

Distribution of 16 EPA-priority polycyclic aromatic hydrocarbons (PAHs) in sludges collected from nine Tunisian wastewater treatment plants

S. Khadhar^{a,*}, T. Higashi^b, H. Hamdi^a, S. Matsuyama^b, A. Charef^a

^a Water Research and Technology Center, Borj Cédria Technopark, PO Box 273, Soliman 8020, Tunisia

^b Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan

ARTICLE INFO

Article history:

Received 16 March 2010

Received in revised form 25 June 2010

Accepted 28 June 2010

Available online 1 August 2010

Keywords:

PAHs

Sewage sludge

Wastewater source

Distribution pattern

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds which may be present as contaminants in wastewater sewage sludge. Due to their toxicity and persistence in the solid phase, information should be gathered relating to their presence in sewage sludge in order to determine their contamination risks after land application. In this study, sewage sludge samples from nine Tunisian wastewater treatment plants (WWTPs) were characterized for the total content in 16 EPA-priority PAHs using an optimized extraction protocol. These WWTPs differ in the type of applied treatment and the wastewater source. Through this first assessment of PAHs in Tunisian sludges, their total concentration varied from 96 to the highest level of 7718 ng g⁻¹. Regardless of the source of wastewater, the highest PAH content was found in sludges deriving from untreated wastewater (natural lagooning). In addition, some correlation was found between the distribution patterns of each PAH, the type of applied treatment on one hand and the wastewater source on other hand.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and other organic pollutants are widely distributed in the terrestrial environment. PAHs are formed by the incomplete combustion of organic materials during natural or anthropogenic processes [1]. They include a large group of compounds with two or more fused benzene rings that are less soluble in water and less volatile with increasing molecular weight, due to the high hydrophobicity and stable chemical structure [2]. Some major characteristics of higher molecular weight PAHs are mutagenic toxicity, high lipophilicity and persistence in the environment. Some ecotoxicological studies showed that PAHs may have phytotoxic effects on lettuce germination and hinder ostracod survival [3]. The ultimate fate of PAHs in solid phase is controlled almost exclusively by strong adsorption onto the surface of mineral and organic matrices, rendering them less bioavailable to degradation over time [4].

The US EPA and the EU have selected 16 PAHs as priority pollutants of concern: naphthalene (Naph), acenaphthylene (AcyI), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phen), anthracene (Anth), fluoranthene (F), pyrene (Py),

benzo[*a*]anthracene (B[*a*]A), chrysene (Chr), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), dibenzo[*a,h*]anthracene (D[*a,h*]A), benzo[*g,h,i*]perylene (B[*g,h,i*]P), and indeno[1,2,3-*cd*]pyrene (I[*c,d*]P) [5,6].

Sewage sludge is a complex organic biowaste resulting from wastewater treatment. As for the solid phase in general, PAHs can be rapidly adsorbed onto the sewage sludge particles in wastewater [7]. On the other hand, sewage sludge has a conventional use in agriculture and can be used as a soil fertilizer. Its positive effect is mainly on the crop yield by improving soil properties through the enrichment with organic matter. However, the negative effect due to the presence of heavy metals, PAHs and/or other contaminants in sewage sludge has to be controlled [8].

In recent years, the re-use of sewage sludge and other biowastes as fertilizers has become an inevitable agronomic practice in Tunisia due to organic matter deficiency in agricultural soils [9]. To date, only heavy metal pollution has been addressed in Tunisian biowastes and biowaste-amended soils [9–11]. In accordance with the directive draft of the EU (2000), the total content of hazardous PAHs in sewage sludge appointed for agricultural re-use (including Ace, Phen, Fl, F, Py, B[*b*]F, B[*j*]F, B[*k*]F, B[*a*]P, B[*g,h,i*]P and I[*c,d*]P) has to be less than 6.0 mg kg⁻¹ [7,12].

The aim of this study was to investigate the distribution of the 16 EPA-priority PAHs in nine Tunisian sewage sludges using an optimized extraction technique. It is worth to mention that the actual investigation is the first record of PAHs in Tunisian sludges.

