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ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOIL WITH AN ENZYME-LINKED IMMUNOSORBENT ASSAY KIT AND WITH GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROSCOPY

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) exist as pollutants in soil on sites where for example wood impregnation or coal gasification have occurred[1,2]. In the remediation of such sites it is of crucial importance to map the distribution of the PAHs, as well as to determine the remediation success as it progresses. With this in view, a fast, accurate and field adapted tool for analysis is needed. The enzyme-linked immunosorbent assy kit, the PAH RIS$^{c®}$ soil test, fulfils these demands[3] and has been validated for field screening purposes in Germany[4,5]. With the aid of this immunoassay it is possible to decide if the PAH level in the soil is above or below a predetermined limit, which is sufficient in a screening situation. However, for quantitative measurements of PAH content and for verification of immunoassay test results, instrumental analysis such as gas chromatography coupled to mass spectroscopy (GC-MS), is recommended[5].

Due to legislative reasons, Swedish PAH data is often generated through instrumental analysis. Usually, the levels of priority-pollutant PAHs, as listed by the US Environmental Protection Agency (US-EPA), are reported[6]. Therefore, it is interesting to know how the results obtained with the PAH RIS$^{c®}$ soil test compare with for instance GC-MS data.

The aim of this study was to investigate the quantitative performance of the PAH RIS$^{c®}$ soil test kit. For that reason, eleven soils were analysed with the test kit and with GC-MS. The results were compared and a correlation factor between the methods was considered. Furthermore, the impact of different soil extraction methods on the PAH concentration determinations was examined.

Materials and Methods

Eleven soils were collected at wood impregnation and coal gasification sites in Sweden. Portions of the samples were extracted by shaking with methanol (MeOH) for one minute and with pressurised liquid extraction (PLE) with hexane[7]. Two of the soils were also extracted by sonication with methanol for five minutes at 30 ºC and by shaking with ethanol (EtOH) for one minute.

The PAH RIS$^{c®}$ soil test procedure

A dilution series of each soil extract was prepared in methanol. The tests were carried out according to the protocol supplied with the kit. In short, 1 ml of buffer and 75 µl of soil extract dilution were added to each conjugate tube, and they were gently shaken. The antibody tubes were

then attached to the conjugate tubes, and the connected tubes were inverted, initiating competitive binding of the PAHs (originating from the soil) and phenanthrene horseradish peroxidase conjugate to the antibodies. After 10 min incubation at room temperature, the antibody tubes were washed and drained. Then, 200 µl of substrate A followed by 200 µl of substrate B, were added to each test tube. After 2.5 min incubation, 200 µl of stop solution was added. Finally, the absorbance at 450 nm was measured. Each test series included a 75 µl phenanthrene standard (9 ng/g) and a 75 µl methanol blank. The phenanthrene standard was used as reference in all of the absorbance measurements.

GC-MS analysis
All samples were analysed by GC-MS as described elsewhere[7]. Prior to GC-MS analysis, the soil samples were extracted with PLE in a similar way as prior to the immunoassay analysis.

Results and Discussion
Following immunoassay analysis, the absorbance value was plotted against the logarithm of the dilution factor for each soil extract. The linear relationship between absorbance and dilution factor of PLE extract, soil sample 7, is shown in Figure 1. The absorbance of all extracts was within the linear range, and data from linear regression analysis was therefore used to derive the intercept with the x-axis. The dilution factor at the intercept corresponds to a phenanthrene concentration of 9 ng/g, and this value was used to determine the PAH content of the soil, as phenanthrene equivalents. The PAH results from immunoassay analysis together with GC-MS data for 24 PAHs are presented in Table 1.

To evaluate how well immunoassay and GC-MS data agree, a correlation factor between the methods was determined for each soil extract. The immunoassay results from PLE and methanol extracts were divided with the corresponding GC-MS results (Table 2).

