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Fluorescence Spectroscopy of Polynuclear Aromatic Compounds in Environmental Monitoring

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The occurrence of polynuclear aromatic compounds (PAC) in the environment and experimental techniques suitable for the detection of PAC in environmental compartments are briefly reviewed. The specific requirements for on-site and *in situ* environmental analysis are outlined. Particular emphasis is given to fluorescence spectroscopic techniques for the investigation of humic acid- and soil-containing samples. Some examples of studies in the literature on Shpol'skii and jet spectroscopy and on laser-induced fluorescence (LIF) measurements of PAC and mineral oils are highlighted. Contaminants in the environment are usually encountered as multicomponent mixtures in very complex matrices. Total fluorescence analysis in combination with the chemometrical technique of rank annihilation factor analysis (RAFA) was employed for the evaluation of a six-component PAC mixture in toluene. It was shown that even in the presence of strong spectral overlap the qualitative identification of all compounds and the reliable quantification of five substances was possible. Results are presented from our stationary and time-resolved fluorescence investigations of the interactions between pyrene and humic acid in water. The Stern–Volmer analysis showed a significant effect of pH on the static quenching efficiency which can be explained by the pH-dependent macromolecular structure of humic acids. Preliminary results from studies of the deactivation of triplet PAC and quenching of delayed fluorescence by humic acid are reported. LIF measurements of mineral oils directly from soil surfaces and of a model oil in a soil column were performed with a fiber-optic coupled multichannel spectrometer. The fluorescence intensity/concentration relationships were established for a crude and a fuel oil; the corresponding lower limits of detection (LOD) were determined to be 0.025 and 0.125% m/m (mass/mass percentages). These detection limits are compared with realistic oil contaminations of soils. In a soil column designed to mimic fixed-bed bioreactors the distributions of fluorescence signal intensities from a perylene-doped model oil before and after water flooding were determined. These results from *in situ* measurements can provide a quantitative basis for the modelling of temporal and spatial contaminants' distributions in reactor design.

KEY WORDS: Environmental analysis; fluorescence spectroscopy; laser-induced fluorescence (LIF); polynuclear aromatic compounds (PAC); mineral oils; humic substances; soil.

INTRODUCTION

In the last decades, the prime importance of environmental issues and the ever-increasing concern about environmental problems—on both global and local scales—have induced an almost unprecedented growth of environmental analytical research. Consequently, a tremendous number of studies encompassing a broad spectrum of experimental techniques are currently being published. For example, for the biannual review on *Environmental Analysis* in *Analytical Chemistry*, more than 20,000 abstracts were examined, and some 160 review

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articles and more than 1100 original papers published in 1991 and 1992 are listed [1]. These numbers indicate that the individual scientist can seldom follow in detail the developments in his special area of research, let alone in the whole field of environmental analysis. It is therefore particularly important for the specialist to be aware of current developments in the larger field of environmental research and continuously to assess the advantages and limitations of the experimental and conceptual approaches in use.

Analytes, methods, and matrices are the important coordinates with which experimental work in environmental research can conveniently be classified. In the present paper we concentrate on polynuclear aromatic compounds (PAC)[3] as analytes. The occurrence of these ubiquitous pollutants in the environment is summarized under PAC in the Environment. Next, we will attempt to briefly review some principal aspects of environmental analysis with particular reference to recent developments in *in situ* and on-site investigations (On-Site and *in Situ* Environmental Analysis). Among the various experimental techniques employed, the role of fluorescence spectroscopy is illuminated, and special attention is paid to the techniques of total fluorescence analysis (TFA) and laser-induced fluorescence (LIF) under Fluorescence Spectroscopy for Environmental Analysis.

Fluorescence spectroscopy has successfully been applied for the detection and analysis of PAC in the air and in water, but only a few studies on the investigation of PAC in soil-containing environmental compartments have hitherto been conducted. One focus of this paper is therefore the interactions of fluorophores with humic substances (Interactions Between Fluorophores and Humic Substances) and on the fluorescence detection of PAC from soil surfaces. Results of our studies of mineral oil contaminated soils are presented under Fluorescence Measurements of Mineral Oils on Soil Surfaces.

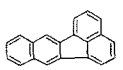
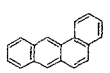
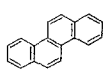
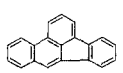
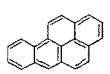
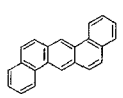
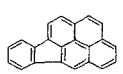
[3] *Abbreviations used:* DF, delayed fluorescence; EEM, excitation–emission matrix; EPA, U.S. Environmental Protection Agency; FIA, flow injection analysis; FOCS, fiber optical chemical sensors; FTIR, Fourier transform infrared; LAMMA, laser microprobe mass analysis; LIDAR, light-induced detection and ranging; LIF, laser-induced fluorescence; LOD, limit of detection; MALDI, matrix-assisted laser desorption/ionization; MPI, multiphoton ionization; OSA, optical spectrum analyzer; PAC, polynuclear aromatic compound; PAS, photoelectrical aerosol sensor; RAFA, rank annihilation factor analysis; RTP, room-temperature phosphorescence; SDW, soil dry weight; SERS, surface enhanced Raman spectroscopy; SIMS, secondary ion mass spectroscopy; SIT, silicon intensified target; TDGC/MS, thermal desorption-gas chromatography/mass spectrometry; TFA, total fluorescence analysis; THEES, total human environmental exposure study; TTA, triplet–triplet annihilation; UMC, uncorrected matrix correlation; WHO, World Health Organization.

PAC IN THE ENVIRONMENT

The occurrence, distribution, and fate of PAC as xenobiotics in the environment have been summarized in several reviews (e.g., Refs. 2 and 3). Human exposure to PAC is of particular concern since many PAC show mutagenic and/or carcinogenic activity in screening tests and animal experiments. The U.S. Environmental Protection Agency (EPA) has included 16 PAC on its list of priority pollutants. Some basic photophysical properties illustrating the absorption and fluorescence behavior of these compounds in solution are summarized in Table I. Particular reference is given to those six PAC of the EPA list which are probable or suspected human carcinogens [4–6]. The data in Table I show that all compounds can conveniently be photoexcited in the UV/visible part of the spectrum and—with the exception of acenaphthylene—that they have good fluorescence quantum yields (Φ_F). Their fluorescence emissions can thus be detected by standard stationary and time-resolved experimental techniques (Fig. 1), unless specific interactions result in strong fluorescence quenching. It is therefore obvious that in order to evaluate the assets of fluorescence spectroscopy in environmental analysis, the occurrence of specific fluorescence quenching effects in environmental matrices requires a detailed investigation.