* Corresponding author. Tel.: +216 79 325 044; fax: +216 79 325 802.

E-mail addresses: samia.khadhar@certe.nrnt.tn, samiakhadhar@yahoo.fr (S. Khadhar).

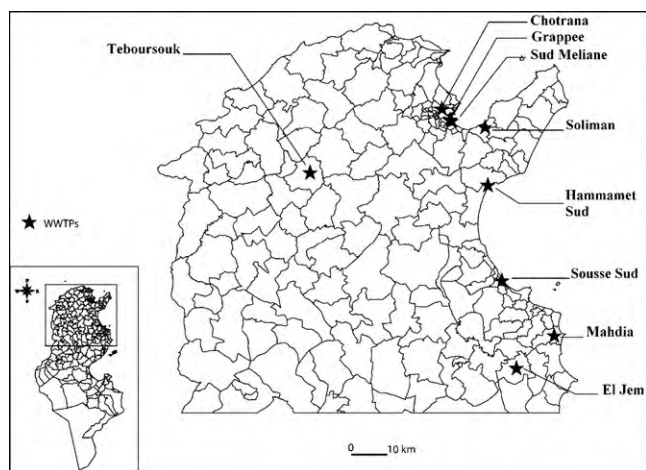


Fig. 1. Location of the nine sewage sludge sampling sites.

2. Materials and methods

2.1. Sewage sludge collection

Sewage sludge samples were collected from nine wastewater treatment plants (WWTPs) distributed in different sites in northern and central Tunisia (Fig. 1). These WWTPs receive various types of wastewater and apply different treatment techniques varying from almost no treatment (natural lagooning at El Jem WWTP) to advanced technologies including oxidizing ditch (OD), or activated sludge plus biofilm processing (AS+BP) (Table 1). On the other hand, Grappee WWTP is the sole treatment plant to receive exclusively industrial wastewater. Sludge sub-samples were first collected from the final storage pond of each WWTP following a Z-shaped path, mixed thoroughly then divided into three composite samples. Sludge samples were air-dried at room temperature (25 °C) in darkness, crushed and sieved through 2-mm mesh [13], then stored at –4 °C until analysis.

Table 2

PAH concentration (ng g⁻¹ dry sludge) in El Jem and Soliman sewage sludge samples using the three extraction protocols.

PAH	WWTP (El Jem)			WWTP (Soliman)			Ratio PII/PI		Ratio PII/PIII	
	Soxhlet DCM/acetone (PI)	Soxhlet toluene (PII)	Ultrasonication acetone (PIII)	Soxhlet DCM/acetone (PI)	Soxhlet toluene (PII)	Ultrasonication acetone (PIII)	El Jem	Soliman	El Jem	Soliman
Naph	230.9	926.7	280.8	26.45	41.42	15.8	4.01	1.56	3.30	2.62
Acyl	nd	nd	nd	nd	nd	nd	–	–	–	–
Ace	nd	nd	nd	nd	nd	nd	–	–	–	–
Fl	49.98	327.5	252.3	nd	nd	nd	6.55	–	1.29	–
Phen	2629	2906	2773	9.267	9.001	4.195	1.10	0.97	1.04	2.14
Anth	nd	nd	nd	nd	nd	nd	–	–	–	–
F	158	844.7	661.5	14.46	15.44	9.524	5.34	1.06	1.27	1.62
Py	1209	1346	1461	30.93	34.89	24.51	1.11	1.12	0.92	1.42
B[a]A	122.9	235.1	162.3	nd	nd	nd	1.912	–	1.44	–
Chr	554.9	781.1	496.2	4.981	4.623	3.722	1.40	0.92	1.57	1.24
B[b]F	168.5	212.2	174.6	nd	nd	nd	1.25	–	1.21	–
B[k]F	nd	nd	nd	nd	nd	nd	–	–	–	–
B[a]P	133.9	139.2	133.1	nd	nd	nd	1.03	–	1.04	–
I[cd]P	nd	nd	nd	nd	nd	nd	–	–	–	–
D[ah]A	nd	nd	nd	nd	nd	nd	–	–	–	–
B[ghi]P	nd	nd	nd	nd	nd	nd	–	–	–	–
Total	5257	7718	6394	86.09	105.4	57.75	–	–	–	–

nd: not detected. Results are means of triplicate.

