	2000-200	200-50	50-20	20-2	< 2	
2 rings PAHs	2.5	65.3	8.1	0.4	0.4	0.0	77
3 rings PAHs	47.1	1 120.0	141.5	1.3	7.2	3.6	1 321
4 rings PAHs	41.0	1 000.4	129.7	3.3	8.7	7.6	1 191
5 rings PAHs	6.8	179.2	23.3	0.4	1.9	1.6	213
6 rings PAHs	2.5	79.0	9.7	0.2	1.2	1.0	94
Total PAHs	100	2 444	312	6	19	14	2 895
Distribution of PAHs	3.45%	84.42%	10.78%	0.19%	0.67%	0.48%	100%
TOC	6 325	77 617	5 146	335	396	181	90 000
2-4 / 5-6 ratio	9.78	8.47	8.48	8.55	5.31	4.37	8.44
PAHs / TOC (%)	1.58	3.15	6.07	1.68	4.90	7.65	3.22

**Table 4.** PAH and total organic carbon concentrations in each of soil S3T.

concentration (mg kg <sup>-1</sup> of soil)	soil fraction (µm)						Soil = Σ fractions
	> 2000	2000-200	200-50	50-20	20-2	< 2	
2 ring PAHs	1.1	19.3	2.5	0.2	0.2	0.0	23
3 ring PAHs	4.1	48.7	4.5	0.2	0.3	0.2	58
4 ring PAHs	8.0	141.2	15.9	0.4	1.2	0.8	168
5 ring PAHs	2.6	51.2	6.6	0.1	0.5	0.4	62
6 ring PAHs	1.2	22.8	10.4	0.1	0.2	0.2	35
Total PAHs	17	283	40	1	2	2	345
distribution of PAHs	4.94%	82.03%	11.55%	0.30%	0.70%	0.48%	100%
TOC	4 448	43 263	7 617	900	490	282	57 000
2-4 / 5-6 ratio	3.47	2.83	1.35	3.96	2.17	1.68	2.58
PAHs / TOC (%)	0.39	0.67	0.54	0.12	0.50	0.60	0.62