



ELSEVIER

Journal of Chromatography A, 774 (1997) 177–192

JOURNAL OF
CHROMATOGRAPHY A

Determination of polycyclic aromatic compounds and heavy metals in sludges from biological sewage treatment plants

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Abstract

The procedure of the analysis of polycyclic aromatic hydrocarbons (PAHs) and their derivatives in the sludges from biological sewage treatment plants has been worked out. The analysis included isolation of organic matter from sludges, separation of the extract into fractions of similar chemical character, qualitative–quantitative analysis of individual PAHs and their nitrogenated and oxygenated derivatives. Liquid–solid chromatography, solid-phase extraction and semipreparative band thin-layer chromatography techniques were used for the separation. Capillary gas chromatography–mass spectrometry analysis of the separated fractions enabled identification of more than 21 PAHs, including hydrocarbons which contained 2–6 aromatic rings as well as their alkyl derivatives, 10 oxygen derivatives, 9 nitroarenes, aminoarenes and over 20 azaarenes and carbazoles. Using the capillary gas chromatography–flame ionization detection technique the content of 17 dominant PAHs was determined. The content of heavy metals was determined in investigated sludges with the use of atomic absorption spectrometry. The concentrations of the respective metals could be ranked in the order $Cd < Co < Ni < Pb < Cr \leq Cu < Mn < Zn < Fe$. The sludges were analysed for the first time in Poland in view of their possible utilisation in agriculture and in cultivating dumps of coal mine wastes, taking into consideration the contents of toxic organic pollutants and heavy metals.

Keywords: Sewage sludge; Polynuclear aromatic hydrocarbons; Heavy metals

1. Introduction

The utilisation of sewage sludges, a by-product of wastewater treatment, is a serious problem of many sewage-treatment plants. New methods of utilisation are urgently searched for because of growing amounts of the product. The use of sewage sludges in agriculture, and in reclamation of lands devastated by industry, is an economical method of utilisation which does not require much financial outlay. The utilisation, however, causes the release into the environment of toxic substances such as: heavy

metals (Pb, Cd, Cr, Cu, Ni, Hg, Zn), organic compounds, i.e. polycyclic aromatic hydrocarbons (PAHs) and their derivatives. Many of them show strong muta- and carcinogenic activity [1].

The sources of PAHs in sludges are industrial wastes and domestic sewage, atmospheric rainfalls, precipitation airborne pollutants, road surface and tyre abrasion products, and also biotransformation of organic matter into PAHs [2].

PAHs are almost completely removed (up to 90%) from sewage during their treatment and concentrated in sludges because of their poor solubility in water and adsorption on solid particles. PAHs concentration range in sewage sludges of different types is

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from several micrograms to about 2000 mg/kg of dry sludge mass [3–5], which may be dangerous for the environment if the sludges are to be utilised. There are no literature data on determination of oxygen and nitrogen PAH derivatives in sewage sludges which often show stronger biological activity than original PAHs.

The controlled sludge management is an important problem in Upper Silesia, the most industrialised and the most polluted region of Poland. Large amounts of industrial wastewaters with high concentrations of various chemical pollutants must be purified and thus a great mass of contaminated sludges is generated in this region.

Keeping in mind that uncontrolled management of sludges with considerable organic micropollutants content may affect secondary contamination of the environment, the work has been started in order to work out determination methodology of PAHs and their derivatives in Polish sewage sludges, as well as evaluation of sludge contamination with these compounds.

2. Experimental

2.1. Materials

The object of the investigations were samples of sewage sludges collected in some of the sewage treatment plants in Upper Silesia. Methodology, and especially separation procedures by extraction, were first checked on a standard mixture.

2.1.1. Sewage sludges samples

Samples of sewage sludges were obtained from 5 sewage treatment plants localised in Upper Silesia. These are typical mechanic-biological sewage treatment plants which purify only municipal wastes or their mixture with industrial wastes.

The plants differ from each other with respect to sludge digestion techniques. The sludge samples were collected at different points along the technological line of sewage sludge digestion. The primary, activated, recirculated digested, digested thickened and lagoon dried sludges were investigated. The sludges were collected twice at each point and the samples were stored in glass vessels:

- liquid sludges (primary, recirculated, digested, digested thickened) were collected as single samples;
- the lagoon dried sludges were collected in some points and stirred to acquire homogeneity.

The samples were stored at about 4°C for no longer than 2 days.

2.1.2. Standard mixture

A standard mixture was used for testing the methodology, especially extract ion separation procedure. The standards were selected taking into account investigation on sewage sludge composition carried out outside Poland [6–8] and our previous works on PAHs determination in airborne particulate matter [9,10]. The standard mixture contained:

- aliphatic hydrocarbons: tetradecane, eicosane, octacosane;
- aromatic hydrocarbons with different numbers of aromatic rings and different degrees of condensation: naphthalene, anthracene, pyrene, chrysene, perylene, benzo[*a*]pyrene, benzo[*e*]pyrene, dibenz[*a,h*]anthracene, coronene;
- PAH derivatives with functional nitrogen and oxygen groups: 1,5-dihydroxynaphthalene, 9-hydroxyphenanthrene, 9-fluorenone, xanthone, anthraquinone, 1,8-dihydroxyanthraquinone, carbazole, acridine, 4-hydroxyacridine, 1-nitropyrene.

The mass composition of the standard mixture was: 13.6% aliphatic hydrocarbons, 40.9% PAHs, 45.5% PAH derivatives.

2.2. Methodology

2.2.1. Preliminary sludges investigations

The preliminary investigations were performed in order to determine the basic properties that are characteristic for examined sludges, i.e. pH, humidity and mineral residue.

pH measurement

pH measurements were carried out by the potentiometric method. For liquid sludges, the decanted liquid over the centrifuged sludges was used. In the case of lagoon dried sludges, 100 g of sample was mixed with 100 ml of distilled water; the further procedure was the same as for liquid sludges.

Humidity and total solid determination

Water contents in sludges (humidity) and the total dry solids were determined from the difference between a raw sludge mass and the dry residue obtained at 105°C.

Mineral residue

A content of mineral substances in sludges was calculated as the difference of dried (at 105°C) sludge mass and burned in a muffle furnace at 550°C.

2.2.2. Measurement of heavy metals content

For heavy metals determination the dry samples (after humidity determination) homogenised in ceramic mortar and sieved (0.5 mm) were used. The samples mineralisation was performed according to the procedure described previously [11].