Table 1

Wastewater source and type of treatment applied in the nine Tunisian WWTP.

WWTP	Type of treatment	Source of wastewater (% in volume)		
		Domestic	Touristic	Industrial
Grappee	AS + CT	0	0	100
Sud Meliane	OD	49	0	51
Chotrana	AP + D	87	1.0	12
Soliman	OD	38	0	62
Teboursouk	OD	93	2.0	5.0
Hammamet Sud	LT	60	22	18
Sousse Sud	AS	80	8.0	12
Mahdia	AP	49	0	51
El Jem	NL	81	11	8.0

WWTP: wastewater treatment plant; AS: activated sludge; CT: chemical treatment; AP: aerated pond; NL: natural lagoon; OD: oxidizing ditch; D: digestion; LT: lagoon treatment.

2.2. Reagents

The standard solutions of 16 EPA-priority PAHs used in this study had a purity of 99% (100 µg mL⁻¹ each in dichloromethane, DCM). The internal standard solution used to spike all the samples prior to solvent extraction was prepared at 5 µg single PAH mL⁻¹ DCM. Calibration solutions were prepared by diluting the standard stock solution with DCM before storage at –4 °C. Solvents used for extraction trials include analytical grade hexane, toluene, acetone, and DCM. Ultra-pure water was obtained from a Milli-Q® water purification system. Silica gel (200 mesh, 60 Å, Wako Pure Chemical Industries, Ltd., Japan) was activated overnight at 130 °C, then kept in a desiccator prior to use.

2.3. PAH analysis

2.3.1. Extraction procedures

As a preliminary investigation, sludge samples from El Jem and Soliman WWTPs (applying NL and OD treatments, respectively) were chosen to optimize PAH extraction by evaluating three extraction protocols. For all extraction procedures, a dried sludge sample (2 g) was first mixed with 1 g of anhydrous sodium sulphate. Protocol I (PI) used Soxhlet extraction with 160 mL of a DCM/acetone mixture (9:7, v/v) for 24 h. In protocol II (PII), Soxhlet extraction was carried out with toluene (120 mL) for 8 h. Protocol III

(PIII) involved a three-time repetitive extraction by ultrasonication (16 kHz, 300 W) with 25 mL of acetone for 30 min.

Solvent extracts from the three procedures were concentrated to approximately 2 mL using a rotary evaporator, and then further reduced to 1 mL under nitrogen flow. In parallel, the same dry sludge samples were spiked with 1 mL of the 16 EPA-priority PAHs standard solution then extracted the same way to evaluate the recovery rates using the three protocols. All samples were analyzed in triplicate.

2.3.2. Clean-up

A clean-up step was carried out on extracts to remove other co-extracted materials from sludges such as biogenic macromolecules, lipids and pigments that may interfere with PAH analysis. Concentrated sludge extracts were added to a mixture of hexane/NaCl solution (30 mL, v/v) in a separating flask. The mixture was shaken for 20 s and only the hexane phase was collected. In order to remove sulphur and water, the hexane phase was stirred overnight with dried Cu bars and anhydrous sodium sulphate. The hexane extracts were then concentrated to 2–3 mL using a rotary evaporator then to 1 mL under nitrogen flow. To ensure a better separation, the extracts were further passed through a wet-packed silica gel column then eluted separately with 20 mL of hexane to remove aliphatic compounds, followed by the addition of 30 mL of hexane/acetone (9:1, v/v). The latter fraction was concentrated to 1 mL at room temperature prior to analysis.

