

UHPLC ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) COMPOUNDS FROM THE SOIL BY QuEChERS AOAC METHOD FROM MANESAR INDUSTRIAL AREA, HARYANA, INDIA

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<https://doi.org/10.55251/jmbfs.5861>

ARTICLE INFO

Received 19. 2. 2022

Revised 10. 6. 2022

Accepted 4. 8. 2022

Published 1. 10. 2022

Regular article



ABSTRACT

An Ultra-High-Performance Liquid Chromatography (UHPLC) method was developed and validated as per ICH guidelines for the analysis of 16 PAH compounds as per USEPA and various soil samples were analyzed in Manesar located 60 km away from Delhi, capital of India 28.3542° N, 76.9400° E in different seasons. The sampling was done in the industrial areas, agricultural fields, and residential plots all within 10 km radius of the industrial area. In the present study, the QuEChERS method was used for sample preparation, which has shown good recoveries around 80 to 120% The extraction time was only 10 minutes, followed by the UHPLC technique of run time of just 16 minutes. Average PAH concentration in Manesar soil was found highest in the winter session of industrial areas. Σ 16 PAH was 141 ppb in the industrial area in winter, 64 ppb in agricultural fields, and 54.7 ppb in residential plots. The lowest PAH levels were observed in the monsoon season. These values are very less if compared to Brazil, UK, Germany, and Korea.

Keywords: Soil, Manesar, PAH, QuEChERS, UHPLC

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAH) are known carcinogenic and mutagenic compounds which are formed by burning organic matter with less oxygen presence and heavy vehicle emissions (Sorensen, 1994; Nam *et al.*, 2003). Such compounds need to be monitored and checked from time to time in industrial areas. Apart from mutagenicity, they are responsible for many other lungs and respiratory diseases (Grimmer *et al.*, 1983; Yang *et al.*, 1991; Rost and Loibner, 2002). In soil, these compounds are retained in humus and organic matter for a very long time as they are hydrophobic (Krauss *et al.*, 2000). Hence soil is the best place to trace out and check for PAH contamination (Ockenden *et al.*, 2003). Few studies have already been done in India, like in Delhi (Kannan and Kapoor, 2004), Mumbai (Sahu *et al.*, 2001), and Ahmedabad (Raiyani and Shah, 1993). PAHs are one of the most dangerous pollutants found in soil as per IARC. Higher ring PAHs are more carcinogenic Yang *et al.*, 1991; Massei and Ollivon, 2004). The study of PAH becomes very essential in areas where the industries are very close to residential apartments and agricultural fields. Manesar located 60 km away from Delhi, the capital of India 28.3542° N, 76.9400° E is one of the fastest-growing industrial areas and townships. As per Human Rights Documentation Indian Social Institute, (India) Manesar has fertile lands which are used for farming. It's becoming more important to check the PAH levels in Manesar as it's a hub of farming lands, residential, industries, and all within 10 km area. The PAH-contaminated soil can be harmful to farming as PAH can percolate from soil to vegetables (Wennrich *et al.*, 2002). Many studies have shown that if vegetables are grown in PAH-contaminated soil, it may lead to the uptake of PAH in them (Wennrich *et al.*, 2002). High concentrations of PAH compounds were found in vegetables like a tomato at 0.2 µg/kg, spinach at 6.6µg/kg, and cabbage 20.4µg/kg (M. Kluska.,2003) So, the study of soil is very crucial to trace out PAH. Currently available and reported PAH analysis methods are long and time taking and use a classical sample preparation and analysis approach. Classical Soxhlet extraction technique takes 8 to 12 hours of extraction of PAH from soil and uses a huge amount of extraction solvents which is then analyzed by HPLC technique whose run time was around 30 to 40 minutes for analysis of 16 PAH. This study has identified the use Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) AOAC method which is very quick and rugged. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is an acetonitrile-based extraction technique followed by dispersive solid-phase extraction (SPE) (Anastassiades *et al.*, 2003). Followed

by this extraction technique a new UHPLC method was also developed and validated as per ICH guidelines which separate 16 PAHs in just 16 minutes.