Limits for certain PAC in industrial effluents, drinking water, soils, etc., have been recommended by the World Health Organization (WHO) and many other institutions. Particularly important are not only the concentrations of PAC in the various environmental compartments but also, of course, the bioavailability of and the human exposure to PAC. The exposure to PAC at the work place and in everyday living has been obtained in selected cases from total human environmental exposure studies (THEES) [7,8]. Table II presents a selection of data related to PAC in the environment reflecting occurrences in different compartments, limiting values and human exposure. Relevant in the context of environmental analysis and, in particular, for our fluorescence investigations of contaminated soil are the limiting PAC concentrations above which soil cleanup is recommended or required. Important reference values for selected PAC can, e.g., be found in the Dutch list of soil contaminants [9]: The limiting concentrations above which further investigations are recommended (so-called "B levels") for single substances range from 1 to 10 mg PAC/kg soil dry weight (mg_{PAC}/kg_{SDW}). A soil cleanup is usually demanded if the overall PAC contamination exceeds 200 mg_{PAC}/kg_{SDW}, or if the concentration of selected single PAC exceeds between 10 (benzo[*a*]pyrene)

Table I. Photophysical Properties of the 16 PAC Classified as Priority Pollutants by the EPA[a]

Structure	Name	Φ_F[b]	τ_F (ns)[b]	λ_{abs}^{max} (nm)	λ_{em}^{max} (nm)	ε^{max} $(M \cdot cm)^{-1}$
	Naphthalene	0.23	96	319 302	322	20 300
	Acenaphthylene	$5.8 \cdot 10^{-4}$[c]	0.9[c]	456[c] 324[c]	541[c]	40 9,500
	Acenaphthene	0.50	46	320 300	347	1,800 4,650
	Fluorene	0.80	10	300	310	10,000
	Phenanthrene	0.13	57.5	346 330	364	220 230
	Anthracene	0.36	4.9	374 356	399	8,900 9,100
	Fluoranthene	0.30	53	359	462	7,840
	Pyrene	0.65[d]	450[e]	372 336	383	140 55,800
	Benzo[*k*]fluoranthene	1.0[f]	11.3[f]	402 308	402	25,000 68,300
	Benzo[*g,h,i*]perylene	0.29[f]	54.3[f]	406 300	419	284 59,700
	**Benz[*a*]anthracene**	0.23[f]	32.5[f] 49.4[e]	385 300	385	101 10,300
	**Chrysene**	0.14	44.7	362 321	381	393 12,000
	**Benzo[*b*]fluoranthene**	0.53[f]	44.3[f]	369 302	446	7,020 40,600
	**Benzo[*a*]pyrene**	0.60[f]	42.9[f] 57.5[e]	404 385	403	4,300 30,600
	**Dibenz[*a,h*]anthracene**	0.11[g]	37.5[e]	394 322	394	1,130 19,700
	**Indeno[1,2,3-*cd*]pyrene**	0.18[f]	7.2[f]	460 302	503	1,400 33,900

[a]Probable or suspected human carcinogens are listed at the bottom (boldface). Φ_F, fluorescence quantum yield; τ_F, fluorescence lifetime; λ_{abs}^{max} and ε^{max}, wavelengths of lowest-energy absorption maximum (upper) and of maximum absortion in the range $\lambda \geq 300$ nm and corresponding molar extinction coefficients; λ_{em}^{max}, wavelength of maximum fluorescence intensity. Data for absorption and emission spectra were taken from standard references, e.g., W. Karcher *et al.* (1985) *Spectral Atlas of Polycyclic Aromatic Compounds,* Kluwer, Dordrecht, or I. B. Berlman (1971) *Handbook of Fluorescence Spectra of Aromatic Molecules,* Academic Press, New York.

[b]If not denoted otherwise, these data were taken from I. B. Berlman (see above) and were measured in deoxygenated cyclohexane at room temperature.

[c]A. Samanta and R. W. Fessenden (1989) *J. Phys. Chem.* **93,** 5823–5827; A. Samanta, C. Devadoss, and R. W. Fessenden (1990) *J. Phys. Chem.* **94,** 7106–7110.

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[f]G. Heinrich and H. Güsten (1980) in A. Björseth and A. J. Dennis (Eds.), *Polynuclear Aromatic Hydrocarbons: Chemistry and Biologic Effects,* Batelle, Columbus, OH (*n*-heptane).

[g]W. R. Dawson and M. W. Windsor (1968) *J. Phys. Chem.* **72,** 3251–3260 (ethanol).

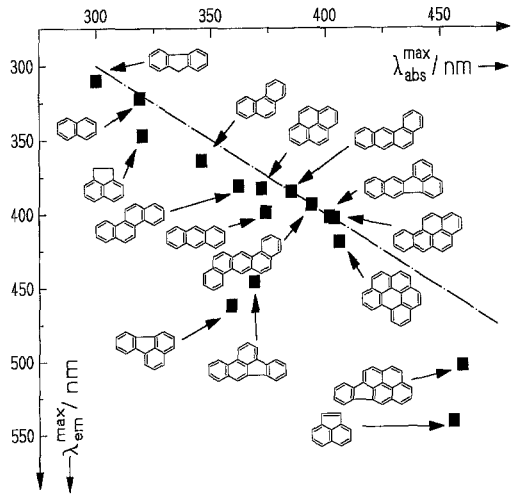

Fig. 1. Wavelength of lowest-energy absorption maximum (λ_{abs}^{max})vs wavelength of maximum fluorescence intensity (λ_{em}^{max}) for the PAC of the EPA list (see also Table I).

Table II. PAC in the Environment: Some Data on Occurrences, Regulation Limits, and Human Intake[a]

Air		1–50 ng/m³
Water	Surface	1–1000 ng/L
	Drinking	0.1–10 ng/L
	Limit	200 ng/L[b]
	Accepted imission	2.5 μg/L[b]
Soil	Rural	0.01–0.1 mg/kg
	Urban	0.6–3 mg/kg
	Contaminated	8–400 mg/kg
	Cleanup limit	200 mg/kg[c]
Sediment	Extreme	400 mg/kg
Human intake	Average, nonsmoker	3–15 μg/day
Cigarette	Average intake	0.1–0.25 μg/each

[a]If not denoted otherwise, typical concentrations were taken from Ref. 8.
[b]From the German drinking water regulations.
[c]From the Dutch list [9].

and 100 (e.g., anthracene) mg_{PAC}/kg_{SDW} (''C levels''). The ''natural background'' in rural soils is estimated to be smaller than 0.1 mg_{PAC}/kg_{SDW}, while at highly contaminated sites (e.g., in sediments) concentrations above 400 mg_{PAC}/kg_{SDW} are not unusual [7]. These figures indicate that analytical techniques suitable for *in situ* investigation of PAC contaminated soils should provide a lower limit of detection (LOD) of at least ca. 1 mg_{PAC}/kg_{SDW} for single substances with a—preferably linear—dynamic range up to ca. 100 mg_{PAC}/kg_{SDW} or more.