2.3.3. Analysis conditions

PAH analysis was performed using gas chromatography (Hewlett Packard 6890N) equipped with a splitless injector and coupled to a mass spectrometer (JEOL JMS 600H) operated in electron impact mode. Separation was carried out in a HP-1MS column (30 m × 0.25 mm ID) coated with diphenyl-polydimethylsiloxane (0.25 μm). Oven temperature programme started at 50 °C, held for 1 min and finally increased to 300 °C for 10 min. Carrier gas was helium (1 mL/min) and the temperature of the injector and transfer line was kept at 280 °C. PAH analyses were performed with SIM mode using one target and two qualifier ions. The quantification of PAHs by GC–MS was based on the area of their molecular ion peak as compared to PAH standards.

Table 4

Concentration of 16 EPA-priority PAHs in sewage sludge samples from nine Tunisian WWTP (ng g⁻¹ dried sludge).

PAHs	Grappee	Sud Meliane	Chotrana	Soliman	Teboursouk	Hammamet Sud	Sousse Sud	Mahdia	El Jem	LOD
Naph	24.92	54.33	61.49	41.43	190.5	210.9	321.3	180.3	926.7	0.61
Acyl	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.10
Ace	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.12
Fl	nd	nd	4.382	nd	1.652	65.31	nd	9.867	327.5	0.03
Phen	6.711	35.04	56.06	9.009	12.08	411.8	30.04	71.01	2906	0.04
Anth	1.204	nd	1.138	nd	nd	nd	3.201	6.946	nd	0.06
F	8.932	37.39	29.55	15.45	12.03	209.4	32.98	43.26	845.4	0.17
Py	37.65	143.6	125.1	34.89	16.01	212.9	99.01	69.57	1346	0.24
B[a]A	nd	nd	nd	nd	nd	41.34	6.587	6.977	235.2	0.08
Chr	16.14	72.28	50.91	4.623	6.588	92.23	28.56	18.89	780.1	0.07
B[b]F	nd	39.83	23.25	nd	nd	82.27	24.41	24.22	212.2	0.04
B[k]F	nd	nd	nd	nd	nd	nd	11.76	nd	nd	0.5
B[a]P	nd	nd	nd	nd	nd	nd	8.701	nd	139.2	0.08
I[cd]P	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.08
D[ah]A	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.05
B[ghi]P	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.02
Σ16 PAHs	95.56	382.4	351.9	105.4	238.9	1326	566.5	431.1	7718	–
Σ7 PAHcarc.	16.14	112.1	74.16	4.623	6.588	215.8	80.01	50.08	1366	–
% Σ7 PAHcarc.	16.88	29.31	21.07	4.386	2.757	16.27	14.12	11.61	17.69	–
% 2–3 rings	34.34	23.37	34.97	47.85	85.48	51.88	62.58	62.19	53.90	–
% 4–6 rings	65.60	76.63	65.02	52.14	14.49	48.12	37.42	37.79	46.10	–
R _{≤3} /R _{≥4}	0.523	0.304	0.537	0.917	5.897	1.078	1.672	1.645	1.169	–

nd: not detected; LOD: limit of detection; PAHcarc.: PAH carcinogenic. Results are means of triplicate.

Table 3

Recovery rate (%) of PAHs from El Jem and Soliman wastewater sludge samples using the three extraction protocols.

PAHs	Soxhlet (DCM/acetone)		Soxhlet (toluene)		Ultrasonication (acetone)	
	El Jem	Soliman	El Jem	Soliman	El Jem	Soliman
Naph	29.5	32.4	47.5	41.7	19.1	23.5
Acyl	48.2	46.8	85.5	87.8	27.6	34.6
Ace	44.8	52.7	89.9	91.2	29.9	21.9
Fl	40.5	63.5	79.8	76.8	25.6	28.5
Phen	37.9	48.9	87.5	89.9	19.3	25.8
Anth	35.1	45.6	83.3	93.7	21.3	27.6
F	41.1	53.7	108.5	98.6	20.0	19.3
Py	35.5	45.3	93.2	97.6	18.5	20.8
B[a]A	37.4	51.8	99.3	92.4	23.5	27.3
Chr	28.6	39.8	79.9	87.5	17.4	21.2
B[b]F	40.3	54.9	91.4	89.7	27.3	25.6
B[k]F	36.7	43.4	90.8	89.6	22.9	31.3
B[a]P	43.3	61.8	92.1	93.7	29.8	37.8
I[cd]P	65.1	72.7	95.6	92.2	53.1	49.1
D[ah]A	65.9	63.5	93.5	90.9	54.1	57.7
B[ghi]P	70.8	67.9	101	95.7	57.5	52.3

Results are means of triplicate.

