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Abstract

We analysed by an optical method (the small volume method) the composition and the vertical distribution of an organic soil which accumulated over an old petroleum tar deposit. The study site was an oil refinery now colonised by woody vegetation since the time of abandonment (1964), located at Merkwiller-Pechelbronn (Alsace, France). Comparisons were done with a nearby unpolluted control plot under similar vegetation. The humus form over the tar deposit was described as an Hemimoder. It was characterised by fine fragmentation of litter, darkening of tree leaves with depth and a dense mycelial mat associated with an ectomycorrhizal root system. Faunal activity was dominated by enchytraeids. The Mesomull described at the control plot was characterized by fast recycling of litter and earthworm activity.

Keywords: Polycyclic Aromatic Hydrocarbons; Humus micromorphology.

1. Introduction

Studies on metal-polluted soils showed that the decomposition of organic matter was affected by a high rate of contamination by trace elements, so that plant debris

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accumulate undecayed over the mineral soil, forming Mor (Coughtrey at al., 1979; Balabane at al., 1999; Gillet and Ponge, 2002). This change in humus form was attributed to the impact of heavy metals on soil organisms, in particular earthworms (Nahmani and Lavelle, 2002; Gillet and Ponge, 2002). We may wonder whether a similar phenomenon occurs when a site polluted by hydrocarbons is abandoned then colonised by forest vegetation, providing an organic matter input to the soil system. Similar changes in humus forms are expected, given detrimental effects of hydrocarbons on soil animal communities and decomposition processes (Erstfeld and Snow-Ashbrook, 1999; Blakely et al., 2002; Shakkir Hanna and Weaver, 2002).

To analyse humus forms, we used a micromorphological method, based on the optical characterization and quantification of biological debris and biogenic structures (Peltier at al., 2001; Sadaka and Ponge, 2003). The same method has been used to study the effects of heavy metal contamination (Gillet and Ponge, 2002). Our work hypothesis was that disturbance of soil microbial and animal activity resulting from hydrocarbon pollution will affect the distribution of organic matter in the upper soil profile, thus in the humus form. As a corollary, the humus form could be used for the early detection of pollution, which will be the purpose of a further study.

2. Materials and methods

2.1. Study site

The study was conducted at the site of the old petroleum refinery of Merkwiller-Pechelbronn, about 50 km north of Strasbourg (Alsace, France). The refinery had an intensive activity until 1964, afterwards it was progressively dismantled then totally abandoned. The site is located on the Pechelbronn oil field (bituminous sand) on the western edge of the Rhine rift valley. The surface soil material (5 m deep) is composed

of recent fluvial deposits overlaying 1400 m thick sediments above the granitic substratum (Sittler, 1985).

Today, the site area (20 ha) is characterised by the presence of old buildings and vegetation including a great variety of semi-natural ecosystems (woodland, grassland, ponds) with zones polluted by hydrocarbons, in particular tar deposits. A polluted plot was selected by visual inspection of the deciduous woodland in October 2002. It was considered to be representative of tar spots in the study site, as ascertained by the visual inspection of numerous soil trenches in the course of a three-day peer-around of the whole site. Only woodland vegetation was considered, since only woody areas remained untouched after cessation of industrial activity. The polluted plot (ca 25 m^2) was characterised by a 3-5 cm thick organic layer overlying a shallow (30-50 cm) pasty petroleum tar deposit. Vegetation was characterized by a discontinuous field layer, composed of *Hedera helix*, *Geranium robertianum*, *Carex pilosa*, *Solidago canadensis* and *Taraxacum officinale*, a shrub layer composed of *Fraxinus excelsior*, *Rubus fruticosus* and *Salix capraea* and a tree layer composed of *Acer campestre*, *Betula pendula* and *Quercus robur*. A nearby, control plot without any visible sign of pollution by hydrocarbons but with similar vegetation, was selected 15 m south of the polluted plot, for the sake of comparison. It was characterised by a much greater plant biodiversity and an earthworm Mull humus form. The field layer was composed of *Hedera helix*, *Arum maculatum*, *Carex pilosa*, *Fragaria vesca*, *Geranium robertianum*, G*eum urbanum*, *Potentilla reptans*, *Stachys sylvatica*, *Solidago canadensis* and *Taraxacum officinale*, the shrub layer was composed of *Acer pseudoplatanus*, *Carpinus betulus*, *Cornus mas*, *Cornus sanguinea*, *Crataegus monogyna*, *Fraxinus excelsior*, *Ligustrum vulgare*, *Prunus avium*, *Rosa canina* and *Rubus fruticosus*, and the tree layer was composed of *Acer campestre*, *Prunus avium* and *Quercus robur*.