ON-SITE AND *IN SITU* ENVIRONMENTAL ANALYSIS

Specific Requirements

A detailed analytical investigation of contaminated environmental compartments imposes often considerable experimental challenges. Usually complex multicomponent mixtures of contaminants with diverse physical and chemical properties are found in complex environmental matrices, often with a high degree of spatial heterogeneity, and a wide range of different analyte/matrix interactions is encountered. Moreover, many important environmental processes take place in dynamic natural and artificial compartments, such as aquatic systems and bioreactors, so that a rapid analysis is required for, e.g., the *in situ* elucidation of transport and migration processes. Traditional analytical methods usually depend on time-consuming and expensive sampling, extraction, and separation sequences. It is obvious that due to restrictions mainly in time and costs, these techniques are often not suitable for the desired characterization of the system investigated. Therefore, currently a considerable interest is devoted to the implementation of modern experimental techniques for on-site and *in situ* measurements in environmental compartments and, in particular, for field screening of contaminated sites [10,11]. In a purely operational sense, experimental methods are labeled here in the following way: On-site techniques have generally to be suitable for measurements under field conditions. They may depend on limited sampling, such as, e.g., for the chromatographic or electrochemical analysis of aqueous samples. Strictly speaking, *in situ* techniques have to be capable of detection and characterization of analytes without their removal from the environmental compartment. Typical examples are the analysis of contaminants in subsurface waters or from soil surfaces, or the remote sensing of air pollutants by LIDAR (light-induced detection and ranging) techniques.

In addition to the usual performance requirements for analytical methods, such as accuracy and precision, experimental techniques for on-site and *in situ* measurements should meet several special criteria, which include the following.

1. High sensitivity: The relinquishment of extensive sampling precludes the possibility of up-concentration of the analytes.

2. High selectivity: The desired minimization of separating steps prior to analysis requires excellent selectivity factors and the capability for multicomponent analysis.
3. Capability to discriminate against background signals and diminution of interferences from the environmental matrix.
4. High speed of experimental procedures combined with the potential of long-term measurements.
5. Preferably the capability of nonintrusive measuring at larger distances (remote sensing) with good spatial resolution.
6. Possibility of compact and rugged experimental design for mobile field use.

Experimental Techniques

Here our attention is restricted to instrumentation for spectroscopic on-site investigation of PAC contaminants in water and on soil surfaces. It seems that currently the most promising analytical methods for field applications can be classified in two groups:

(1) techniques based upon ion detection, often in combination with mass spectrometry, and
(2) fluorescence spectroscopic techniques.

Not included here are other important experimental techniques such as Fourier-transform infrared (FTIR) [12] and Raman spectroscopy (e.g., the employment of surface-enhanced Raman scattering (SERS) for sensitive detection of organic analytes on metal surfaces [13,14]), and optoacoustic or optogalvanic methods [15]. While these methods certainly provide specific advantages they often require complicated instrumentation and have thus not been prioritized in field use. Moreover, the large fields of remote sensing (e.g., for the detection of oil spills on water surfaces by LIDAR [16,17]), flow injection analysis (FIA) [18], and chemical sensors such as, e.g., fiber optical chemical sensors (FOCS) [11,19–23] are not covered in this paper (although fluorescence detection is frequently used in variations of these experimental techniques).

Fluorescence spectroscopy as a special case of luminescence techniques is dealt with in the following section. The potential of phosphorescence spectroscopy has recently been significantly increased with the introduction and elaboration of room temperature phosphorimetry (RTP) [24], but a survey of the current literature reveals that phosphorescence spectroscopy does not yet play a significant role in environmental analysis.

The ion detection techniques, which are competing with luminescence measurements, are briefly surveyed here. In [25] a fiber optic detector employing laser multiphoton ionization (MPI) and photocurrent measurements for the detection of PAC in solution was described and a survey of this experimental technique was presented. In a similar experimental approach laser MPI has been used to detect PAC from metal surfaces in ambient air [26]. While these methods are distinguished by their experimental simplicity and excellent detection limits (e.g., down to pg/ml for pyrene [25]), their use for environmental measurements is probably limited due to large background current signals to be expected in natural waters or from soil surfaces. An interesting application of ion detection is the photoelectrical aerosol sensor (PAS) elaborated by Niessner et al. [27]. With this sensor PAC-coated aerosols can be monitored, e.g., in combustion exhausts and cigarette smoke. Obviously, the detection of total ion currents does not allow the identification of individual compounds and thus these techniques can provide only limited selectivity.

In principle, analysis of pollutants on surfaces can be performed with a variety of sensitive techniques available for the investigation of solid samples, such as secondary ionization mass spectrometry (SIMS) or laser microprobe mass analysis (LAMMA). For environmental samples, however, significant problems are usually encountered with analyte fragmentation and interfering ion signals from the matrices (e.g., organic colloids, soils) themselves. Therefore, several experimental approaches have been put forward with two-step processes in which, first, desorption of the neutral molecules from the surfaces and, second, their subsequent ionization takes place. Presently, thermal desorption-gas chromatography/mass spectrometry (TDGC/MS) seems to be the most advanced technique. In recent studies TDGC/MS was evaluated for field screening of organic pollutants in water [28] and for on-site PAC detection in soils from hazardous waste sites [29]. Excellent dynamic ranges and high precision in interlaboratory comparisons were demonstrated for 10 PAC with minimum amounts detected in the parts per billion (ppb) range [29]. Alternatively, laser desorption–laser ionization mass spectrometry [30] has also successfully been employed for the analysis of PAC in meteorites and contaminated soils [31,32]. Of particular current interest is the technique of matrix-assisted laser desorption/ionization (MALDI), which was, e.g., employed for the investigation of high molecular weight biopolymers [33,34]. These studies indicate that with thermal or laser desorption schemes in combination with mass-selective ion detection, an outstanding performance can be achieved in on-site investigations with highly selective and sensitive PAC analysis directly from soil surfaces.

FLUORESCENCE SPECTROSCOPY FOR ENVIRONMENTAL ANALYSIS

General Overview

Reviews of luminescence analysis of biological and environmental systems—based mainly on investigations not employing lasers—can be found in Refs. 35 and 36.

In the last decades, the availability and widespread use of powerful and tunable lasers have led to important fluorescence spectroscopic advances, e.g., in the fields of remote sensing [16] or trace analysis [37]. The large variety of different cw- and pulsed lasers provides ideal tools for spectroscopic investigations with unique properties (e.g., high intensities, low divergences, short pulses, etc.). Today, the employment of lasers as excitation sources is common in advanced fluorescence instrumentations. Most LIF experiments are performed in usual configurations for fluorescence measurements and it is just capitalized on the outstanding advantages of using lasers as light sources.[4] Some applications of these "normal" LIF experiments to the environmental analysis of PAC are reviewed under LIF Measurements of PAC and Mineral Oils in Environmental Compartments (below).

Other fluorescence spectroscopic techniques which combine the usage of lasers with some special experimental aspects have found considerable applications in the environmental analysis of PAC. These "special" techniques include

(1) fluorescence LIDAR [17,38],
(2) fluorosensors [11,19,20,38],
(3) Shpol'skii fluorometry [39–42],
(4) supersonic jet fluorescence spectroscopy [43,44], and
(5) multidimensional spectroscopy such as synchronous or total fluorescence analysis (TFA) [45–48].

As already indicated we do not consider the first two techniques here. Rather, a very brief overview of literature results from applications of Shpol'skii fluorometry and supersonic jet spectroscopy, as well as an example of our investigations with TFA, is given.

[4] In order to avoid confusion with "induced fluorescence" (which is often used synonymously with stimulated fluorescence) as a special radiative transition, it would seem preferable to use the expression "laser-excited fluorescence" instead of "laser-induced fluorescence." However, the latter terminus and its acronym "LIF" are so widely used that they are retained in this paper.