3. Results and discussion

3.1. PAH analysis optimization

The target PAHs extracted from El Jem and Soliman samples are presented in Table 2. The three extraction protocols succeeded at detecting the same PAHs in both sludge samples. However, the highest extraction levels for the total 16 EPA-priority PAHs was obtained by Soxhlet technique using toluene as solvent [14]. Consequently, the ratio of Soxhlet extraction with toluene to the other protocols were higher than 1 for most of PAHs (1.03–6.55 for PII/PI and 0.92–3.30 for PII/PIII in case of El Jem sludge, for instance) (Table 2). The extraction efficiency of PII was also confirmed by higher recovery rates for PAH-spiked El Jem and Soliman samples (48–100% and 42–98%, respectively) (Table 3).

On the other hand, recovery rates for naphthalene were the lowest under all of the three extraction procedures (data not shown), which is consistent with previous studies [15–17]. Among PAHs, naphthalene is the only PAH made up of two fused benzene rings

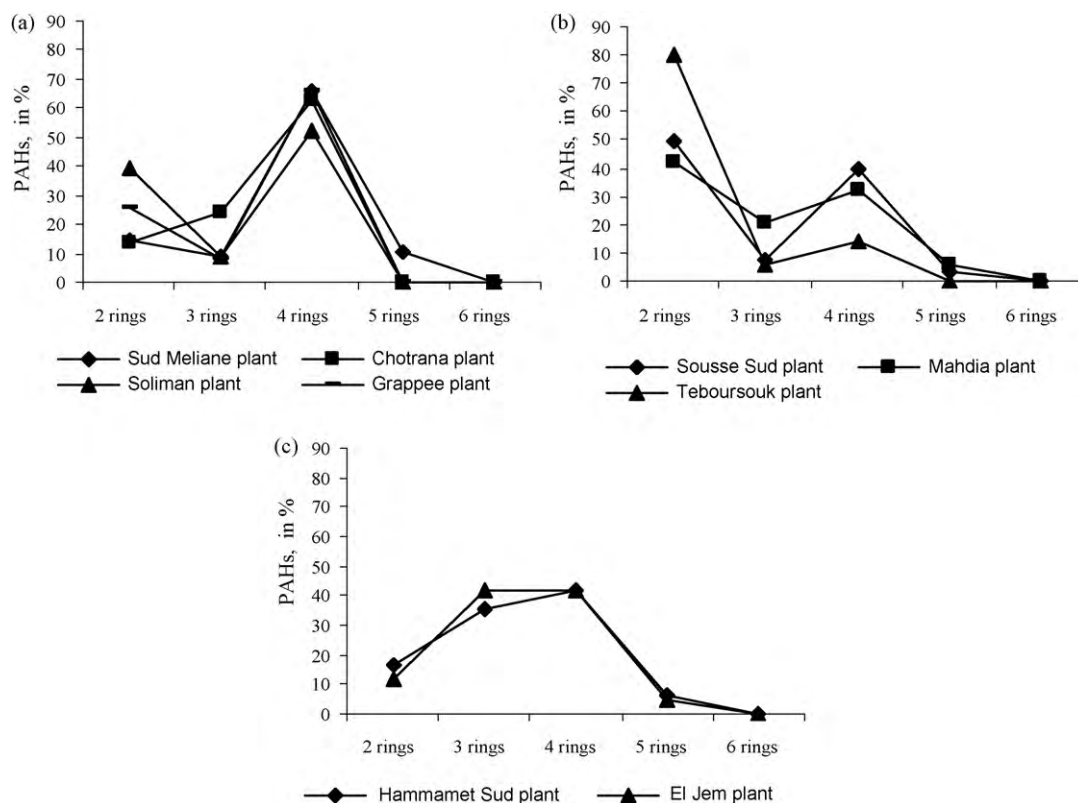


Fig. 2. EPA 16 priority PAH distribution pattern according to the number of rings in nine Tunisian WWTP. (a) Grappee, Sud Meliane, Chotrana, and Soliman, (b) Sousse Sud, Teboursouk and Mahdia, and (c) Hammamet Sud and El Jem.