2.2. Soil micromorphology

Topsoil profiles (litter included) were sampled and analysed by the micromorphological method of small volumes (Bernier and Ponge 1994). A block 5x5 cm section, with variable depth, was collected at each plot with as little disturbance as possible. A thorough visual inspection of the two compared plots confirmed that sampled humus profiles were representative of the biological soil functioning at both plots. Different layers were distinguished in the block by eye and were directly separated then fixed in 95% (v/v) ethyl alcohol. Layers were identified according to the nomenclature of soil horizons by Brêthes et al. (1995) as OL (entire leaves), OF (fragmented leaves with faecal pellets) and A (hemorganic horizon). When several layers where collected in the same horizon, they were sub-labelled as OL1, OL2,… The thickness of each layer was measured to the next mm.

In the laboratory, each layer was spread gently in a Petri dish, then covered with 95% ethanol, taking care not to break the aggregates. The different components were identified under a dissecting microscope at 40x magnification, with a reticle in the eye piece and quantified by a point-count method. A transparent film with a 200-point grid was placed over the preparation. At each grid node, using the reticule as an aid for fixing the position, the litter/soil component beneath it was identified and classified according to vegetation type, organ, decomposition stage and colour for plant organic matter and according to zoological group, colour and degree of organo-mineral mixing for animal faeces. The various kinds of plant debris were identified visually by comparison with a collection of main plant species growing in the vicinity of the sampled topsoil profiles. Animal faeces were classified by the size, the shape, the degree of mixing with organic matter and colour according to animal groups when possible (Ponge, 1991; Topoliantz et al., 2000). Afterwards, the relative volume

percentage of each component was estimated by summing the corresponding counts then dividing this total by the number of points inspected.

2.3. Chemical analyses

Samples were collected in April 2003 at the Merkwiller-Pechelbronn site to determine the amount of extractable polycyclic aromatic hydrocarbons (EPA list of 16 PAHs) in (1) the 10 top cm of the control soil (2) the tar deposit at 10-20 cm depth and (3) the organic soil overlying the tar deposit. Five samples were taken randomly at each plot then bulked for analysis. They were kept in glass jars then rapidly transported to the laboratory. At the laboratory, soil samples were homogenised and sieved at 1 cm then kept in glass jars at -18C° until analysis. Each sample was defrozen, dried, then sieved at 2 mm. To check the validity of our control plot, samples were collected in May 2003 in the park of the laboratory, in the 10 top cm of a similar soil (rich earthworm mull at neutral pH) under deciduous woody vegetation.

PAHs were extracted from each bulk sample with the automatic system ASE 200 (Accelerated Solvent Extraction) DIONEX, using a mixture of dichloromethane and acetone (50/50) for soil or acetonitrile for tar. The extract was concentrated under forced air with a TuroVap LV (Zymark), then PAHs were separated by HPLC (High Power Liquid Chromatography) with UV detection (alliance 2690 chain, PDA 996 detector, column LC-PAH Supelco). Unfortunately, after sifting the contaminated soil, which was mainly made of badly decomposed tree litter, only a few amount of fine matter was available for analysis, thus only 7 major PAHs could be analysed on this material: naphthalene, phenanthrene, fluoranthrene, pyrene, benzo(a)anthracene, chrysene and benzo(ghi)perylene, the others being under detection levels. All measurements were done in triplicate.

Five soil samples were collected in the same time at both plots from the Pechelbronn site, then they were air-dried and stored in plastic bags for pH measurement. Soil pH was measured in water and in potassium chloride suspensions according to ISO 10390 (AFNOR, 1999). The soil was suspended in deionized H_20 and 1M KCl (1:5 soil:water v:v) for pH H_20 and pH KCl, respectively. Each suspension was shaken for five minutes, then pH was measured in the supernatant after sedimentation for 2 h with a glass electrode. The difference between these two measurements (δpH) was taken as a rough estimate of exchange acidity.

3. Results and discussion

The distribution of the 16 PAHs of the EPA list and the total amount of PAHs were quite similar in the control mineral soil from the Pechelbronn site and the park of the laboratory (Table 1). As a consequence we estimated that the plot chosen as a control at Pechelbronn was valid, even though it was selected within an ancient industrial site. The total amount of the seven PAHs analysed in the soil over the tar deposit at Pechelbronn (5.95 mg/kg) was six times higher than the corresponding amount at the control plot (0.93 mg/kg). At the species level, the concentration of benzo(ghi)perylene was eleven times higher than the control, while the amount of fluoranthrene was only three times higher.