MATERIALS AND METHODS

Regional site description

Manesar located 60 km away from Delhi, the capital of India 28.3542° N, 76.9400° E has become the hub of industrialization and real estate. The faster-growing industrialization needs the huge movement of heavy vehicles run on diesel. NH8 is the highway that connects Manesar to Gurugram and Delhi. Apart from the main highway NH8 various other roads pass through agricultural fields. One such road is Pataudi road which is the easiest and most popular road considered as an alternative to NH8. The frequency of heavy and small vehicles increased drastically throughout the last 5 years due to the sudden outburst of industrialization and real estate construction in Manesar. This road is surrounded by farmlands. PAH study is very essential to check the levels as heavy vehicle exhausts are a potential source of PAHs. Manesar itself has many factories which release smoke from chimneys. Chimneys are potential sources for PAH releases. During winter seasons, many organic materials are burnt like wood and coals as a source of fuel, which is again a potential source of PAH. Hence, PAH study has become essential in Manesar and surrounding areas. The climate of Manesar is dry and hot with a temperature range from 32°C to 48°C. In winter, the temperature ranges from 4°C to 30°C.

Sample collections

For sample collection, the entire Manesar area was categorized into three parts industrial area, an agricultural area, and a residential area. For analysis, 5 cm topsoil was taken through an auger and sieved through 20-mesh sieve, and stored in airtight plastic tubes in a refrigerator at 4°C. Total of 240 samples were collected, 80 samples from each location.

Extraction and analysis of PAH

QuEChERS AOAC technique was used for the extraction of PAH from the soil. In a 50 ml tube, 5gm soil samples were taken, followed by 5ml water and sonicated

for 2 minutes, then 10ml acetonitrile was added and again sonicated for 2 minutes. Agilent QuEChERS AOAC pouch (PN-5982-0755) was added to the mix and vortexed for 15 minutes and the contents were centrifuged for 5 minutes at 10000

RPM. The volume of about 5ml of the supernatant was transferred to the dispersive SPE 15ml tube and vortexed for 2 minutes.

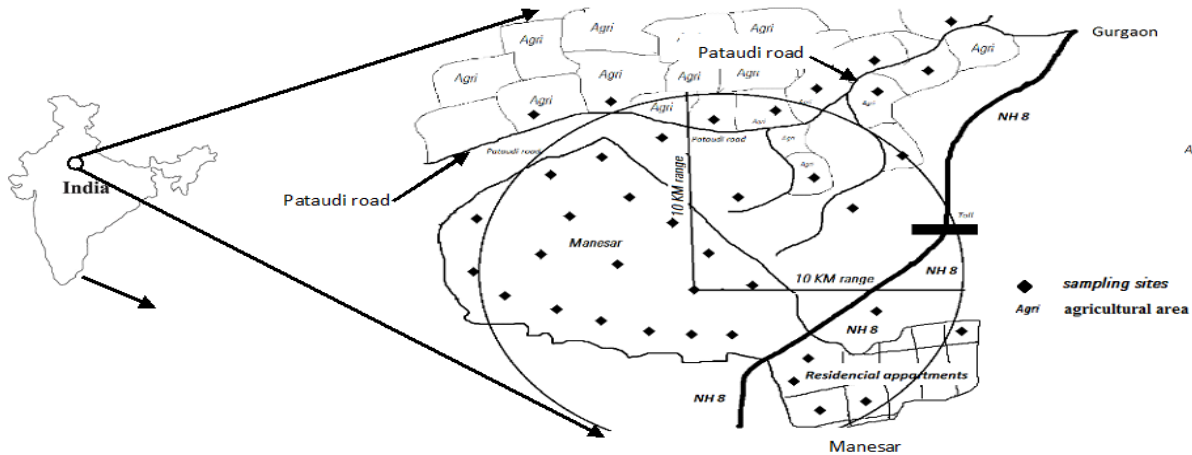


Figure 1 Sampling points at Manesar

The supernatant was filtered through 0.2µm syringe filter and injected into UHPLC (Ultra-High-Performance Liquid Chromatography). The analysis of PAH was done by Agilent 1290 Binary UHPLC with Agilent PAH column (2.1mm x 100mm x 1.8µm). Milli Q water was used in mobile phase channel A and HPLC grade acetonitrile was used in mobile phase channel B, flow was optimized at 0.35ml/min with a stop time of 18 minutes. The gradient elution program was given as 55% mobile phase B at the start, ramp till 95% mobile phase B at 9.5 minutes, 95% mobile phase B at hold till 18 minutes, 1 minute post time for column equilibration at an initial gradient, the column was kept at room temperature. For trace level detection DAD (Diode array detector) and FLD (Fluorescence detector), both were used with UHPLC. DAD at 230nm was only used for detection of *Acenaphthalene*, for others, FLD was used with a fixed excitation at 260nm and multiple emission was used at 350nm, 420nm, 440nm, and 500nm.