The measurement of heavy metals content was carried out by atomic absorption spectrometry (AAS). An atomic absorption spectrometer (AAS-3, Carl Zeiss–Jena) was equipped with replaceable hollow cathode lamps (HCL), which emitted radiation of wavelength (nm) specific for the elements in question (213.9–Zn, 324.8–Cu, 240.7–Co, 232–Ni, 217.0–Pb, 228.8–Cd, 357.9–Cr, 279.5–Mn, 248.3–Fe). Analysed solutions were thermally dissociated in the flame atomiser. The burner, in which atomisation was carried out, was supplied with air as oxidising gas and acetylene as flammable gas. More details about optimal analysis conditions were presented in a previous work [11].

2.2.3. Extraction of organic matter from sludges

Organic matter from the investigated sewage sludges was extracted using N,N-dimethylformamide (DMF). The rate of recovery of extracted substances was tested by adding a known amount of standard PAHs to the extracted sewage sludges. The extraction process control was based on the absorbance value measurement in the UV range and extract mass determination after solvent evaporation.

The methodological details concerning extraction process conditions are presented in a previous work [12].

2.2.4. Isolation of the polynuclear aromatic hydrocarbons fraction from organic matter

Three methods were used in parallel to isolate the PAHs fraction from the raw extracts: solid-phase extraction (SPE), liquid–solid chromatography (LSC) and semipreparative thin layer chromatography.

The diagram of the separation procedures is presented in Fig. 1. In each of the above mentioned methods silica gel was used as the stationary phase. The elution was carried out using solvents which enabled isolation of the PAHs fraction from the paraffin's one and from more polar compounds than aromatic hydrocarbons (Fig. 1). Isolation conditions were selected according to the analyses of the standard mixture. A comparison of SPE and LSC isolation results showed that the results are consistent. Recoveries of standard PAHs averaged about 80%. More information about the methodology of PAHs isolation were presented in our earlier work [13].

2.2.5. Isolation of oxygenated and nitrogenated PAHs derivatives fractions from organic matter

SPE and LSC methods enabled (Fig. 1) us to obtain concentrates of polar and moderately polar compounds containing oxygen and nitrogen PAHs derivatives due to properly selected eluents. Because of the very complex composition of these concentrates they were further subjected to isolation by semipreparative (band) thin-layer chromatography (TLC; Fig. 2, part A). The conditions of band TLC were worked out by performing a separation of the standards mixture. The details on semipreparative (band) TLC conditions are presented in Table 1. More details are presented elsewhere [14,15].

For isolation of nitro-, aza- and aminoarenes from the sewage sludge extracts, the same procedure was used as for airborne particulate matter extracts [16,17]. The technique of column adsorption chromatography on Al₂O₃ and silicic acid hydrate (SiO₂·aq) as stationary phases was used (Fig. 2, part B). The silicic acid column chromatography in connection with reduction of the separated nitroarenes and their derivatization with pentafluoropropionic acid anhydride (into pentafluoropropylamides) yielded an enriched nitrogen compounds fraction.

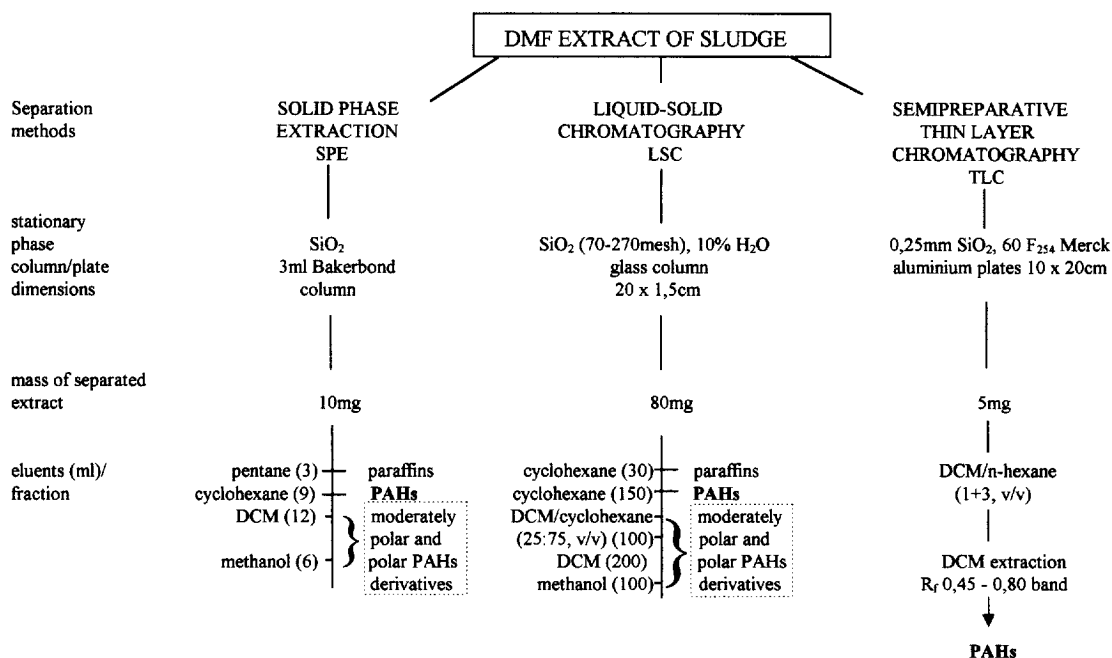


Fig. 1. Analytical scheme for the isolation of PAH fractions from sewage sludge.