The pH measured in water was nearly neutral, while the pH measured in potassium chloride was one unit lower in both Pechelbronn sites (Table 1). Exchange acidity, expressed by δpH, was higher on the unpolluted site.

One hundred and sixty one categories of litter/soil components were identified in the soil matrix. They were bulked into forty one gross categories for further treatment of the data (Appendix). The humus form at the unpolluted (control) plot was a Mesomull

(Brêthes et al., 1995). Six layers were sampled, which were grouped in two horizons, OL (0-2.7 cm) and A (2.7-6.5 cm). The humus form at the polluted plot was a thin Hemimoder overlying the tar deposit. Seven layers were sampled in three horizons, OL (0-0.7 cm), OF (0.7-1.6 cm) and A (1.6-3.2 cm).

The diagrammatic presentation of the data showed marked differences between plots (Figure 1). In the polluted zone, leaf decomposition was mainly characterised by fine fragmentation and by darkening of limbs (from white to dark black), both processes increasing with depth. In the unpolluted zone, light-brown entire leaves were dominant in the upper part of the OL horizon then leaves were skeletonized and fragmented in the lower part of this horizon, before being totally incorporated into hemorganic assemblages in the A horizon.

The animal activity was dominated by enchytraeids at the polluted plot, where their dark-coloured faeces abounded in OF and A horizons. On the contrary, earthworm activity was prominent in the A horizon from the unpolluted plot, where the hemorganic matter was made of earthworm faeces (of the same colour as the surrounding soil), which became incorporated in a hemorganic mass of similar colour in the lower part of this horizon.

At the polluted plot a dense mycelial system was present in OF and A horizons, which was concomitant to the development of an ectomycorrhizal root system. The unpolluted plot was characterised by non-mycorrhizal root systems (without any visible ectomycorrhizae) in OL and A horizons (OF being absent) and a little mycelial network.

At the polluted plot the lower part of the A horizon was characterised by tar masses and assemblages of tar and mineral matter, which we considered as a probable artifact of the sampling method.

In spite of a slight distance between plots, a dissimilarity in humus forms was observed. The decomposition of organic matter was reduced at the plot polluted by hydrocarbons. This was confirmed by a lower exchangeable acidity, indicating incomplete humification of the accumulated organic matter. However, faunal activity was shown by the presence of springtail and enchytraeid faeces, and skeletonization of leaf litter by the latter group (Ponge, 1999). The finer size of foliar debris at greater depth could be attributed to mechanical fragmentation by frost-and-thaw events, since few earthworm activity was observed at this plot. The absence of an earthworm structure could be attributed to the shallow soil overlying the tar deposit, which prevented animals from burying during winter frost and summer drought. Toxicity of the environment, known to severely affect soil decomposer activity (Blakely et al., 2002), could not explain this local collapse in earthworm populations, since at our PAHs concentrations no toxic effect was observed by authors (Dorn at al., 1998; Erstfeld and Snow-Ashbrook, 1999).

Over the tar deposit we observed a pronounced darkening of leaves, which was reflected in the dark colour of enchytraeid faeces, while on the control, organic matter decomposition was characterised only by early fragmentation and skeletonization of leaves. No bleaching of oak leaves was observed, which could be explained by fast incorporation of litter by earthworms into the mineral part of the soil. On the contrary, at the polluted plot, where white rot activity was similarly absent, fragmented leaves accumulate and come into contact with the pollution source (tar), making possible a transfer of hydrocarbons to decaying leaves, acting as a sink, which could explain their darkening. Strong affinity between organic matter and hydrocarbons has been demonstrated (Xing, 2001).

We showed that roots were ectomycorrhizal at the polluted plot whereas at the control plot no sign of ectomycorrhizal development was observed. Leyval and Binet (1998) demonstrated the advantage of mycorrhizal over non-mycorrhizal plants in the presence of a pollution of the soil by hydrocarbons. They showed that mycorrhizal fungi contributed to the establishment and maintenance of plants in PAH-polluted soils. Unlike Blakely et al. (2002) working on a creosote-polluted soil, we observed at the tar-polluted plot the development of a dense mycelial network. According to observations done by Ponge (1990) on forest litter, and in the absence of fungal decomposition of oak litter on our polluted plot, we suspect that the mycelial mat was mainly formed by mycorrhizal fungi. It is noteworthy that, in addition to facilitate the establishment of vegetation, ectomycorrhizal fungi are able to degrade PAHs with three to five benzene rings (Gramss at al., 1999).