The new UHPLC method was validated as per ICH guidelines and calibration was done for different levels. To check the robustness of the UHPLC method 2% deviation was introduced to the flow rate and injection volume and 5% deviation in the column temperature and found that the retention time (RT) deviation was within 3% limit. For repeatability, 10 replicate injections were done, and % RSD (Relative Standard Deviation) for area and RT were calculated. To validate the sample prep spiking studies were performed at low, mid, and higher concentration levels, and recoveries were found between 80% to 120%. The chromatogram for UHPLC is shown in Figures 2& 3.

Table 1 shows which emission wavelength was used for what PAH, with the limit of detections

Serial no.	Detector	PAH name	LOD concentrations, µg/L [ppb]
1	FLD, Em γ 350nm	Napthalene	1.9
2	DAD 230nm	Acenaphthalene	1.9
3	FLD, Em γ 350nm	Acenaphthene	1.9
4	FLD, Em γ 350nm	Fluorene	0.195
5	FLD, Em γ 350nm	Phenanthrene	0.078
6	FLD, Em γ 420nm	Anthracene	0.039
7	FLD, Em γ 440nm	Fluoranthene	0.097
8	FLD, Em γ 420nm	Pyrene	0.195
9	FLD, Em γ 420nm	Benzo(a)anthracene	0.097
10	FLD, Em γ 420nm	Cyrysene	0.097
11	FLD, Em γ 440nm	Benzo(b)fluoranthene	0.039
12	FLD, Em γ 420nm	Benzo(K)fluoranthene	0.039
13	FLD, Em γ 420nm	Benzo(a) pyrene	0.096
14	FLD, Em γ 420nm	Dibenzoanthracene	0.39
15	FLD, Em γ 420nm	Benzoperylene	0.156
16	FLD, Em γ 500nm	Indeno pyrene	3.12