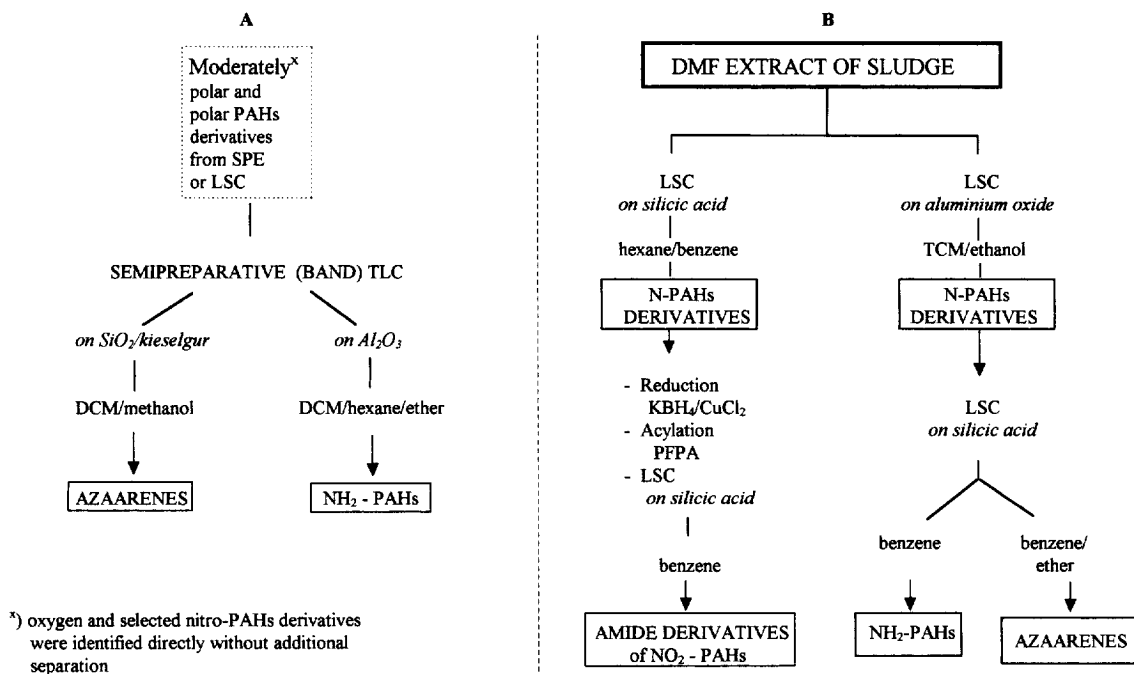


Fig. 2. Analytical scheme for the isolation of PAH derivatives from sewage sludge.

Table 1

Condition of isolation (by semipreparative band TLC) and identification (by TLC) of azaarenes and aminoarenes (in “DS” types sandwich chamber, Modin Lublin, Poland)

Parameters of separation	Azaarenes		Aminoarenes	
	Isolation	Identification	Isolation	Identification
Plates (stationary phase)	20×20 cm, 0.2 mm layer SiO ₂ /Kieselguhr 60 F ₂₅₄ Merck, Germany		10×10 cm, 0.2 mm layer aluminium oxide 60 F ₂₅₄ , Merck, Germany	7.5×5 cm, 0.2 mm layer RP-18 F ₂₅₄ , Merck, Germany
Mobile phase	DCM–methanol (1:1, v/v)		DCM– <i>n</i> -hexane–diethyl ether (1:1:1, v/v/v)	
R _F (detection limit in µg ^a)	0.52–0.80	Acr ^b (0.56 (0.05)) B(h)Q 0.80 B(f)Q 0.53 Ph 0.52 AF 0.57	0.1–0.47	AQ 0.55 (0.1) 6-AChr 0.61 (0.04) 1-APyr 0.66 (0.04) 6-AFlth 0.70 (0.05) 2-AFl 0.73 (0.05) 2-APhl 0.93 (0.10)

^a Detection limits were checked for chosen standards.

^b Abbreviations: Acr=acridine, B(h)Q=benzo[*h*]quinoline, B(f)Q=benzo[*f*]quinoline, Ph=phenanthridine, AF=azafluorene, AQ=aminoquinoline, AChr=aminochrysene, AFlth=aminofluoranthene, AFl=aminofluorene, APhl=aminophenol.

Detection of spots: (1) UV illumination, λ=254 and 365 nm, (2) dyeing reagents /14.15/. Azaarenes: Dragendorff reagent (Bi(NO₃)₃ + CH₃COOH + KI); aminoarenes: (I) Sulfanilamide + HCl + C₄H₁₀ + NaNO₃, (II) 4-nitroaniline + NaNO₂ + K₂CO₃, (III) Blue salt.

2.2.6. Qualitative and quantitative determination of PAH and PAH derivatives by capillary gas chromatography and gas chromatography–mass spectrometry

Identification of the individual components of PAHs fractions was performed using gas chromatography (GC) coupled with mass spectrometry (MS). The quantitative analysis of dominant compounds

was carried out using GC with flame ionization detection (FID). The GC–MS and capillary GC (cGC)–FID conditions of PAHs analysis are presented in Table 2. The GC–MS results interpretation was done on the basis of data collected in a computer library (NBS/NIH/EPA Mass Spectra Database) containing 43 000 spectra as well as the results of standards separation. The quantitative measurements

Table 2

Conditions of qualitative (GC–MS) and quantitative (GC) analysis of PAHs

Parameter	Method of analysis	
	GC–MS	cGC–FID
Apparatus and temperatures	Shimadzu, QP-2000 –ion source EI, 70eV, 300°C –injector splitless 270°C –interface 300°C	Shimadzu, GC-14A –detector FID 300°C –injector splitless 280°C
Scan range/scan time	1–2800/1 s	
Capillary column	HP, Ultra 2, 25 m×0.25 mm, 0.25 µm	Supelco PTE 5 30 m×0.32 mm, 1 µm
Temperature programme	60°C (3 min), heating 25°C/min to 220°C, 4°C/min to 300°C, (held for 30 min)	100°C (2 min), heating 15°C/min to 180°C, 6°C/min to 300°C, (held for 20 min)
Helium flow-rate	1.2 ml/min	

Table 3
GC–MS conditions of PAH derivatives analysis

Fraction	Temperatures (°C)			
	Ion source	Injector	Interface	Temperature programme
Azaarenes, aminoarenes and oxygen PAH derivatives	270	280	280	60°C, heating 5°C/min to 135°C, 2°C/min to 185°C, 5°C/min to 280°C (5 min)
Nitroarenes	270	280	280	60°C/min (2 min), 4°C/min to 280°C (5 min)

Equipment: Shimadzu QP 2000, ion source $E_i = 70$ eV, column: HP Ultra 2 (25 m × 0.25 mm, 0.25 μm).

were carried out on the basis of calibration curves and by coinjection of an internal standard (2,2-dinaphthyl).

The qualitative composition of the concentrates containing PAH derivatives was determined, similarly to PAHs composition, by GC–MS. The measurements conditions determined on the basis of the analysis of standard aza-, nitro- and aminoarenes mixtures and oxygen derivatives, are presented in Table 3.

2.2.7. Application of TLC to the identification of PAH derivatives

Identification of functional groups by TLC was described earlier [14].

The optimal conditions of azaarenes and aminoarenes identification were selected using adequate standard mixtures. The analyses conditions are presented in Table 1. The chromatograms were first observed under UV illumination and next sprayed with specific reagents for aza compounds and amino groups [18].

The results showed the presence of hydroxyl, ketone, carboxyl, nitro, amino groups and compounds of azaarenes type, next the results were used in identification of mass spectra of PAH derivatives.