Shpol'skii and Jet Spectroscopy

Shpol'skii fluorometry takes advantage of narrow fluorescence excitation and emission linewidths that can be achieved with the incorporation of large fluorophores (e.g., PAC) in suitable matrices (e.g., *n*-octane) at low temperatures (77 K or lower) [49]. With this so-called Shpol'skii effect highly resolved spectral features can be obtained for species that are usually characterized by much broader spectra. The main asset of this technique is therefore its outstanding selectivity, which is paid for by considerable experimental complexity (narrow bandwidth lasers, low-temperature equipment). Interesting applications of Shpol'skii fluorometry in environmental analysis include the trace analysis of pyrene in extracts from marine sediments and organisms as well as from bird meat [39,40].

Laser spectroscopy of isolated, ultracold molecules in supersonic molecular beams (jets) is another experimental technique capable of providing high spectral resolution by avoiding rotational and vibrational congestions [43,44]. In combination with either ion or fluorescence detection, high sensitivity and selectivity can thus be achieved. The determination by LIF measurements of jet-cooled pyrene and benzo[*a*]pyrene directly evaporated from solid environmental samples, such as, e.g., marine sediments, has recently been reported [50]. The detection limits of pyrene and benzo[*a*]pyrene in environmental samples were 200 ng (0.4 ppm) and 900 ng (1.8 ppm), respectively. As Shpol'skii fluorometry, jet spectroscopy requires quite sophisticated experimental equipment and has therefore not yet found many applications in field screening or *in situ* measurements for environmental analysis.

LIF Measurements of PAC and Mineral Oils in Environmental Compartments

LIF spectroscopy has successfully been applied for the detection and analysis of PAC in the air, mainly on aerosols [51], and in natural waters. Closely related to the trace analysis of PAC is the detection of oil pollution in the environment. PAC are the major fluorescent constituents of mineral oils and can make up to 30% of crude or heavy oils [52]. The application of fluorescence spectroscopy to the investigation of oil pollutants in the environment became of major interest in the early 1970s and the experimental results obtained prior to 1981 and 1984 have been summarized in the extensive reviews by Eastwood [53] and Østgaard [54], respectively. More recent investigations have, e.g., also focused on the fluo-

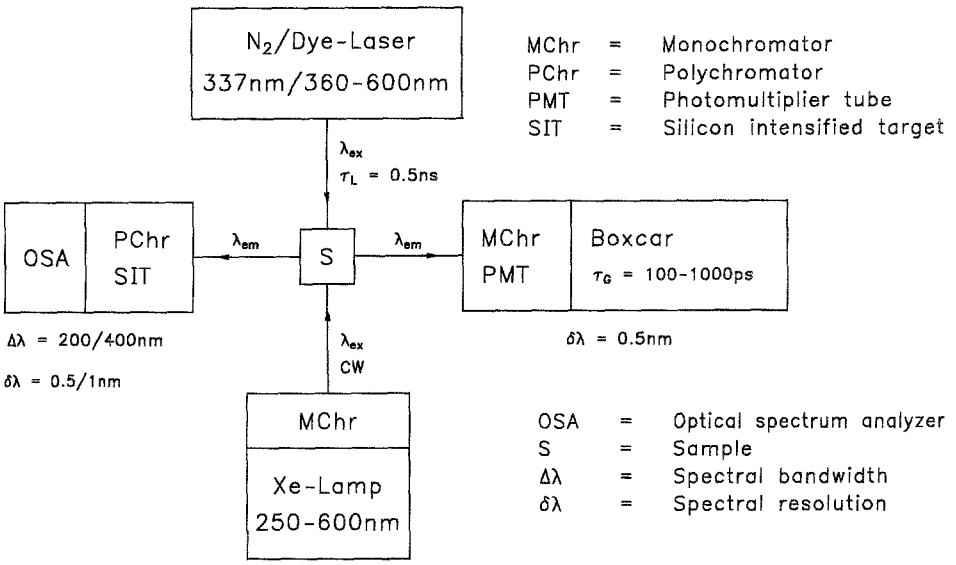

Fig. 2. Schematic description of the experimental setup used in our laboratory.

rescence identification of different mineral oils [38,55] or on the influence of weathering on oil fluorescence spectra in oil spills [56]. A particularly active field of research is the employment of LIF in the trace analysis of PAC and mineral oils in natural waters and in soil, and some examples of recently reported studies are highlighted in the following.

Fluorescence Trace Analysis of PAC and Mineral Oils in Water. The application of LIF analytical techniques for the determination of hydrospheric pollutants has attracted considerable attention [57]. For field use, the latest experimental developments have focused on nitrogen lasers as excitation sources with usually a combination of temporally and spectrally resolved detection schemes [58–60]. In natural waters, reported detection limits were ca. 10 μg/L for PAC [60] and 0.5 mg/L for mineral oil hydrocarbons [61].

PAC and Oil Fluorescence Trace Analysis of Soil Pollutants. A fiber optic-based laser fluorimeter for field screening and subsurface detection of mineral oil pollution has been developed by Lieberman et al. [62–64]. The measurements of stationary fluorescence signals were performed with a nitrogen laser through UV/vis transmitting optical fibers (length up to 100 m) contained in a probe which could be pushed ca. 50 m into the ground with a truck-mounted cone penetrometer. The mobile system has been successfully operated to locate subsurface oil plumes at contaminated hazardous waste sites and to map vertical fluorescence profiles of hydrocarbon contaminations in various soils. A similar mobile experimental system making use of time-resolved fluorescence spectroscopy of oil pollutants on soil surfaces has recently been introduced by Schade and Bublitz [61]. For mineral oil-contaminated soils these authors report a detection limit of 5 mg hydrocarbons/kg soil [61].

LIF Analysis of PAC and Oils After Graphite Furnace Vaporization. The combination of graphite furnace vaporization and LIF analysis for rapid screening and fingerprinting of PAC in complex samples was elaborated by Mellone and Winefordner [65]. Investigated were PAC-containing tobacco, food, and vegetable and mineral oil samples. The great advantage of the technique is the high temperatures (≥1100°C) than can be reached with graphite furnaces, which allow the direct analysis of complex liquid and solid samples with multidimensional fluorescence techniques.

Experimental Details

A scheme of the experimental setup used in our laboratory is shown in Fig. 2. Measurements of fluorescence lifetimes were performed with a nitrogen laser or a nitrogen-pumped dye laser (MSG 800, Laser Technik Berlin) as excitation source. The pulswidth was ca. 500 ps FWHM; the energy of the nitrogen laser at 337.1 nm was approximately 400 μJ. For single-channel time-resolved measurements, the fluorescence light emitted from the samples was passed through a monochromator (maximum spectral resolution, 0.5 nm) and detected with a fast photomultiplier tube (Hamamatsu R2496), with a

typical rise time of 0.7 ns. Time resolution was obtained by gated detection with a boxcar integrator. The minimum gatewidth of the fast sampler SR255 (Stanford Research) is 100 ps. Alternative to the boxcar integrator, a digital storage oscilloscope (Gould DSO 4062, 150 MHz sampling rate) could be used for time-resolved measurements. Multichannel, stationary fluorescence detection was performed using a polychromator (LTI, Model 01-001) and an optical spectrum analyzer (OSA 500 B&M spectronic) with a SIT camera (spectral resolution, 0.5 or 1 nm/channel, depending on the selected grating). In addition to the pulsed laser, a cw 150-W Xe lamp could be used as excitation source. For measurements in bioreactors and from soil surfaces bifurcated quartz optical fibers (LOT Oriel) were used for excitation and fluorescence detection.