4. Conclusion

Although limited by lack of replication our study showed that strong changes in soil foodwebs could be observed under the influence of pollution by hydrocarbons, forty years after abandonment of industrial activity. Despite a decrease in PAH concentration in the soil accumulated over tar deposits, the inability of earthworm populations to colonize pollution spots, and the development of a superficial ecto-mycorrhizal root mat caused profound changes in the environment of animal as well as microbial communities, as suggested by Blakely at al. (2002). According to the integrated model by Ponge (2003), changes in humus forms can reveal a damage to the ecosystem, and thus could be used for the detection of diffuse as well as spot pollution.

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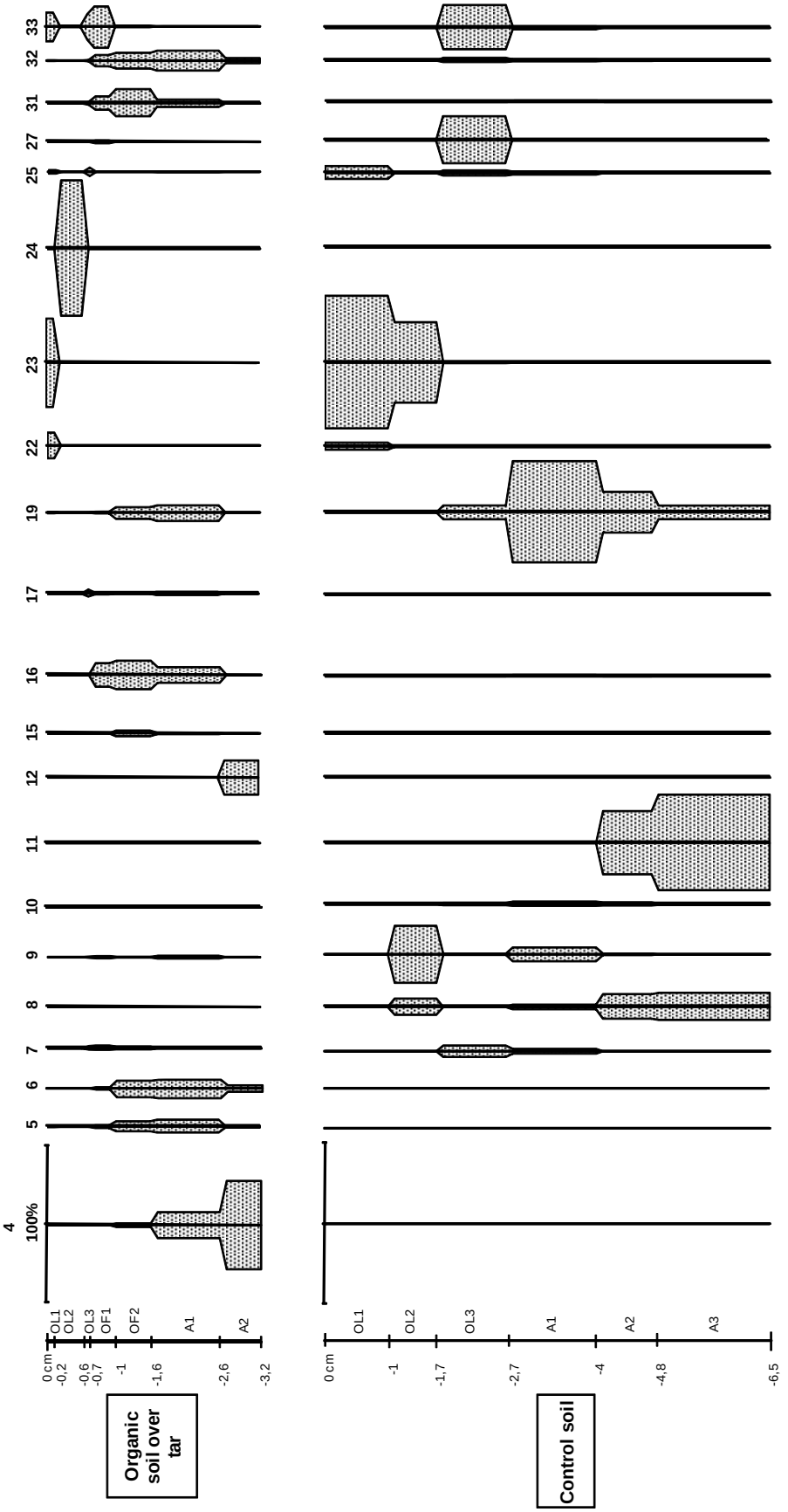
Figure captions

Fig 1. Vertical distribution of main litter/soil components in percent total volume of solid matter (abscissa) at polluted and unpolluted plots (class numbers as in Table 2).