3. Results and discussion

Table 4 presents the data characterising general properties of examined sewage sludges. pH values for most of the samples were within the range 6.7–7.3. The total solid and mineral substances contents were considerably differentiated in values

for samples from various points along the technological line.

Table 5 presents the content of metals in the investigated sludges. The data include the results for the sludges collected in different points of the treatment process of a given treatment plant. The sludges from the sludge drying beds before utilisation always had the highest values. All analysed sludges from the Upper Silesia region contained as majority elements: Fe (from 7 to about 60 g/kg of dry mass) and Zn (from 1 to 12 g/kg of dry mass). The least concentrated elements were Cd (from 0.4 to 55 mg/kg) and Co (from 0.1 to about 100 mg/kg). The concentration distribution of metals in sludges can be presented in the order:

$$\text{Cd} < \text{Co} < \text{Ni} < \text{Pb} < \text{Cu} < \text{Mn} < \text{Zn} < \text{Fe}$$

When comparing the determination results of metals in sludges from Upper Silesia and sludges from other regions of Poland [19] one must notice that sludges from Silesia contain much more (sometimes 2–3 times) Fe, Mn and Cu. The content of other metals differs depending on the sludges origin, but they are often of the same order of values as sludges from sewage treatment plants outside Silesia.

Table 6 presents the content of organic substance isolated by extraction with N,N-dimethylformamide in sludges of various origins. The examined sludges contained from 4.3 to 13.8 g of organic substance calculated for 1 kg of dry mass (i.e. from about 4% to about 14%).

The total content of PAHs and PAH derivatives fractions was determined in the extracted organic substance by LSC and SPE methods. The PAHs fractions amounted to only 0.1–0.5% of organic extract (Table 6); the content of PAHs derivatives

Table 4
General characteristic of the examined sewage sludges

Sewage sludge plant location	Type of sludge ^a	pH	Dry mass (g/l)	Mineral residue (g/l)
Zabrze–Mikulczyce	LD	–	–	–
Dąbrowa Górnicza–Strzemieszyce	P	6.44 ^b	76.7	52.2
	R	7.34	10.8	8.6
	D	7.27	72.1	45.3
	LD	6.65	489.9	153.1
Siemianowice–Centrum	A	7.04	5.7	2.7
	R	7.13	9.6	4.9
	D-ODS	7.12	37.1	19.4
	D-th	7.05	50.5	26.0
	LD	6.64	452.2	251.8
Tychy	M	5.88	16.4	11.8
	A	6.94	2.7	2.0
	R	6.85	4.5	3.1
	D	7.26	18.4	11.0
	LD	6.92	351.3	112.7
Piekary Śląskie	A ⁰ I	7.06	4.1	2.8
	A ⁰ II	6.86	4.6	3.2
	R	6.79	17.0	12.3

^a Explanation of abbreviations: A = activated, D = digested, D-th = digested thickened, D-ODS = digested in open sludge digester, LD = lagoon dried, M = mixed, R = recirculated.

^b Data averaged for two samplings.

with different functional groups was higher and averaged up to several percent.

3.1. Quantitative and qualitative PAHs composition

The content of aliphatic hydrocarbons from 0.3 to 1% of dry mass was determined. Identification of the

hydrocarbons led to detecting *n*-alkanes with carbon atoms numbering between 16 and 32; *n*-alkanes C₂₀, C₂₂ to C₂₈ and C₃₀ were predominant. Besides *n*-alkanes, isoalkanes of various structure were found. No detailed analysis of these compounds was performed because they do not show biological activity.

Table 5
Concentration of heavy metals in sewage sludges from Upper Silesia region (in mg/kg of dry mass)

Metal	Sampling place			
	Dąbrowa Górnicza	Siemianowice	Tychy	Piekary Śląskie
Zn	2015–8384	7202–12064	1068–3794	2447–2670
Cu	139–745	211–442	89.5–529.7	114.5–755
Co	14.2–46	16.5–114.5	0.4–12.2	0.07–2.98
Ni	8–88	21–260	55.5–127.6	–
Pb	59.5–638	94–930	88–337	225.1–354
Cd	1.5–13.7	5.2–55.7	3–9.5	0.4–8.4
Cr	3.2–50.3	44.5–752	56–1228	34.3–43.4
Mn	56.6–1565	583–3165	122–2136	238–285.6
Fe	16 474–58 328	31 510–47 551	10 623–48 492	7249–8156

Data for different sludges in a given plant.

Table 6
Content of DMF extract, PAHs and PAH derivatives fractions in investigated sewage sludges (in % of dry mass)

	Sampling Place				
	Zabrze ^b	Dąbrowa G.	Siemianowice	Tychy	Piekary Śl.
Content of organic DMF extract ^a	4.3	10.2	6.7	12.4	13.8
Content in extract of:					
PAH fraction	0.1	0.3	0.5	0.3	0.4
PAH derivatives fraction	4.2	9.9	6.2	12.1	13.4

^a Data averaged for sludges collected in different points of their digestion in a given plant.

^b Sludge sampled only in drying lagoons.

Table 7 presents the results of cGC and GC–MS analyses of PAHs concentrates separated by the three methods, i.e. LSC, SPE and semipreparative TLC (Fig. 1). Comparing the separation effectiveness of each of these techniques, a good repeatability was found for the results of PAHs determination in cyclohexane fractions obtained from separation by SPE and LSC. Not all PAHs were identified in the concentrate obtained from semipreparative band TLC.

Fig. 3 shows a GC–MS chromatogram of the cyclohexane fraction, containing PAHs from the chosen sewage sludge.

The column with Ultra 2 phase used in GC–MS enabled separation of isomers of molecular mass $M_r = 216$, i.e. benzo[*a*]fluorene and benzo[*b*]fluorene; $M_r = 228$, i.e. chrysene and benz[*a*]anthracene; $M_r = 252$, i.e. benzo[*e*]pyrene, benzo[*a*]pyrene and perylene.

However, we did not succeed in separating chrysene from triphenylene ($M_r = 228$) and benzo[*b*]fluoranthene from benzo[*k*]fluoranthene ($M_r = 252$); these compounds were determined together. Identification of isomers is a very crucial problem since they can show differentiated biological activity.

Twenty one individual PAHs and ten alkyl derivatives were identified in the investigated sludges. Some of them, e.g., benzo[*ghi*]fluoranthene, benzo[*c*]fluorene and dibenzo[*def,mno*]chrysene had not been identified in sewage sludges so far. In the sludges there were found all 16 PAHs, the determination of which, in different environmental samples, is recommended by the Environmental Protection Agency [20]. Their strong negative influence on living organisms had been proved in numerous studies. Many PAHs which are not active or have

poor biological activity were also identified. However, their presence and concentrations level are of interest since they can affect properties of other compounds.