and is characterized by the highest water solubility (30 mg L^{-1}) and volatility ($\text{Log } K_{ow} = 3.4$), which may explain these low recovery rates [2].

3.2. PAHs in Tunisian sludge samples

3.2.1. Total concentration

The validated extraction procedure (PII) with liquid–liquid separation was applied to determine the 16 EPA-priority PAHs in 9 sewage sludge samples collected from different Tunisian WWTPs. Among all the 16 target compounds, the number of detected PAHs in sludge samples varied between 5 (Soliman) and 10 (Sousse Sud) as illustrated in Table 4. On the other hand, the concentration of individual PAHs in each sludge sample varied considerably.

The total concentration of detected PAHs ($\Sigma 16 \text{ PAHs}$) ranged from 96 to the highest level of 7718 ng g^{-1} in sludge samples from El Jem WWTP ($\Sigma 9$ detected PAHs). The general variation of total PAH concentration was very similar to PAH levels reported in various sludge samples from Kuwait (up to 6180 ng g^{-1}) [18]. In particular, Acyl, Ace (2 rings) and all 6 ring PAHs were not detected in the current analyzed sludges. PAHs with 6 benzene rings are known for their insolubility in aqueous phase ($<0.5 \text{ ng L}^{-1}$) and extreme recalcitrance in the solid phase rendering these compounds almost unextractable with solvents [2].

The most abundant PAHs found in all Tunisian sewage sludge samples belonged to ≤ 4 ring PAH range, namely: Phen followed by Py, Naph, Fl, and Chr with the average concentrations of 393, 232, 224, 137, and 119 ng g^{-1} , respectively (Table 4). The preponderance of the same compounds was also noticed in sludge samples from Madrid [7]. On the other hand, Anth, B[a]P, B[k]F and B[a]A were the less frequently detected PAHs in Tunisian sludges and had much lower concentrations in comparison to those, for instance, found in Beijing sludges ($22.5\text{--}214 \text{ ng g}^{-1}$ for Anth, $226\text{--}6174 \text{ ng g}^{-1}$ for

B[a]P, $115\text{--}2138 \text{ ng g}^{-1}$ for B[k]F, $170\text{--}2171 \text{ ng g}^{-1}$ for B[a]A) [19]. In terms of possible PAH sources in sludge, a recent study revealed that domestic inputs provide the major contribution to PAH load [20]. Other research studies reported that lower molecular weight PAHs typically Naph, Ace, fl, Phen, F and Py were present in household wastewater and light industrial estate [21,22]. PAH levels are generally higher in wastewater from the light industries, probably as a result of wastewater from engine or other automotive washing [23]. On the other hand, the predominance of high molecular weight compounds such as PAHs with 5 and 6 rings indicates the presence of pyrolytic origins [24]. Thus, wastewater treatment technology should be adapted to the quality and the origin of the wastewater to be treated.

In this study, the total concentration of the seven reported carcinogenic PAHs [$\Sigma 7 \text{ PAH}_{\text{carc.}}$: B[a]A, Chr, B[b]F, B[k]F, B[a]P, D[a,h]A, and I[c,d]P] ranged from 16.14 to the highest level of 1366 ng g^{-1} in El Jem sludges. $\Sigma \text{PAH}_{\text{carc.}}$ accounted for less than 29% of the total PAH content in all nine Tunisian sludges, which is relatively low compared to higher values reported in literature in some more industrialized countries: 62% in Greece [26] or 88% in China [25]. Despite the fact that El Jem sludges were predominately of urban origin, they were the most contaminated samples (Table 4). These sludges derived from wastewaters that did not undergo any prerequisite treatment (Table 1). It is likely that all different treatments applied in the rest of WWTP resulted in a significant effect on PAH dissipation in water and sludges [25]. This may partly explain the lower PAH content in supposedly contaminated sludges of industrial origin sampled from Grappee and Soliman WWTPs.