The total fluorescence measurements of multicomponent PAC mixtures in solution were performed in 1×1-cm optical cuvettes with the Xe lamp as excitation source. The excitation wavelengths were scanned from 320 to 480 nm, with increments of ca. 3 nm (spectral bandwidths, 10 nm). The fluorescence emission was detected with the OSA (spectral bandwidth, 400 nm). Since only 60 evenly spaced of the total 500 channels of the diode array were selected to represent the emission spectra, the overall spectral resolution was ca. 6.6 nm. For one multicomponent sample usually 58 emission spectra were recorded. The resulting 60 × 58 excitation–emission matrices (EEM) were analyzed with a self-designed program using the matrix algorithm toolbox of a commercial software package (MATLAB).

The humic acid employed was obtained as sodium salt from Aldrich and used as received. Various PAC and other chemicals were obtained commercially and also used as received. Deionized water was used as solvent in the experiments of quenching prompt fluorescence, whereas methanol was the solvent in the experiments for the detection of delayed fluorescence and triplet–triplet absorption. The pH of the solutions was varied using HCl and NaOH and controlled with a pH electrode. All experiments were performed at room temperature.

In the fluorescence quenching experiments the PAC were used as fluorophores; Aldrich humic acid was employed as quencher. The quencher concentrations were increased by dropwise addition of aqueous stock solutions. Stationary measurements were performed with the multichannel spectrometer in the setup described above or with a Perkin Elmer MPF 44A fluorescence spectrometer.

For the fluorescence measurements on soil surfaces a loamy sand sieved through a 2-mm sieve was used. Moisture was 13% m/m (''moist soil''), content of humic substances was 4.2% m/m (mass/mass percentages), and pH was 7.3. For all fluorescence measurements the soil was mixed with a model oil (doped with perylene as fluorophore) or with mineral oils. The mineral oils were excited with the nitrogen laser at 337 nm and the perylene was excited with the dye laser at 380 nm. Measurements of the fluorescence intensities as a function of oil concentrations were performed in petri dishes. Twenty grams of soil were mixed with various aliquots of the oils and filled into the dishes. Typically, a surface area of ca. 20 mm^2 was illuminated (laser intensities were ca. 400 μJ at 337 nm and ca. 60 μJ at 380 nm) and the fluorescence signal was collected from approximately that spot size.

The soil column was contained in a glass tube with a height of 200 mm and an inner diameter of 74 mm. The tube was filled with layers of pure soil (''uncontaminated soil'') and of soil mixed with model oil (''contaminated soil''). Water (with 10% m/m NaN_3 added to ensure sterile conditions) could continuously be pumped through the column. The optical fiber was directly mounted to the surface of the tube. For fluorescence measurements at different positions the optical fiber could be moved up and down and the column could continuously be turned around its central axis.

Further experimental details are given in Refs. 66–68.

Total Fluorescence Analysis of Multicomponent PAC Mixtures

The detection of PAC in the environment is usually accompanied by the necessity of multicomponent analysis because most real contaminations consist of complex mixtures, such as, e.g., mineral oils or oil products. From stationary measurements the optimum spectroscopic information for the evaluation of such multicomponent mixtures is obtained if total fluorescence analysis (TFA) is used. With this technique both the fluorescence and the absorbance characteristics of a sample are determined. The data are recorded by measuring emission spectra at various excitation wavelengths and the fluorescence intensities are represented in the so-called excitation–emission matrix (EEM) with the corresponding wavelengths as the row and column designees. The EEM contains all information that can be gained from other stationary fluorescence techniques such as, e.g., measurements of emission and excitation spectra, constant-wavelength and constant-energy synchronous fluorescence spectra, etc. [46–48].

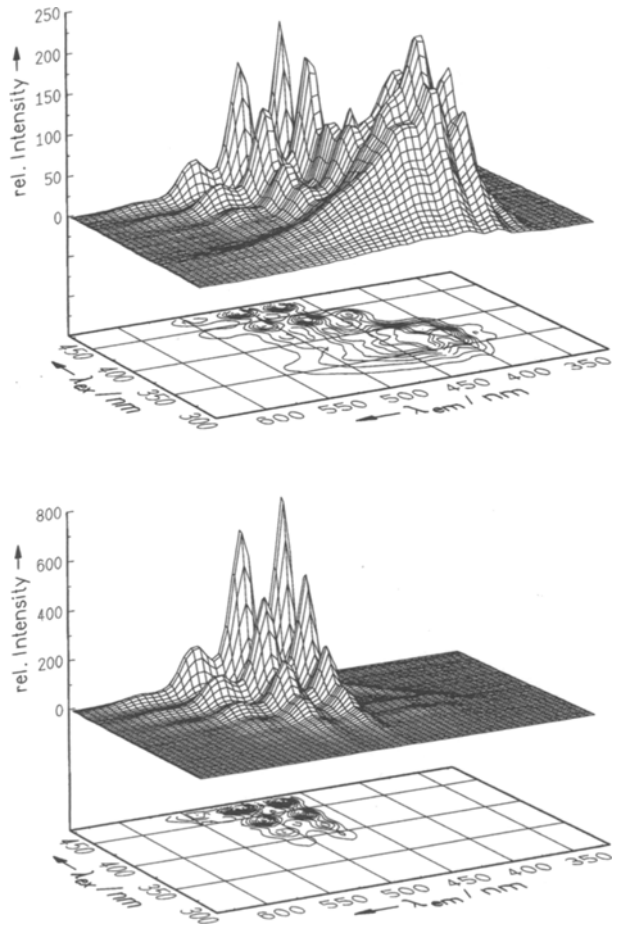

Fig. 3. Total fluorescence spectra of a PAC mixture (top) and of tetracene (bottom) in toluene.

Table III. RAFA Results for a Six-Component PAC Mixture in Toluene

Substance	c^a/M	$c_{rel}{}^b$	$UMC_{AA}{}^c$
Anthracene	1×10^{-5}	0.86	0.9985
Benzo[*a*]pyrene	4×10^{-7}	0.30	0.9675
9,10-Diphenylanthracene	1×10^{-6}	0.76	0.9970
Fluoranthene	4×10^{-6}	1.01	0.9962
Perylene	1×10^{-7}	0.95	0.9970
Tetracene	2×10^{-6}	0.89	0.9979

[a]Concentration in the mixture.
[b]Concentration calculated from RAFA relative to concentration in the mixture.
[c]Spectral overlap between standard and calculated emission spectra.

The evaluation of matrices of such high complexity as the EEM of multicomponent mixtures has become possible with the availability of fast personal computers and the development of multivariate chemometrical procedures. We used the method of rank annihilation factor analysis (RAFA) for the quantitative determination of the components in mixtures, and for the calculation of the fluorescence emission and excitation spectra of the various single components relative to standard solutions. Good overviews of mixture analysis with chemometrical methods including the application of the RAFA technique for EEM analysis can be found in Refs. 69 and 70.