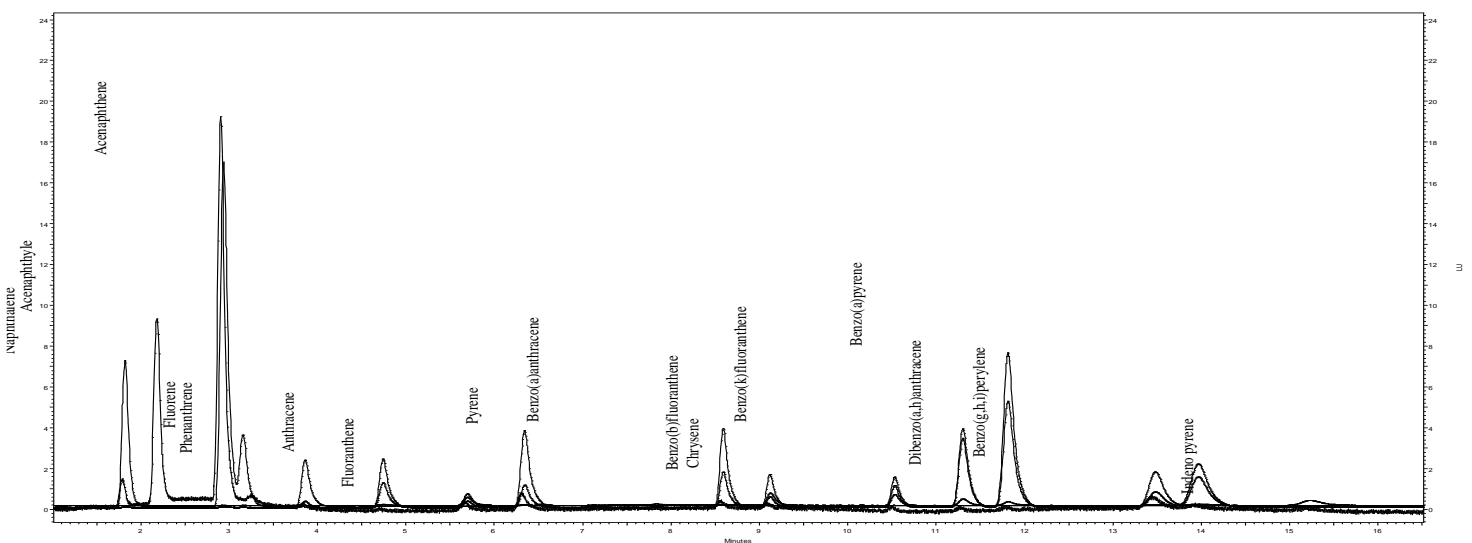


Figure 2 UHPLC separation for 16 PAH through UHPLC

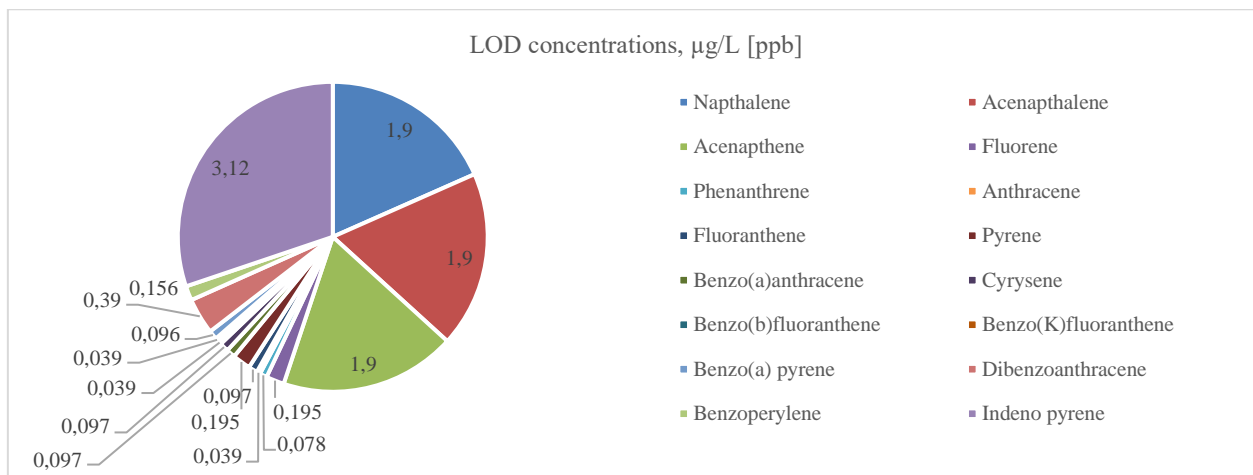


Figure 3 Separation for 16 PAH through UHPLC

RESULTS AND DISCUSSIONS

PAH extraction from soil

Manesar was divided into industrial, agricultural, and residential areas, and samples were collected in summer (S), monsoon (M), and winter (W), average concentrations are reported in table 2 and figure 6. The average level of PAH in the industrial area was an all-time high as compared to agricultural lands and residential areas. This was well justified as the agricultural lands were close to the PAH emission zones. The residential areas were located 10 km far and close to only NH8 which is the highway so the only potential PAH source is vehicle emission.

Seasonal variations

Average concentrations of PAH were plotted in the graph in which the Y-axis is the ppb concentration of PAH. It was found that in winter the PAH levels were higher relatively. In winter it was noticed that the burning of organic wastes was more in village areas near agricultural fields which contributes to higher PAH levels. The entire amount of average PAH in the industrial area is 89.3 ppb in the summer and 141 ppb in the winter. This value is very low if compared to other case studies (Wennrich et al., 2002). The average PAH concentrations are represented for different countries, Table 3.

Table 2 Seasonal average PAH concentrations in soil at the locations of Manesar, Summer – S; Monsoon – M, Winter–W

