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Mutagenic potency in *Salmonella typhimurium* of organic extracts of soil samples originating from urban, suburban, agricultural, forest and natural areas

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ABSTRACT

The purpose of the present work was to assess the mutagenic potency of soil samples presumably not contaminated by industrial wastes and discharges. A set of 51 soil samples was collected from areas considered as not contaminated by a known industrial activity: 11 urban samples (collected in cities), 15 suburban samples (collected in villages), 7 agricultural samples, and 18 forest or natural samples. Each soil sample was collected at the surface (0–5 cm deep), dried, sieved (2 mm), homogenized before organic extraction (dichloromethane/acetone 1/1 (v/v), 37 °C, 4 h, soil/solvent ratio 1/2, m/v), solvent exchange to DMSO and sterilizing filtration. The micro-method adaptation of the standard bacterial mutagenicity test on *Salmonella typhimurium* strain TA98 was performed with and without a metabolic activation system (rat-liver homogenate S9), and thus detected the effect of pro-mutagens and direct mutagens, respectively. The use of a pre-incubation method increased the sensitivity of the assay. The results obtained showed a wide range of effect levels, from no effect to clear mutagenicity. In particular, the extract of all 11 urban soil samples demonstrated mutagenic activity, while the extracts of 10 of the 15 suburban samples showed mutagenicity. On the other hand, the extract of only one of the 7 agricultural samples studied induced mutations, and none of the 18 natural or forest-soil samples investigated produced mutagenic extracts. These findings seem to indicate the crucial influence of the diffuse pollution originating from different human activities on the mutagenic potency of urban soil samples. These findings make it possible to classify the soils according to their mutagenic potency. No clear correlation was found between the mutagenicity detected in soil extracts and the measured polycyclic aromatic hydrocarbon (PAH) content of the soils investigated.

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1. Introduction

A significant part of the chemicals released in the environment by various human activities ends up in surface soil. Considering that many contaminants induce harmful effects and that humans can be exposed to soils through different routes (inhalation, ingestion or skin contact), contaminated soils constitute a potential source of risk for human health. Soil characterization is one of the first steps performed in the assessment of the health risks associated with human exposure to a soil. At present, procedures for soil characterization are based on chemical analyses (i.e. search for and measurement of known contaminants) and individual toxicity values (data only available for a limited number of chemicals). This type of approach is non-exhaustive and informs us neither about the real toxic effects on living organisms nor about the potential synergetic or antagonistic effects induced by mixtures of contaminants in soils.

The occurrence of mutagenic activity in soil extracts from different origins has now clearly been demonstrated by several authors, and reviewed by White and Claxton [1]. When initiating our work on soil mutagenic potency, one of the key questions was that of control soils: "Which soil samples should be chosen in order to obtain soil extracts without detectable mutagenic activity?". The aim of the study reported in the present paper was to evaluate the

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mutagenic potency of presumably uncontaminated soil samples, i.e. soils not contaminated by a known industrial activity of production, storage or discharge of chemicals. To do so, we collected 51 soil samples of different origin and submitted their extracts to the micro-method adaptation of the standard mutagenicity test with the bacterium *Salmonella typhimurium* [2].

2. Material and methods

2.1. Chemicals

All chemicals used in the present study were HPLC grade. The CAS number, the purity and the provider for the solvents used were as follows: dichloromethane (CAS No. 75-09-2, purity 99.5%) and acetone (67-64-1, 99.8%) from Carlo Erba Reagenti (Italy), and DMSO (67-68-5, 99.7%) from Merck (Germany). The chemicals used as reference mutagenic compounds (positive controls) in the *Salmonella* mutagenicity test were 2-nitrofluorene (CAS No. 607-57-8, purity 98.2%) from Merck, and 2-anthramine (613-13-8, 97.4%) from Aldrich.

2.2. Soil samples

2.2.1. Origin

A set of 51 soil samples was studied in the present work. All were considered "control" soil samples, that is to say none were known to be affected by an industrial facility that produced industrial wastes and discharges. The origin and nature of the soil samples collected were various, with the aim to obtain a representative database. The soil samples were mainly collected in the North of France, but also in other regions of France and in other countries.

2.2.2. Classification of soil samples

In a key review on soil mutagenicity, White and Claxton [1] distinguished three categories of sampling sites: (1) industrial sites, located on the grounds of an industrial facility that produces industrial wastes and discharges, (2) urban/suburban sites, located in cities or towns but not directly on the grounds of an industrial facility that produces industrial wastes and discharges, and (3) rural/agricultural sites, located outside urban and suburban communities and including remote park-like settings and agricultural areas. In the present work, no industrial site was studied, and the collected soil samples were classified into four categories:

- Urban soils, collected in cities of more than 20,000 inhabitants, or in densely populated areas.
- Suburban soil, collected in villages or in less densely populated areas.
- Agricultural soils, collected in areas outside urban and suburban communities, but possibly affected by biological or chemical contamination, originating from manure, fertilizer or pesticide spreadings.
- Natural or forest soils, collected in areas that should not be contaminated by direct urban or industrial activities; these sites may, however, be submitted to other sources of contamination: natural characteristics, atmospheric fallout, water transfers through flooding, or scattered wastes.