To demonstrate the high potential of TFA for PAC mixture analysis we have measured the total fluorescence spectra of several six-component mixtures in toluene solution. An example is shown in Fig. 3: The upper spectrum shows the EEM of a mixture in a three-dimensional representation and as a contour plot of the data. The lower spectrum was obtained from a solution of tetracene as one of the components. In this simplified example already visual comparison of both spectral patterns suggests that tetracene is one of the components in the mixture, but the RAFA of the mixture EEM allows the separation and quantification of all six components. For this analysis the EEM for every substance has to be determined and is input as standard in the chemometrical evaluation.

An example of the results obtained with RAFA is given in Table III, which contains the real concentrations of the particular six-component mixtures shown in Fig. 3 and relative concentrations as calculated with RAFA. In order to test the potential of this chemometrical technique, we deliberately took a mixture containing components with strong spectral overlaps thus imposing difficult conditions for the spectral resolution of the individual compounds. In the following we distinguish between the spectral overlap calculated from the uncorrected matrix correlation (UMC) [71,72] of two compounds, A and B (UMC_{AB}), and the spectral overlap obtained from standard and RAFA calculated spectra for a single compound A (UMC_{AA}). For the mixture in Table III the overlaps of excitation spectra ranged from $UMC_{AB} = 0.15$ for the pair tetracene/anthracene to $UMC_{AB} = 0.91$ for benzo[*a*]pyrene/9,10-diphenylanthracene.

Even under these conditions the qualitative RAFA allowed the spectral identification of all six components by the evaluation of the spectral overlaps between the standard spectra and the spectra calculated from the mixture EEM using RAFA for the individual compounds. With the exception of benzo[*a*]pyrene these spectral overlaps were better than $UMC_{AA} = 0.99$ (cf. Table III). The quantitative analysis gave deviations of less than 15% for four of the six components, the concentration of one component (9,10-diphenylanthracene) deviated by 24%, and only the quantitative determination of benzo [*a*]pyrene yielded no reliable result (cf. Table III). The

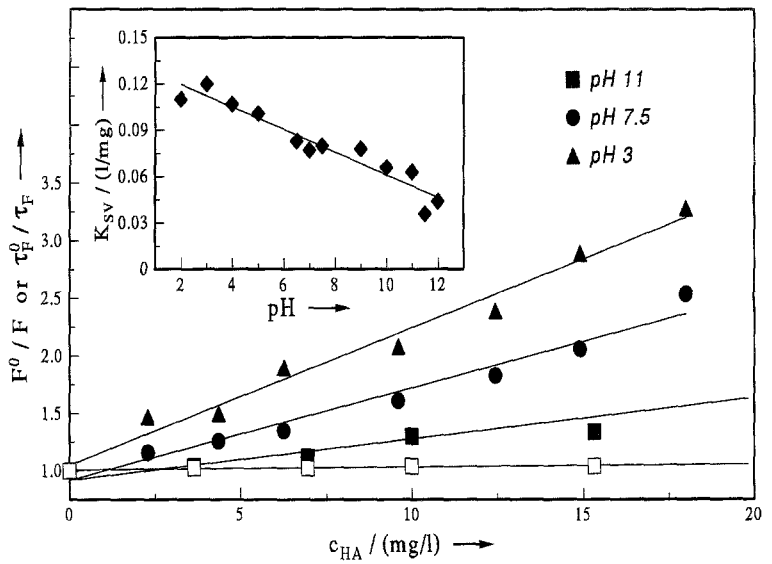

Fig. 4. Stern–Volmer plots of pyrene fluorescence quenching by humic acid. Stationary measurements at different pH (filled symbols) and time-resolved measurements (open symbols). The inset shows the pH dependence of the Stern–Volmer constant K_{SV} for static fluorescence quenching. c_{HA}, concentration of humic acid; F^0, F (τ_F^0, τ_F), fluorescence intensity (lifetimes) in the absence and presence of quencher.

deviations of the real and calculated concentrations of the latter two compounds are obviously due to their strong spectral overlaps (UMC_{AB}).

INTERACTIONS BETWEEN FLUOROPHORES AND HUMIC SUBSTANCES

For the application of luminescence analysis in soil-containing samples a fundamental understanding of fluorophore/soil-interactions is indispensible. Fluorescence quenching studies can yield valuable information about such interactions and can, e.g., allow us to distinguish between static and dynamic quenching mechanisms if stationary and time-resolved measurements are combined. For our investigations selected PAC were used as model chromophores to study the fluorescence quenching by humic acid and to derive information about changes in the humic acid structure under different experimental conditions relevant, e.g., for soil decontamination processing [66,67,73].

Quenching of PAC Fluorescence by Humic Acid

The quenching of PAC fluorescence by different humic materials has been investigated before [74–78]. However, the interpretation of the experimental results was not unequivocal and recently there has been some debate in the literature about how stationary fluorescence measurements can help to distinguish static and dynamic fluorescence quenching processes [79,80]. We have therefore performed both time-resolved and stationary fluorescence measurements to investigate the quenching of pyrene, anthracene and phenanthrene fluorescence by a commercial humic acid (from Aldrich) in water. In the following the results of the investigation of the pyrene fluorescence quenching are briefly summarized; a full account of the experimental details and of the interpretation of the results is given in Ref. 67.

In stationary measurements a strong decrease in the pyrene fluorescence intensity with increasing concentration of humic acid and a significant pH dependence of the quenching efficiencies was observed (Fig. 4). The Stern–Volmer constants K_{SV}, as taken from the slopes of the linear Stern–Volmer plots, ranged from K_{SV} = 0.12 l/mg at pH = 3 to K_{SV} = 0.03 l/mg at pH = 11.5 (estimated experimental uncertainties of the K_{SV} values: ± ca. 10%).

The fluorescence decay of pyrene in water was found to be monoexponential both in the absence (fluorescence lifetime in air-saturated water: τ_F^0 = 145 ns) and in the presence of humic acid. The Stern–Volmer analysis of the measured fluorescence lifetimes gave a straight line with an intercept of unity and a Stern–Volmer constant of $K_{SV} = 1.5 \cdot 10^{-3}$ L/mg (estimated experimental uncertainty: ± ca. 25%) independent of pH. It is obvious that in comparison to the fluorescence intensities from stationary measurements, the pyrene flu-

orescence lifetime is much less effected by the humic acid (Fig. 4).

These results indicate that both static and dynamic quenching of pyrene fluorescence occurs, but the pronounced difference in time-resolved and stationary measurements also shows that the static processes are strongly dominating in the quenching of pyrene fluorescence by the humic acid employed. The static quenching is due to a ground-state interaction between fluorophore and quencher, which may derive from a binding-type situation or an incorporation of the pyrene into the macromolecular structure of the humic acid. Our results indicate that the "humic acid-bound" pyrene is nonfluorescent, since otherwise nonmonoexponential decay of the fluorescence signal would have been observed. This is in agreement with results from other studies which showed that binding by humic materials completely quenches PAC fluorescence [74,75,78].