Seventeen dominant PAHs were subjected to quantitative determination. The content of the total of determined PAHs averaged 330 mg/kg of sludge solids.

Hydrocarbons with 4 and 5 aromatic rings in a molecule were major components of the sludges. The content of benzo[*a*]pyrene, considered to be a standard carcinogenic, was about 8% of the total of determined PAHs.

The detection level has been obtained from 0.2 to 0.8 ng for individual compounds which corresponded to about 0.3 to 1 $\mu\text{g}/\text{kg}$ dry mass.

3.2. Identification of oxygenated and nitrogenated PAHs derivatives

The total content of the concentrates of PAHs derivatives containing different functional groups (oxygen, nitrogen and mixed) considerably exceeded PAHs content (Table 6). It turned out that concentrates consisted of a wide variety of different compounds as determined by the GC–MS and TLC methods.

Table 8 shows the results of identification of oxygen PAHs derivatives among which compounds with a carbonyl group prevail. Table 9 contains a list of nitrocompounds determined as amido derivatives of pentafluoropropionic acid after their separation by column chromatography on silicic acid (Fig. 2).

The GC–MS analysis was performed in order to compare retention times and mass spectra of the

Table 7
PAH determined in Upper Silesia by cGC and GC–MC

Compound	Ring number	Molecular mass (M_r)	Retention time (GC–MS) (min)	Concentration range ^a (mg/kg dry mass)
Naphthalene	2	128	7.71	nd
Dimethylbiphenyl	2	182	10.63	nd
Fluorene	3	166	10.43	0–6
Methylfluorene	3	180	11.33	nd
Dimethylfluorene	3	194	12.23	nd
Phenylene	3	166	11.40	nd
Phenanthrene	3	178	12.05	0.3–4.4
Methylphenanthrene	3	192	13.36	nd
Dimethylphenanthrene	3	206	14.71	nd
Anthracene	3	178	12.11	b–7
Methylanthracene	3	192	13.36	nd
Fluoranthene	4	202	15.13	2–19
Dihydrofluoranthene	4	204	13.83	nd
Pyrene	4	202	15.85	2–15
Methylpyrene	4	216	16.68	nd
Dimethylpyrene and/or Dihydrobenz[<i>a</i>]anthracene	4	216	15.88–16.45	nd
Benzo[<i>a</i>]fluorene	4	216	17.11	3–23
Benzo[<i>b</i>]fluorene	4	216	17.50	1–12
Benzo[<i>c</i>]fluorene	4	216	18.00	nd
Benz[<i>a</i>]anthracene	4	228	20.78	2–16
Chrysene + triphenylene	4	228	20.98	5–30
Methylchrysene	4	242	20.20	nd
Benzo[<i>a</i>]fluoranthene	5	252	26.15	6–40
Benzo[<i>e</i>]pyrene	5	252	27.35	3–31
Benzo[<i>a</i>]pyrene	5	252	27.56	3–25
Dihydrobenz[<i>a</i>]pyrene	5	254	23.35	nd
Perylene	5	252	27.90	b–15
Dibenz[<i>a,h</i>]anthracene	5	278	33.25	b–27
Dibenz[<i>def,mno</i>]chrysene	6	276	32.88	nd
Indeno[1,2,3- <i>cd</i>]pyrene	6	276	33.10	b–20
Benzo[<i>ghi</i>]perylene	6	276	34.53	1–2

^a Data obtained for all of the sewage sludges which were studied.

nd = Not quantitatively determined.

b = Below detection limit: from 0.3 to 1 mg/kg dry mass dependent on the analysed compound.

determined compounds with the data obtained for the standards.

Fig. 4 presents mass spectra of PAHs derivatives found in the examined sludges and of standard compounds. Fig. 4a presents the spectra of a chosen oxygen compound (xanthone) and Fig. 4b of a nitrocompound, i.e. nitropyrene.

When a standard was not available the identification was carried out by finding the molecular ions, M^+ , and fragmentation ions characteristic for a given group of compounds.

Compounds, occurring in sludges, were identified among others on the basis of the following fragmentation ions:

- nitroarenes: $(M-30)^+$, $(M-46)^+$, $(M-47)^+$, $(M-48)^+$;
- semiquinones and hydroxyarenes: $(M-28)^+$, $(M-29)^+$, $(M-30)^+$, $(M-31)^+$;
- quinones: $(M-28)^+$, $(M-56)^+$, $(M-57)^+$.

The identification of amide derivatives was carried out on the basis of the molecular ion of amide.

In the mass spectrum of the amide derivative is

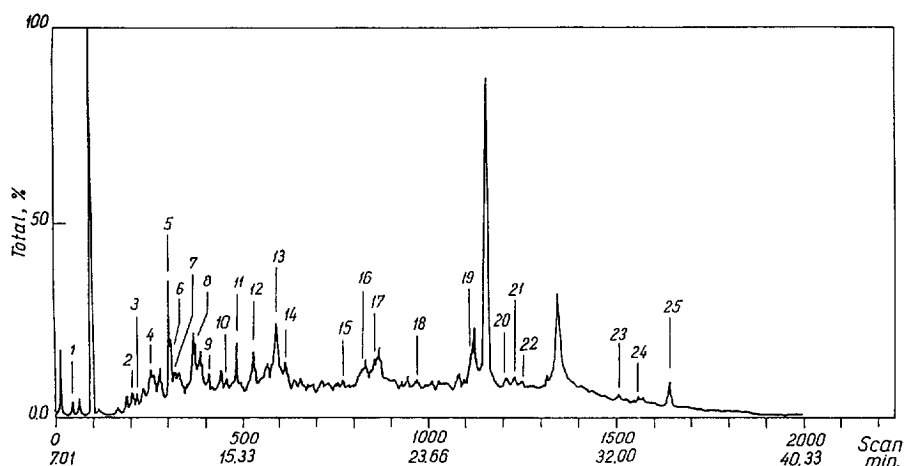


Fig. 3. GC–MS total ion chromatogram of PAH fraction separated by SPE method from sewage sludge extract from Zabrze–Mikulczyce. GC–Capillary column: Hewlett–Packard, Ultra 2 2.25 m×0.25 mm, 0.25 μ m. (1) naphthalene, (2) fluorene, (3) dimethylbiphenyl, (4) methylfluorene, (5) phenanthrene, (6) anthracene, (7) dimethylfluorene, (8) methylanthracene or phenanthrene, (9) dihydrofluoranthene, (10) dimethylphenanthrene, (11) fluoranthene, (12) pyrene, (13) benzo[*a*]fluorene, (14) benzo[*b*]fluorene, (15) benzo[*ghi*]fluoranthene, (16) benz[*a*]anthracene, (17) chrysene, (18) dihydrobenzo[*a*]pyrene, (19) benzo[*k*]fluoranthene, (20) benzo[*e*]pyrene, (21) benzo[*a*]pyrene, (22) perylene, (23) indeno[1,2,3-*cd*]pyrene, (24) dibenz[*a,h*]anthracene, (25) benzo[*ghi*]perylene.