Serial no.	PAH	Industrial area			Agricultural land			Residential area		
		[ppb]			[ppb]			[ppb]		
		S	M	W	S	M	W	S	M	W
1	Napthalene	10.2	7.5	15.6	7.2	5.2	8	4.6	3.6	5.2
2	Acenaphthylene	6.2	3.5	8.6	4.6	2.1	5.2	3.5	1.2	4.2
3	Acenaphthene	2.3	1.5	5.6	1.5	ND	2.5	1.2	0.5	2.3
4	Fluorene	2	1.8	6.3	1.4	ND	2.1	1.1	ND	2.5
5	Phenanthrene	8.2	5.6	12.5	6.2	2.1	7.6	5.5	3.5	4.6
6	Anthracene	3.2	1.2	6.2	0.8	ND	2.1	0.6	ND	2.2
7	Fluoranthene	7.5	5.5	8.3	2.3	0.8	3.1	2.1	0.8	3.6
8	Pyrene	11.3	8.5	15.2	5.2	3.1	6.7	4.2	3.1	4.9
9	Benzo(a)anthracene	8	4.6	11.3	4.2	0.5	5.9	3.5	2	5.5
10	Cyrysene	4.5	1.2	6.5	1.6	ND	3.2	0.8	ND	2.1
11	Benzo(b)fluoranthene	7.5	4.6	9.6	2.3	0.4	2.4	2.1	0.5	3
12	Benzo(K)fluoranthene	7.2	4.5	8.6	3.2	ND	3.8	3	0.6	3.2
13	Benzo(a) pyrene	9.2	3.5	10.2	4.6	1.2	5.3	3.5	1.2	3.8
14	Dibenzoanthracene	3.2	2	4.6	1.6	0.5	2.1	1.2	ND	2.1
15	Benzoperylene	3.8	1.5	5.6	0.8	ND	1.6	0.5	ND	2
16	Indeno pyrene	5.2	4.1	6.3	2.6	0.4	3	1.5	0.7	3.5
	Total	89.3	61.1	141	50.1	16.3	64.6	38.9	17.7	54.7

Table 3 Comparison of PAH concentration.

Study area	No.	PAH, ppm	Reference
Agricultural			
Brazil	20	0.096	Wilcke et al. (1999a)
UK	12	0.19	Wild and Jones (1995)
Germany	6	1.9	Tebaay et al. (1993)
India, Agra	11	6.7	Masih, Amit, Taneja, Ajay (2006)
India, Manesar	16	0.064	Present study
Residential			
Bangkok	20	0.38	Wilcke and Muller (1999b)
Brazil	20	0.39	Wilcke et al. (1999a)
Germany	6	1.8	Tebaay et al. (1993)
UK	12	4.2	Wild and Jones (1995)
India, Agra	11	9.3	Masih, Amit, Taneja, Ajay (2006)
India, Manesar	16	0.054	Present study
Industrial			
UK	12	4.5	Wild and Jones (1995)
Germany	6	16	Tebaay et al. (1993)
Austria	18	79	Weiss et al. (1994)
India, Agra	11	13.7	Masih, Amit, Taneja, Ajay (2006)
India, Manesar	16	0.141	Present study

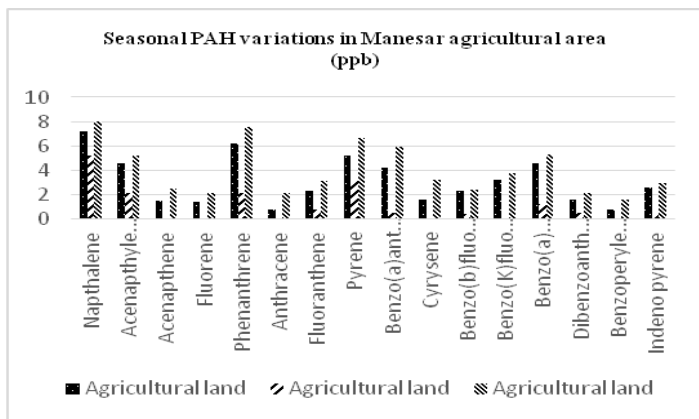


Figure 4 Seasonal PAH variations in Manesar agricultural area (ppb)