Experimental evidence has been presented that the structure of humic acids changes from coil-type to extended chain-type forms when the pH of the solution is increased [78,81,82]. Evidently, the coiled forms of humic acid induces stronger static quenching of pyrene fluorescence. In the coils probably more cage-like structures ("pockets") and thus more sites for the incorporation of PAC are present, and therefore static fluorescence quenching is very efficient. On the other hand, as a result of increased electrostatic interactions the number of incorporating sites in the chains and thus the static quenching efficiency decreases at higher pH.

The Deactivation of Triplet PAC by Humic Acid

Fluorescence quenching studies can provide information about the fluorophore/quencher interactions that take place during the fluorophores' fluorescence lifetimes, which are typically 1–100 ns for most singlet excited PAC. However, for the incorporation of PAC into macromolecular humic acids experimental investigations on much longer time scales may be important. This is possible by the exploitation of the long lifetimes of many PAC in their lowest excited triplet state (T_1). The PAC triplet lifetimes measured in non-viscous solvents at room temperature are usually of the order of 0.1–10 ms (often limited by impurity quenching). The concentrations of triplet molecules can conveniently be monitored by the detection of the delayed fluorescence (DF) from the S_1 state, which is repopulated either by thermally activated reverse intersystem crossing (E-type DF) or by triplet–triplet annihilation (TTA; P-type DF). Alternatively, the triplet molecules can be monitored with time-resolved triplet–triplet absorption spectroscopy. Detection of the DF makes available the advantages of emission spectroscopy, e.g., the high sensitivity, and this technique has been used to study photophysical processes in polymers [83] and the properties of eosin/protein complexes [84]. Measuring the transient triplet absorption signal seems to be the more generally applicable approach, since DF is often not observable and since many PAC have distinct triplet–triplet absorption bands.

In order to study the interactions of PAC with humic acids we are currently performing measurements of the DF and of the triplet–triplet absorption of several PAC in the presence of humic acid. In the following preliminary results of these ongoing investigations will be reported for the first time. Shown in Fig. 5 is the quenching of the DF signal resulting from the homo-TTA of anthracene by the humic acid in methanol. The distinct quenching effect is clearly discernible and the reduction of triplet lifetime can be evaluated with the usual Stern–Volmer analysis. Such a Stern–Volmer representation including results from triplet–triplet absorption measurements of pyrene and tetracene is given in Fig. 6. Obviously, the efficiency of triplet quenching by the humic acid is different for the PAC examined and seems to correlate with their triplet-state energies.

FLUORESCENCE MEASUREMENTS OF MINERAL OILS ON SOIL SURFACES

Concentration Dependence of LIF Signals

For the quantitative analysis of PAC and mineral oils on soil surfaces the dependence of the fluorescence intensities on the fluorophore concentrations has to be established. Obviously, high dynamic ranges preferably with linear intensity/concentration relationships and low limits of detection (LOD) are most desirable. Previously we have reported the results of our LIF investigations of a PAC-doped model oil on soil surfaces [68]. It was shown that for the detection of single PAC, both the linear dynamic range and the lower LOD are in good agreement with what is required in soil decontamination processing. With a perylene-doped model oil the fluorescence signal was linear in the whole concentration range investigated (0.5–58 mg_{Per}/kg_{SDW}) [68]. This compares favorable with realistic PAC concentrations encountered in contaminated soils and with monitoring (e.g., 1–10 mg_{PAC}/kg_{SDW}) and cleanup limits (e.g., 100 mg_{PAC}/kg_{SDW}) defined by the Dutch list (cf. above).

If PAC are found in real soil contaminations, they are usually only one class of compounds in often extremely complex mixtures of a large variety of other

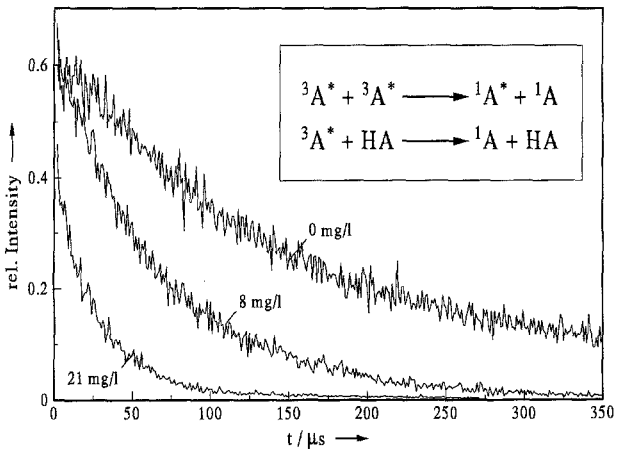

Fig. 5. Quenching of anthracene delayed fluorescence by humic acid in methanol. The reaction scheme in the inset shows the competition between the annihilation of anthracene triplets ($^3A^*$) and the quenching by humic acid (HA), leading to the formation of singlet excited molecules ($^1A^*$). τ_T^0, τ_T: triplet state lifetimes in the absence and presence of quencher in the given concentrations.

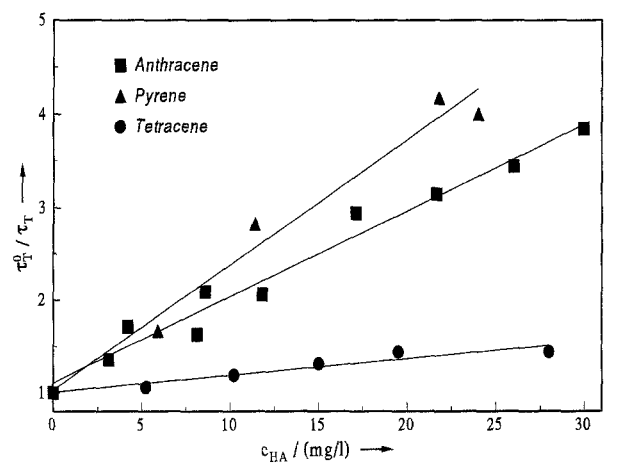

Fig. 6. Stern–Volmer plots of PAC triplet quenching by humic acid in methanol. Results from measurements of delayed fluorescence (anthracene) and triplet-triplet absorption (pyrene, tetracene).

substances such as, e.g., encountered in crude or fuel oils. Even the use of the most advanced analytical tools—based on spectroscopic or other experimental techniques—does usually not allow to separate such mixtures in environmental matrices into individual compounds. Nevertheless, fluorescence spectroscopy can be used for the quantitative analysis of oil products on and in soils if the overall fluorescence of the oil is taken as the analytical parameter. We have measured the fluorescence of a crude oil (Statfjord) and a fuel oil (Mobil) directly from a soil surface using nitrogen laser excita-

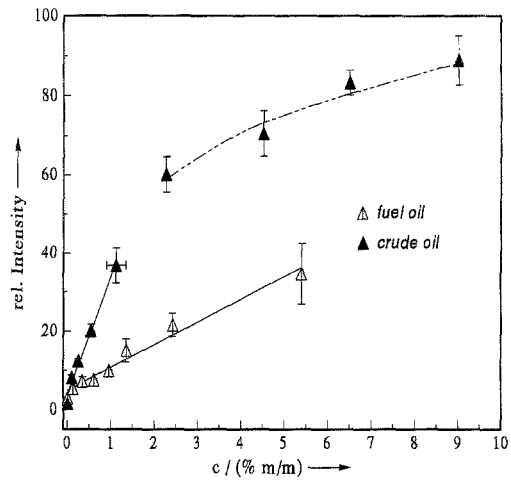

Fig. 7. Dependence of crude and fuel oil fluorescence intensities on the oil concentrations measured in petri dishes on "moist soil" with nitrogen laser excitation at 337 nm.

tion at 337 nm. The dependence of the fluorescence intensities on the concentrations of these two oils is shown in Fig. 7. Evidently, the intensity/concentration relationship for the fuel oil is linear in the whole concentration range under investigation (0.1–5% m/m).[5] In contrast, the fluorescence intensity of the crude oil increases linearly with concentration in the range 0.1–1.5% m/m but levels off at higher concentrations. The absorption and fluorescence properties of the investigated oils and the dependence of the fluorescence signals on the oil concentrations in the soil are further elucidated in Ref. 68.