It should be noted that soil-category assignments were based on site descriptions only, and were performed prior to analysis of mutagenicity or PAH data.

2.2.3. Collection of the soil samples

The soil samples were collected according to the following procedure: 500 g to 1 kg of surface soil were collected in open soil area, avoiding recently moved soil as well as possibly polluted areas (e.g., barbecue ash, parking places, former manure heaps). Only surface soil samples, i.e. 0–5 cm deep, were collected because it was demonstrated that soil mutagens are airborne mutagens that accumulate in the top layer of the soil [3]. All soil samples were identified, placed in a glass container protected from light, and maintained at 4 °C at the laboratory until drying and extraction.

2.2.4. Treatment of the soil samples

2.2.4.1. Drying. As soon as possible after collection, the soil samples were dried at room temperature under a laboratory hood. Each soil sample was aerated and weighed regularly. After 24–48 h, depending on the sample, the weight of the sample did not decrease any more with time, and the sample was considered dry. The sample was then mechanically broken up using mortar and pestle, and passed through a 2-mm sieve for homogenization and removal of gravel, stones and plant materials. The sieved fractions of each soil sample were mixed and homogenized, and either extracted immediately or placed at +4 °C protected from light until extraction. The drying of the soil sample and the storage at +4 °C were performed in order to minimize chemical degradation as observed, e.g., with soils contaminated by polycyclic aromatic hydrocarbon contaminated soils [4].

2.2.4.2. Extraction. A single extraction procedure was performed on each collected soil sample, as follows: 3 g of soil sample was placed in a 60-mL glass bottle (using a coloured glass in order to prevent photo-degradation) and 6 mL of organic extraction solvent (dichloromethane/acetone, 1/1, v/v) was added. The mixture was placed in a water-bath shaker (120 rpm) for 4 h at 37 °C. At the end of the extraction time, the glass bottle containing the mixture was left standing at room temperature during at least 15 min for sedimentation of the large particles. The liquid phase was then recovered and submitted to centrifugation (10 min, $863 \times g$) in order to remove the smaller soil particles that may have been recovered with the solvent.

2.2.4.3. Solvent exchange. The mixture of organic solvents used for extraction is not compatible with the *Salmonella* mutagenicity test due to intrinsic toxicity. A solvent exchange was thus performed by replacing the extraction solvent mixture with dimethyl sulfoxyde (DMSO), which is compatible with the *Salmonella* test. To do so, the extraction solvent was evaporated at 37 °C under nitrogen, and 1 mL of DMSO was added before complete drying of the extract. The evaporation procedure was then continued until only DMSO was left in the sample. Before use in the *Salmonella* test, all extracts were filtrated through a DMSO-resistant 0.45-μm membrane in order to ensure sample sterility. The solvent-exchange procedure resulted in concentration of the initial organic extract, such that the final concentration in DMSO was 3 g soil equivalent per mL. The doses studied in the *Salmonella* test were expressed as mg soil equivalent per plate, and the highest dose tested was 30 mg soil equiv./plate.

3. Analysis of PAH content of the soil samples

3.1. Extraction

The soil samples were analyzed for their content in the 16 PAHs listed by the USEPA. The extraction step was carried out using an accelerated solvent-extractor system (DIONEX ASE 2000). The soil matrix was treated for 15 min at 100 °C and 2000 psi with a solvent mixture of dichloromethane and acetone (1/1, v/v). The solvent was evaporated under an air stream to nearly dryness, and the sample was diluted with acetonitrile before 0.45-μm filtration and quantification.

3.2. Quantification

The concentrations of the 16 PAHs were determined in the extracts by separation on an HPLC column (Supelco, C18 reverse phase, length 250 mm, diameter 2.1 mm) followed by photo diode-array detection (Waters 996). The mobile phase was a gradient of acetonitrile and degassed water (flow rate 0.3 ml/min). A guard column (Supelco, C18 reverse phase, length 20 mm, diameter 2.1 mm) was positioned in front of the HPLC column. Depending on the identity of the analysed PAH, the limit of quantification ranged from 5 to 21 μg/kg.

4. Micro-method *Salmonella* test

The mutagenicity assay performed in this study was a micro-method adaptation of the standard *Salmonella* test [5,6] using a 96-well micro-titre plate. Briefly, the bacterial suspension from a culture agitated overnight at 37 °C was concentrated 5 times and the concentrate was stored on ice until use. In each well, 10 μl of the soil extract, 100 μl of the concentrated bacterial suspension, and 90 μl of phosphate buffer in the assay without metabolic activation or 90 μl of S9-mix in the assay with metabolic activation were successively added. The plates were incubated at 37 °C under stirring for 90 min. The content of each well was then transferred into 2 ml of top agar, maintained in a state of superfusion at 45 °C. The content of each tube was thoroughly mixed and spread out in a Petri dish containing 20 ml of minimum agar. The number of bacteria applied to each plate was 0.5×10^9 to 1×10^9. The plates were then kept at 37 °C for 48 h, after which the number of revertant colonies was determined for each plate.

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