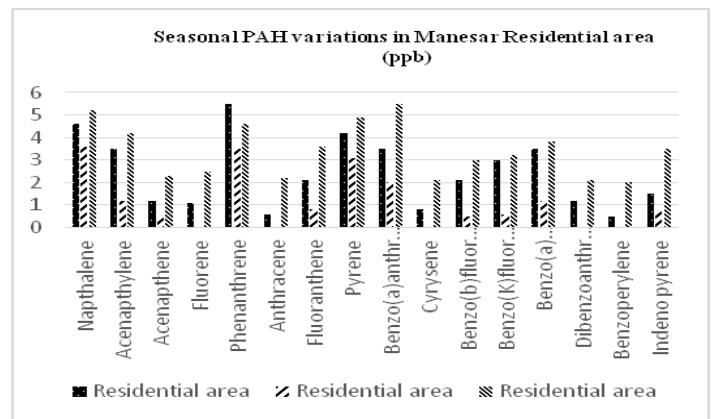


Figure 5 Seasonal PAH variations in Manesar residential area

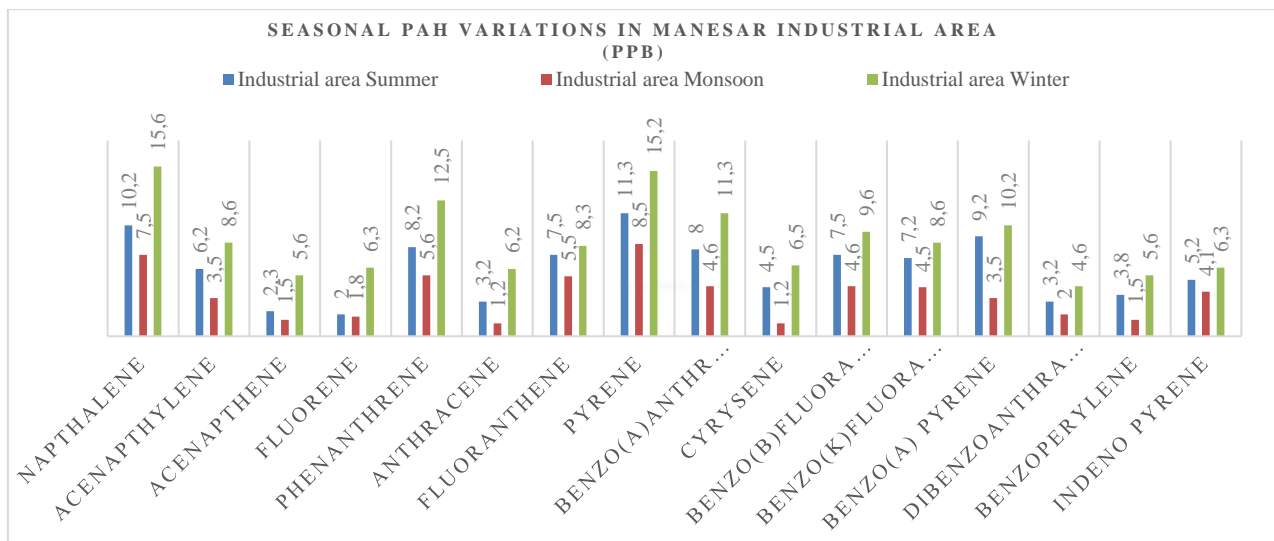


Figure 6 Seasonal PAH variations in Manesar industrial area

PAH presence profile assessment

Till date, the PAH concentration and exposure limit are not directly linked to any disease, but few are categorized as most carcinogenic like benzo(a)pyrene, and classified as (2A) and (2B) human carcinogens by the International Agency for Research on Cancer (IARC, 1987). BaP has 5 rings (C₂₀H₁₂) which have shown potential mutagenic character for human cells in cultures environment (Osborn and Crosby, 1987), and animal assay (Cerna et al., 2000). As per the toxic equivalent factor, BaP is one (1) which is the highest of all. PAH concentration below 1ppm shows the soil of Manesar is non-carcinogenic presently.

CONCLUSION

In the location of Manesar, 16 PAH were observed in the soil. The total PAH concentrations were found to be 141µg/Kg, 64 µg/Kg, 54.7 µg/Kg in industrial areas, agricultural areas, and residential areas in the winter season respectively. Out of summer, monsoon, and winter, the winter season has shown the highest amount of PAH concentration. In the monsoon season the concentrations were very low, 61 µg/Kg, 16.3 µg/Kg, 17.7 µg/Kg respectively in industrial areas, agricultural areas, and residential areas. Anyhow, in winter the concentration of PAH for industrial areas is also very low concerning other countries' industrial areas, the reason could be the establishment of Manesar is new around 10 years old. But such monitoring is very important from time to time. The new UHPLC method was developed and saved a lot of time and solvent, and good separation was achieved. The sample preparation technique also saves a lot of time. The most carcinogenic PAH is BaP which is also reported very low and insignificant in Manesar soil at present as per table 3.

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