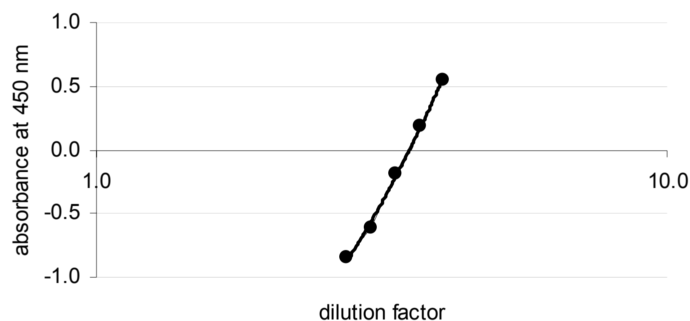

Figure 1. Relationship between absorbance and dilution factor for PLE extract, soil sample 7. The intercept with the y-axis is -3.91, the slope 1.11 and the regression coefficient 0.9971.

Table 1. Total PAH concentration (μg/g) in eleven soil samples according to GC-MS and immunoassay analysis, presented as phenanthrene equivalents.

Sample	GC-MS	Immunoassay			
		PLE	*MeOH*	*MeOH sonication*	*EtOH*
1	39	288	8		
2	70	1102	11		
3	140	352	23		
4	3377	1870	1582		
5	1509	1059	605		
6	1637	2873	1429		
7	2810	4167	1954		
8	818	1043	133		
9	1447	1541	816		
10	9306	12881	3580	2232	2276
11	505	732	134	113	117

Table 2. Correlation factors between GC-MS and immunoassay data.

Sample	Extraction	
	PLE	*MeOH*
1	7.4	0.2
2	16	0.2
3	2.5	0.2
4	0.6	0.5
5	0.7	0.4
6	1.8	0.9
7	1.5	0.7
8	1.3	0.2
9	1.1	0.6
10	1.4	0.4
11	1.5	0.3

The PAH concentrations obtained with the immunoassay test kit after PLE extraction were higher than after shaking with methanol or ethanol and after sonication with methanol (Table 1). This is probably due to the higher extraction efficiency of PLE as compared to the other methods. For soil samples 1 through 3 and 11, which had the lowest PAH content, the discrepancy was considerable. This is probably due to the relatively higher probability for surface sorption at low concentration as compared to high concentration (assuming a limited number of strong sorption sites). Consequently, the PAH concentrations for soils with higher PAH content were in much better agreement, both between GC-MS and immunoassay derived data and between immunoassay data generated with the different extraction methods.

Yet, a consistent ratio between immunoassay and GC-MS data was not possible to establish for the soil samples studied (Table 2). The PAH concentrations obtained with immunoassay after PLE extraction were generally slightly higher than the results achieved by GC-MS, while methanol extraction yielded lower concentrations than GC-MS. This might be due to differences in both extraction efficiency and detection specificity. After PLE, the amount of phenanthrene, cross-reacting PAHs, and other possible cross-reactants, such as polycyclic aromatic compounds (PACs), is elevated as compared to the methanol extracts.

In conclusion, the immunoassay test kit under investigation in this study can be used as a means to semi-quantitatively detect PAHs in soils of various origins. By using linear regression analysis on data from the immunoassay test, it is possible to decide the total PAH concentration in the soils, and even though it is represented as phenanthrene equivalents, this number is close to the total PAH concentration as determined by GC-MS analysis. The simplicity of the methanol extraction as compared to PLE outweigh the lower accuracy in the PAH determinations in most field screening situations.

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References

1. Howsam M. and Jones K.C. (1998) in: The Handbook of Environmental Chemistry, Part I (Hutzinger O., Ed.) Springer-Verlag, Berlin
2. Mueller J.G., Chapman P.J. and Pritchard P.H. (1989) Environ.Sci.Technol. 23, 1197
3. McDonald P.P., Almond R.E., Mapes J.P. and Friedman S.B. (1994) J.AOAC Int. 77, 466
4. Kramer P.M. (1998) Anal.Chim.Acta 376, 3
5. Kramer P.M., Ebert K., Eikenberg O. and Kettrup A.A.(1998) Am.Environ.Lab. 10,16
6. Development of generic guideline values (Report 4639), Swedish Environmental Protection Agency
7. Lundstedt S., van Bavel B., Haglund P., Tysklind M. and Öberg L.G. (2000) J.Chromatogr.A 883, 151