Table 1

Chemical features of tar, organic matter accumulated over tar, control soil from Pechelbronn site and laboratory soil. Concentration of PAH (Polycyclic Aromatic Hydrocarbons) is expressed in µg/g dry soil. Means of three replicated measures on pooled samples (PAHs) or of five replicate samples (pHs) are followed by standard errors. Significant differences among groups at 0.05 level (for pH only) are indicated by different superscript letters (Mann-Whitney test). N.D. = not determined

	Tar	Soil over tar (P)	Control soil (C)	Laboratory soil
pH (water)		6.7±0.3[a]	7±0.4[a]	
pH (potassium chloride)		6.3±0.3[a]	6.2±0.4[a]	
δ pH		0.4±0.04[b]	0.8±0.05[a]	
Naphthalene	52.7±1.0	0.493±0.022	0.096±0.003	0.064±0.005
Acenaphthene	N.D.	N.D.	0.038±0.002	0.023±0.000
Phenanthrene	151.1±2.8	0.776±0.048	0.102±0.006	0.119±0.019
Anthracene	N.D.	N.D.	0.025±0.001	0.024±0.008
Fluoranthrene	N.D.	0.655±0.012	0.238±0.020	0.230±0.024
Pyrene	N.D.	1.551±0.136	0.178±0.020	0.152±0.014
Benzo(a)anthracene	N.D.	0.633±0.033	0.107±0.011	0.080±0.009
Chrysene	35.7±0.4	0.694±0.051	0.108±0.005	0.105±0.013
Benzo(k)fluoranthrene	N.D.	N.D.	0.057±0.004	0.048±0.004
Benzo(a)pyrene	N.D.	N.D.	0.157±0.019	0.147±0.011
Dibenzo(a,h)anthracene	N.D.	N.D.	0.023±0.003	0.016±0.001
Benzo(ghi)perylene	27.2±2.0	1.146±0.031	0.103±0.012	0.074±0.003
Indeno(123cd)pyrene	N.D.	N.D.	0.130±0.008	0.083±0.006
Σ 13 PAHs analysed in C soil			1.363±0.102	1.165±0.094
Σ 7 PAHs analysed in P soil		5.950±0.290	0.934±0.070	0.824±0.083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OL3
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-4
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Control soil

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2 Fig. 1

1

Appendix

List of the 41 classes of litter/soil components identified in our study

N°	Class	N°	Class
1	Fauna	**22**	White entire leaf
2	Moss	**23**	Light-brown entire leaf
3	Lichen	**24**	Dark-brown entire leaf
4	Tar	**25**	Light-brown leaf fragment > 1cm
5	Mycelium	**26**	Light-brown leaf fragment < 1cm
6	Mycorrhizal root	**27**	Dark-brown leaf fragment > 1cm
7	Nerve	**28**	Dark-brown leaf fragment < 1cm
8	Root	**29**	Brown-black leaf fragment > 1cm
9	Twig > 1cm length	**30**	Brown-black leaf fragment < 1cm
10	Twig < 1cm length	**31**	Black leaf fragment > 1cm
11	Light-brown hemorganic mass	**32**	Black leaf fragment < 1cm
12	Hemorganic mass with tar	**33**	Skeletonized leaf fragment
13	Mineral matter	**34**	Skeletonized entire leaf
14	Bud scale and seed	**35**	Brown black entire leaf
15	Brown-black hemorganic faeces	**36**	Leaf covered by mycelium
16	Hemorganic enchytraeid faeces	**37**	Leaf coated with hemorganic faeces
17	Brown-black holorganic enchytraeid faeces	**38**	Flaky mass (dead hyphae+bacteria+organic matter)
18	Hemorganic millipede faeces	**39**	Unidentified organic matter
19	Hemorganic earthworm faeces	**40**	Petiole
20	Light-brown holorganic earthworm faeces	**41**	Stem
21	Light-brown mineral earthworm faeces		

1