found an intensive peak that arises as a result of substitution of hydrogen atom of amino group (which is formed after $-\text{NO}_2$ reduction) with pentafluoropropyl rest. This peak corresponds with the ion $(\text{M} + 116)^+$, where M is a molecular mass of nitro-

arene. It should be mentioned that the GC–MS chromatogram of the amide fraction was very complex and mass spectra of individual peaks contained numerous fragmentation ions. Probably nitrocompounds concentrations in examined sludges were low

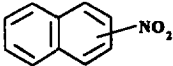
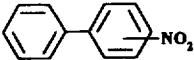
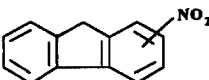
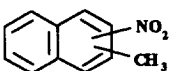
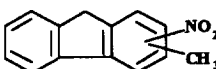
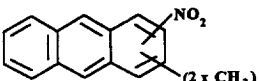
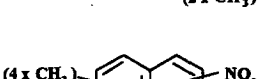
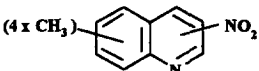
Table 8

Oxygen and mixed (oxygen and nitrogen) compounds identified by GC–MS in moderately polar (DCM) and polar (methanol) fractions from SPE and LSC (see Fig. 1)

Compound	t_R (min)	M^+ (fragmentation ions)
9-Fluorenone	33.01	180 (152)
Xanthone	38.88	196 (168, 139)
Anthraquinone	43.65	208 (180, 152)
1,8-Dihydroxyanthraquinone	50.21	240
Dihyrodimethylnaphthalenedion ^a	56.26	218
Benzo[<i>b</i>]fluorenone ^a	56.40	230 (202)
4-Hydroxyacridine	42.21	195 (167)
Anthracenone or methylfluorenone ^a	42.56	194
Methylanthracenone ^a or methylphenanthrenone	49.36	222 (193)
Methylanthraquinone ^a	49.48	222 (166)
1-Nitrofluoranthene ^a	54.18	247 (217)
1-Nitropyrene	58.43	247 (201, 200, 189)

^a Compounds identified on the basis of standard mass spectra from the computer library.

Table 9
Nitroarenes identified in sewage sludges as their amide derivatives

Compound	t_R (min)	Structure of nitrocompounds	Molecular ion of amide (M) ⁺
Nitronaphthalene	27.26		289
Nitrobiphenyl	42.63		315
Nitrofluorene	45.05		327
Nitronaphthalene (1 × CH ₃)	30.06		303
Nitrofluorene (1 × CH ₃)	38.03		341
Nitroanthracene and/or nitrophenanthrene (2 × CH ₃)	41.65 41.75	 	367
Nitroquinoline	41.76		346

(which is also suggested by the results of functional groups determination by TLC technique) causing difficulties in identification of individual substances.

3.3. Identification of azaarenes and aminoarenes

Table 10 presents the data on selected azaarenes identified in investigated sludges. Fig. 5 presents a GC–MS chromatogram of the azaarenes fraction. The azaarenes fraction was isolated from sludge extracts according to Fig. 2. MS spectra enabled identification, in these fractions several azaarenes containing from 2 to 4 aromatic rings in a molecule. There were also found several alkyl derivatives, mainly alkylquinoline and alkylisoquinoline and methyl derivatives of acridine.

When comparing efficiency of LSC and band TLC techniques in azaarenes isolation, it was seen that

thin layer chromatography is on the one hand a faster and more economical method of obtaining azaarenes concentrates but, on the other hand, in the fraction separated by LSC technique (Al₂O₃/silicic acid) there were identified more azaarenes of higher molecular mass (above 193) [14,21].

The GC–MS method was used for identification of the individual aminoarenes in benzene fractions separated from extracts using the Al₂O₃/silicic acid system. The total ion chromatogram of aminoarenes fraction isolated from selected samples is presented in Fig. 6. The data indicate that the two-steps (Al₂O₃/silicic acid) column chromatography makes possible selective separation of aminoarenes from other nitrogen compounds (nitro- and azaarenes). GC–MS analysis of aminoarenes can be carried out by comparing standards' retention times and mass spectra with those for sample components. The

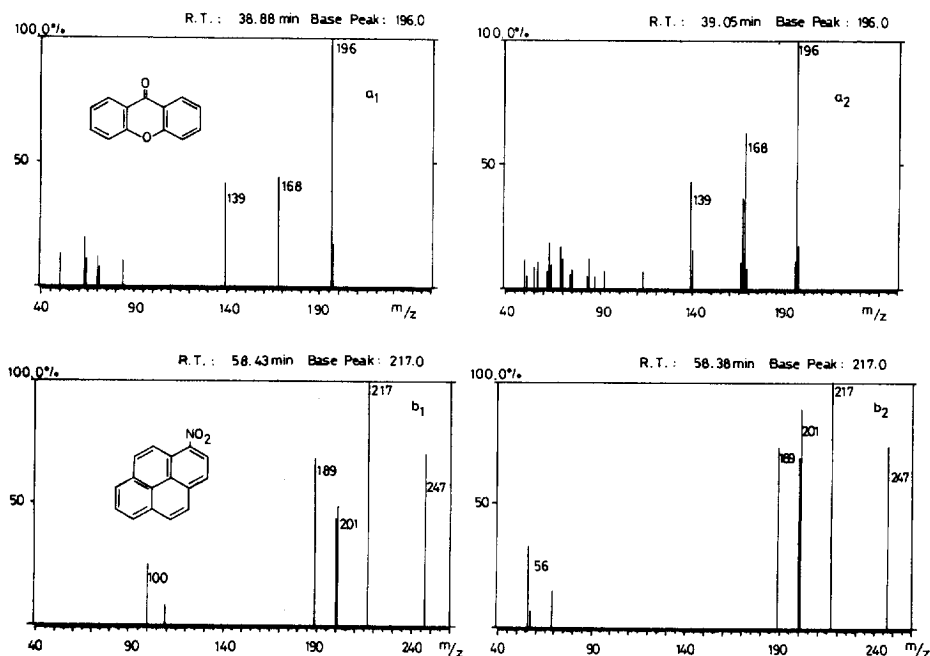


Fig. 4. Mass spectra of PAH derivatives identified in sewage sludges. (a₁) xanthone (sludge), (a₂) xanthone (standard), (b₁) nitropyrene (sludge), (b₂) nitropyrene (standard).

interpretation of mass spectra was based on the presence of molecular ions and characteristic fragment ions ($M-CNH_2$)⁺, ($M-CNH$)⁺. Table 11 presents GC-MS data of the identified aminoarenes confirmed by TLC analysis.