For both oils the lower LOD was determined from the ratios of the tripled standard deviation of fluorescence measurements of uncontaminated soils (blanks) and the initial slopes in Fig. 7 [48]. The lower LODs were found to 0.025% m/m for the crude and 0.125% m/m for the fuel oil. These detection limits were obtained with corrections applied to account only to for the thermal background noise of the SIT camera, and not for the background of the sample—which is mainly the organic matter fluorescence. The error bars shown in Fig. 7 represent the accuracy of repeated measurements (≈4%, determined for crude oil only) and the additional uncertainty introduced by variations in the soil/oil mixing process. The overall experimental uncertainty ranged from 4 to 12% for the crude oil and from 4 to 22% for the fuel oil measurements.

We have compared the linear dynamic range and the lower LOD for fluorescence detection of oil on soil

[5] For convenience we use here the mass/mass percentage representation for concentration referring to the soil dry weight.

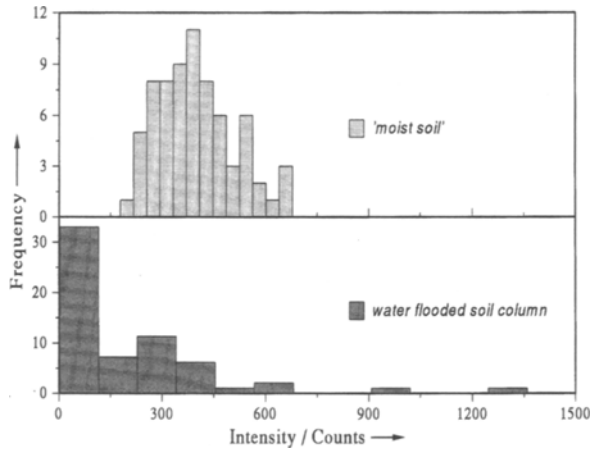

Fig. 8. Probability distribution of fluorescence intensity of a perylene-doped model oil on a soil column.

with the limits also defined by the Dutch list [9]. For mineral oils the concentration above which a soil cleanup is required is 0.5% m/m (''C level''). Further investigation is recommended for oil concentrations above 0.1% m/m (''B level''). Our LIF measurements of the oils gave lower LODs significantly below the C level, and the linear dynamic ranges extended reasonably above that value, so that our experimental technique is very well suited for monitoring the degree of soil contamination, e.g., in bioremediation.

These results clearly show that fiber optic-coupled LIF measurements are a useful analytical tool for the determination of oil contaminations in soil-containing environmental compartments. We have also shown the potential of this technique for the on-line analysis of single PAC and of different oil on and in soils [67,68]. With our experimental setup, on-line measurements directly from soil surfaces can be performed (a single spectral scan takes 32 ms) and the experimental method has a high potential for field screening applications.

Distribution of a Model Oil in a Soil Column

In the design of reactors for bioremediation processes the availability of the contaminants for the microorganisms is very important. In fixed-bed reactors migration processes and the distribution of the contaminants in the soil and on the soil surfaces play an important role. To test our method of mechanically suspending the model oil on the soil and to investigate the influence of water flow on the oil distribution, we performed experiments in a soil column which was designed to model a soil-containing fixed-bed bioreactor for the microbial degradation of PAC containing oil products.

Experiments were made with the soil column containing layers of uncontaminated (i.e., without model oil) and contaminated (i.e., with 5% m/m model oil) soil [68]. In order to suppress mixing between the layers, they were separated by filter tissues. Initially, the soil contained only the natural moisture content of ca. 13% (''moist soil''). To get information about the distribution of the model oil in the contaminated layer, the fluorescence intensity was measured at 72 locations around the column before and after flooding with water. The results of the measurements in the soil column are exemplified in Fig. 8: The probability distribution of fluorescence signal intensities on moist soil was found to be rather narrow, with a median value of 398 counts and an interdecile range I_{80} of 300 counts. The interdecile range I_{80} defines the interval accommodating 80% of all values and can be taken as a measure of the width of the distribution. When water was percolated through the column (''wet soil''), the distribution of the model oil changed significantly as is evident from the histogram presented in the lower part of Fig. 8. The median shifted to a smaller value (168 counts) and the distribution became much broader as indicated by the I_{80} value of 440 counts. These two effects—decrease in the median values and increase in the widths of the distributions on going from moist soil to water-flooded soil columns—were found in all our measurements.

The observed broadening of the distribution results from the formation of oil droplets with very high local concentrations. Measurements in the uncontaminated layer after flooding show spots of high fluorescence intensity, while in other regions no model oil was detectable. These results can provide quantitative information about how flooding of the soil column leads to spreading of the model oil (concentration decrease in the contaminated layer), droplet formation (broadening of the fluorescence intensity distribution), and migration into formerly uncontaminated zones. The detailed knowledge of the various parameters that can be obtained from these investigations, such as concentration profiles, droplet properties, heterogeneity of contaminants' distribution, etc., are prerequisite for, e.g., the successful modelling of migration processes in reactor design.

CONCLUSIONS

In environmental research fluorescence spectroscopic techniques are successfully applied for the detection and analysis of contaminants in different com-

partments. The capabilities of fluorescence detection for on-line analysis of soil-containing samples are rivaled only by ion detection techniques. The employment of advanced instrumentation both for excitation (e.g., new diode lasers) and for detection (e.g., multichannel detectors) in multidimensional stationary and time-resolved fluorescence techniques and the combination with elaborate chemometrical tools will further increase the importance of fluorescence measurements in environmental analysis. Particular emphasis in future developments will be put on compact and mobile fiber optic-coupled spectrometers with high flexibility for field use. Also, remote fluorescence detection and monitoring of contaminants from water and soil surfaces and *in situ* measurements to control biotechnological processes (e.g., bioremediation of contaminated soils) will find increasing applications. In addition to these instrumentation-related research and development prospects, modern fluorescence spectroscopy will continue to serve as powerful probe of fundamental properties in environmental systems. Major goals of current efforts are, e.g., the elucidation of photophysical processes in microheterogeneous environments and on surfaces, and of the interactions of contaminants with humic material or the characterization of their spatial and temporal distributions in environmental compartments.

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