3.2.2. Distribution pattern

Many studies pointed out that the amount and the distribution pattern of individual PAHs depend on sewage sludge origin and

the type of treatment applied in WWTPs [27]. Relative percent of each PAH concentration according to the number of rings in the nine Tunisian sludges is illustrated in Fig. 2. Three groups of sludges could be distinguished according to percent variation: Grappee, Sud Meliane, Soliman and Chotrana samples (Fig. 2a), Mahdia, Sousse Sud and Teboursouk samples (Fig. 2b) and, El Jem and Hammamet Sud samples (Fig. 2c). The sludge collected from El Jem and Hammamet Sud WWTPs contained relatively higher proportion of 3 and 4 ring PAHs, and the percentage of high molecular weight PAHs (≥ 4 rings) was almost comparable to that of low molecular weight PAHs (3 rings) (Fig. 2c). Furthermore, Grappee, Sud Meliane, Chotrana and Soliman sludge samples contained more than 50% of PAHs with 4 rings. However, the Sousse Sud, Mahdia and Teboursouk samples showed the highest proportions of 2 and 4 rings PAHs. The ratios of the relative fraction of 2–3 rings to 4–6 rings PAHs, as denoted by $R_{\leq 3/\geq 4}$ (Table 4), were well in agreement with the grouping variation of the studied samples mentioned above. The ratio $R_{\leq 3/\geq 4}$ varied from 0.30 to 0.92 for the samples in Fig. 2a, 1.64 to 5.90 for that in Fig. 2b, and from 1.08 to 1.17 for the samples in Fig. 2c.

$R_{\leq 3/\geq 4}$ reflects the difference in distribution profiles of 16 EPA-priority PAHs in the addressed sludges. This behaviour may be due to the type of treatment and the wastewater sources [17]. Also, as it is shown in Table 1, El Jem and Hammamet Sud WWTPs employ natural lagooning and lagoon techniques respectively to treat mainly wastewater of domestic or hotel business origins. Grappee, Sud meliane and Soliman WWTPs receive high percentage of industrial wastewater, except for Chotrana sludge which shows higher relative percentage of 3 ring PAHs. Moreover, only Chotrana sludge underwent digestion treatment. The rest of samples originate mostly from domestic and industrial sources with different treatments. Thus, the wastewater source and the type of the treatment are likely to influence the composition of PAHs in the studied samples. Similar results were also found by other authors, who proved certain tendencies of correlation between the distribution pattern of individual PAHs from one side, the applied treatment and the source of wastewater from the other side [17,27].

4. Conclusion

The focus on PAH distribution in Tunisian sewage sludge was mainly due to the risks related to their toxicity and persistence in soil after re-use as organic amendments. Results show that Soxhlet extraction with toluene followed by liquid–liquid separation proved to be an efficient extraction procedure for PAHs in sludge. EPA 16 priority PAH levels were similar to those found in previous studies. PAHs with six rings were absent in the studied sludges likely because they do not originate from fuel industry. Regardless to wastewater origins, the absence of any treatment resulted in higher levels of PAHs as observed in El Jem WWTP, for instance. Nevertheless, the total amount of PAHs in different Tunisian WWTPs was still lower than the limit proposed in the directive draft of the EU (6 mg kg^{-1}). Some significant differences between the sludge were observed accordingly; the wastewater source and the type of applied treatment seem to have an indirect effect on the composition and individual pattern of PAH in the studied samples.

Acknowledgments

The authors would like to thank the Japan International Cooperation Agency (JICA) for supporting this work and the National Office of Urban Sanitation, Tunisia (ONAS) for facilitating sludge sampling.

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