Several amino PAH derivatives, as well as quinoline and isoquinoline, have been identified in investigated sludges.

4. Conclusions

The procedure of the analysis of PAHs and their derivatives in sewage sludges was worked out. The procedure included the following stages: preparation of samples and isolation of organic material from sludges, separation of the extract into fractions of compounds of similar chemical character, identification of individual hydrocarbon compounds, oxygen, nitrogen and oxygen-nitrogen derivatives and quantitative determination of the dominant compounds.

There were compared the results of separation performed by LSC method (with use of silica gel

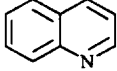
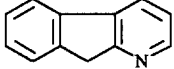
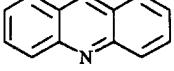
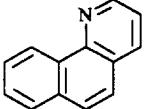
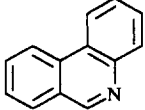
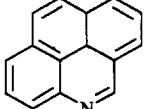
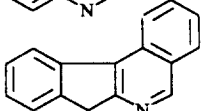
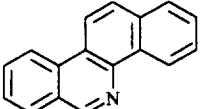
with defined water content) and SPE method (with application of Baker columns with silica gel), and it was concluded that the results were in good agreement with one another.

Taking into consideration additional advantages of the SPE technique, such as short separation time and minimal solvents use, this technique is suggested as an optimal one for separation of organic substance isolated from sewage sludges. The method allows the evaluation of the total content of saturated hydrocarbons, PAHs and PAH derivatives.

The separation of the sludges organic substance was carried out additionally by band TLC technique on plates with silica gel. Several different polyaromatic hydrocarbons and their homologues were identified in the PAHs band (after gel extraction). Although use of mentioned earlier SPE and LSC methods leads to better identification of components, band TLC may be recommended as a quick and economical method in recognitive or routine analyses, especially when the SPE system is not available.

cGC and GC-MS techniques do not need any recommendation. In investigations of organic sub-

Table 10
Azaarenes identified in sewage sludges after LSC or TLC separation

Compound	t_R (min)	Structure of azaarenes	M^+ (fragmentation ions)
Quinoline ^a and/or isoquinoline	13.70 14.33		129 (76, 101, 102)
4-Azafluorene	32.23		167
Acridine	36.05		179 (126, 152, 151)
Benzo[<i>h</i>]quinoline	36.63		179 (152, 151)
Benzo[<i>f</i>]quinoline	36.95		179 (152, 165)
Phenanthridine	37.51		179 (152)
Phenylquinoline ^a	52.33		205 (178)
1-Azafluoranthene and/or 4-azapyrene ^a	52.41 52.68		203 (175, 179)
Benzoazafluoranthene ^a and/or isomer benzo[<i>x</i>]carbazole	52.80		217 (167, 179, 203)
Benzoacridine and/or azachrysene ^a	59.08		229 (201)
Quinoline and/or isoquinoline (1 × CH ₃) ^a (2 × CH ₃) (3 × CH ₃) (4 × CH ₃) and/or (1 × C ₄ H ₉)	15.90 29.28 29.31 34.25 46.45 49.81		143 (115, 104) 157 (129) 157 (115, 104) 171 185 (129) 185
Acridine, phenanthridine, benzo[<i>x</i>]quinoline and/or benzo[<i>x</i>]isoquinoline (1 × CH ₃) (2 × CH ₃) ^a	43.51 43.78 44.96 45.11 44.20 44.65 45.98		193 (179, 166, 165) 193 (179, 166, 165) 193 (179, 165) 193 (179, 166, 165) 207 (179, 165) 207 (193, 179, 165) 207 (193, 179, 129)

^a Compounds identified only in the azaarenes fraction isolated by LSC method (Al₂O₃/silicic acid).

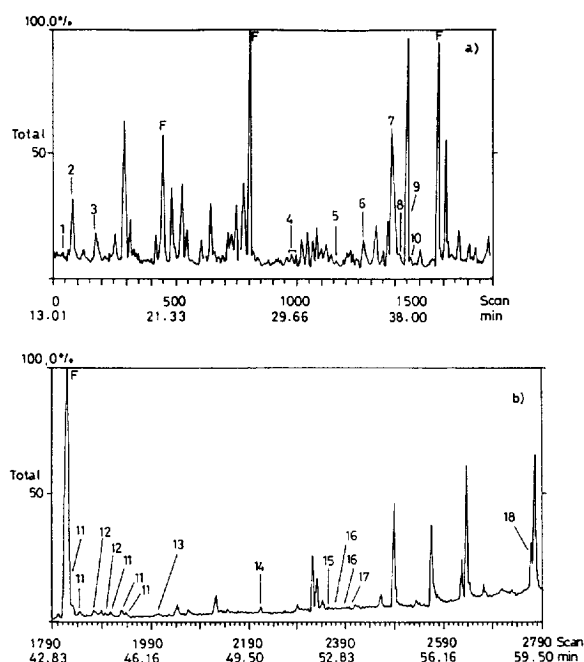


Fig. 5. GC-MS total ion chromatogram of azaarenes fraction isolated from sewage sludge (a) scan range: 1–1790 (b) scan range: 1790–2700. (1) quinoline, (2) isoquinoline, (3) (1×CH₃)-quinoline and/or isoquinoline, (4) (2×CH₃)-quinoline and/or isoquinoline, (5) 4-azafluorene, (6) (3×CH₃)-quinoline and/or isoquinoline, (7) acridine, (8) benzo[h]quinoline, (9) benzo[f]-quinoline, (10) phenanthridine, (11) (1×CH₃)-acridine and/or benzo[x]quinoline and/or benzo[x]isoquinoline, (12) (2×CH₃)-acridine and/or benzo[x]quinoline and/or benzo[x]isoquinoline, (13) (4×CH₃)-quinoline and/or isoquinoline, (14) (2×CH₃)-carbazole and/or -azafluorene, (15) phenylquinoline, (16) azafluoranthene and/or azapyrene, (17) benzoazafluorene and/or benzo[x]carbazole, (18) benzoacridine and/or azachrysene. F - esters of benzenedicarboxylic acid.

stance mixtures from sewage sludges, these techniques successfully enabled identification and qualitative determination of several compounds including those which had not been identified in sludges so far.

With the use of these methods, over 20 PAHs with 2 to 6 condensed aromatic rings in a molecule as well as several alkyl PAH derivatives were identified. Estimating quantitative PAHs composition in the light of literature data, our sludges are characterised by a great variety of compounds. Some of them, e.g., benzo[ghi]fluoranthene, benzo[c]fluorene, dibenzo[def,mno]chrysene and, predominantly, PAH

homologues had not been found in sludges so far [5,6].

Among PAH derivatives, there were identified 10 oxygen compounds (ketones, hydroxycompounds), 9 nitroarenes, aminoarenes and over 20 azaarenes and carbazoles, as well as their alkyl derivatives.

PAH derivatives determination required adopting the procedures worked out earlier for airborne particulate matter and optimising the conditions of isolation method of the fraction enriched in nitroarenes by the LSC technique with the use of silicic acid and further reduction and acylation to amides. The procedure of azaarenes determination by two-stage LSC (Al₂O₃/silicic acid) and band TLC required detailed selection of conditions.

Seventeen dominant PAHs were subjected to qualitative determination by capillary gas chromatography. It was found out that investigated sewage sludges contain mostly compounds with 4 and 5 rings in a molecule, i.e. chrysene (which was determined with triphenylene), benzofluoranthene isomers, benzopyrene isomers and benzo[a]fluorene. The concentrations of the hydrocarbons in question were from several to about 30 mg/kg of dry mass. The total content of 17 PAHs in sludges was about 330 mg in 1 kg of dry mass.

Taking into consideration a sludge type, it was found out that total PAHs content in the sludges collected in different points of the treatment process was usually considerably higher than PAHs content in sludges in sewage sludge beds. In the case of heavy metals this regularity was reversed. Probably due to mineralisation of organic matter during fermentation and lagooning, heavy metals become concentrated in residual, hardly decomposing sludge mass. However, the content of aromatic hydrocarbons, which are the compounds that are able to biodegrade, photodegrade or sublime, may be gradually decreased.

PAHs contents determined in sewage sludges from Upper Silesia were lower than PAHs concentrations in domestic and industrial sludges in USA and Canada [22,23], but higher than concentrations in European countries [3,5,7]. The determined PAHs concentrations, including the strongest mutagenic and carcinogenic ones, correspond approximately to PAHs concentrations in domestic-industrial and domestic-coke plant sludges in Germany [8].

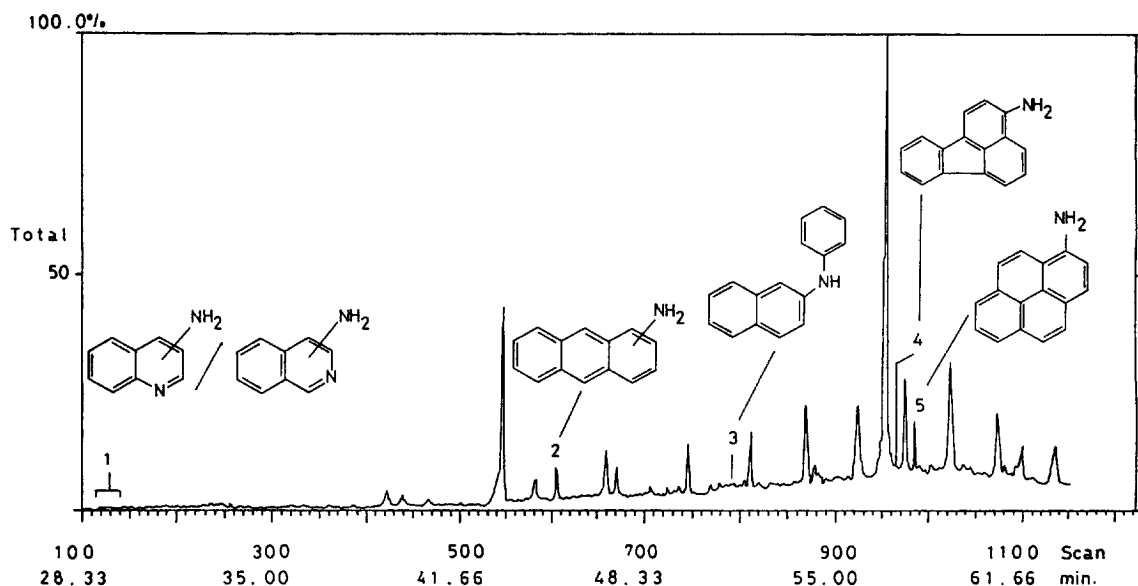


Fig. 6. GC-MS total ion chromatogram of aminoarenes fraction isolated from sewage sludge. (1) aminoquinoline and/or aminoisoquinoline, (2) aminoanthracene and/or aminophenanthrene, (3) aminophenylnaphthalene, (4) 3-aminofluoranthene, (5) 1-aminopyrene.

The investigations which were performed made it possible to find out the quantitative and qualitative composition of organic pollutants, mainly PAHs and their derivatives in sewage sludges in Upper Silesia. In the literature there are no reports on PAH derivatives in sewage sludges. It ought to be emphasised

that PAH derivatives show considerably stronger biological activity than non-substituted hydrocarbons. Some of them, e.g., 2-nitrofluorene and nitroarenes of $M_r = 247$ belong to the strongest genotoxicants [24]. It is likely that these compounds will penetrate into soil, and then to plants, more easily

Table 11
Aminoarenes identified in sewage sludges by GC-MS or TLC methods

Compound	M^+	Fragmentations		Retention time, t_R (min) t_R (min)	Confirmed by TLC
		($M - CNH_2$)	($M - CNH$)		
Aminonaphthalene	143	115	–	32.30	+
Aminoquinoline and/or aminoisoquinoline	144	116	117	29.06–29.56	+
Aminofluorene	181	–	–	43.40	+
Aminoanthracene and/or aminophenanthrene	193	165	–	45.00	+
Aminofluoranthene	217	189	–	56.88	+
Aminopyrene	217	–	–	57.93	+
Aminophenylnaphthalene	219	191	192	50.96	–
Aminochrysene	243	–	–	64.53	+

than hydrophobic non-substituted polyaromatic compounds, due to the presence of polar and moderately polar functional groups.

Acknowledgments

The authors wish to thank Dr. Krystyna Tyrpień for her assistance in performing TLC analyses.

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