

Original Research

Quantification of Selected Polycyclic Aromatic Hydrocarbons (PAHs) in Soil, Water and Blood by an Optimized and Validated HPLC –UV–DAD Method

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are produced as primary environmental pollutants as a result of incomplete combustion of fuels. They harbor mutagenic and carcinogenic properties. Their continuous monitoring is considered a requisite for keeping them into admissible limits. The current study was designed to quantify the levels of PAHs in soil, water and blood samples. One hundred and fifty samples each of soil, water, human and animal blood were collected from highly exposed areas including industrial, highways and incinerators linked areas around Lahore city, Punjab, Pakistan. Amounts of six selected PAHs (Phenanthrene, Biphenyl, Biphenylene, Naphthalene, Anthracene and P-Ansidine) were quantified by reverse phase-high performance liquid chromatography (RP-HPLC) equipped with UV-VIS photodiodes array detector (PDA) at 247 nm. An isocratic method was optimized and validated for use with soil, water and plasma samples. To obtain the reliable results the HPLC method was validated following the ICH/FDA guidelines. It was found that PAHs exist in large quantity in highly exposed areas particularly the soil samples were extremely contaminated with PAHs. The highest average concentration of Naphthalene (260.85 ± 165.64 $\mu\text{g}/\text{kg}$) was detected in samples from industrial areas. Conversely, the lowest average amount of Biphenanthrene (10.31 ± 3.46 $\mu\text{g}/\text{kg}$) was found in samples from highways. Human and animal plasma samples were also found to carry PAHs in comparatively lower levels than soil and water.

Keywords: PAHs, RP-HPLC, soil, water, blood

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) have attracted a great deal of attention as a major persistent organic pollutant, due to their carcinogenic, mutagenic, and teratogenic effects on organisms and humans [1, 2]. Due to fused aromatic rings (2 or >2), they exhibit nonpolar properties and have the ability to persist for a long period of time and also spread into environment [3]. Their most perceptible properties (rheological and photophysical), have been observed in many scientific studies, and almost more than 100 PAHs have been pointed as human health hazards [4].

Keeping in view the health risks of PAHs, sixteen PAHs, containing 2-6 rings were classified as priority control pollutants by the United States-Environmental Protection Agency (US-EPA) that have been commonly studied by researchers from many years [5-9].

Characteristic nature of PAHs plays an important role in their dispersal, deposition and impact within the environment part on the whole ecosystem [10]. Various sources have been reported for their production [11-13]. Furthermore, their synthesis can also be made possible by discharge of electricity in decorative items like paints, glue and other protection products [14].

Variation in PAHs' toxicity was observed and reported that it was directly related to their concentration and duration of exposure, also route of exposure (inhalation, drinking, diet activities and dermal exposure) [15]. On the basis of these factors their effects may be acute or chronic. Prolong exposure may cause chronic effects like metabolic disorder, prostate, breast and other mutagenic effects [16].

Due to their volatile ability as well as long-range transport capacity, these pollutants not only affect the connected areas but also responsible for harmful effects in distant areas. The accumulation and persistence of PAHs in the environment can produce harmful effects, in both aquatic and terrestrial ecosystems. Humans and animals exposed to PAHs through inhalation, drinking water and via food chain may face serious health effects [1, 2]. Accumulation of PAHs in animals and plants depends on their biochemical nature; due to their lipophilic nature they often accumulate in fat tissues of organisms.

Among 16 PAH priority pollutants according to the U.S. Environmental Protection Agency, following PAHs (Phenanthrene, Biphenyl, Biphenylene, Naphthalene, Anthracene and P-Ansidine) were focused in the present study of PAHs in environmental matrices like water, soil and blood samples. In the early 1970's, since their commencement, high-performance liquid chromatography (HPLC) has been used for their qualitative as well as quantitative analysis. Several methodologies have been reported for their estimation. Reversed-phase mode of analysis has become the most popular HPLC mode for the separation of PAHs. The main thrust for this study is to quantify various types of (LMW) PAHs (Phenanthrene, Biphenyl, Biphenylene,

Naphthalene, Anthracene and P-Ansidine) by HPLC in soil, water and blood (human, animal) samples in a reverse phase mode by optimizing and validating (ICH/FDA) an isocratic method instead of gradient method [17]. This method is more sensitive as its LOD and LOQ limits are improved as compared to previously used methods. Validation was done in all three modes like soil, water and blood (plasma).

Materials and Methods

Chemicals

The solvents used in this study included methanol, acetonitrile (HPLC grade), acetone and n-hexane (analytical grade) from Merck. HPLC-grade water was obtained in a Milli-Q system (Quality Operations Laboratory, UVAS Lahore). Analytical standards of PAHs (Phenanthrene, Biphenyl, Biphenylene, Naphthalene, Anthracene and P-Ansidine) were supplied by Sigma Aldrich.

Sampling

To check the prevalence of PAHs, 50 samples of soil (250 gm), industrial waste water (500 mL) and blood (5 mL) sample of both human (male, non-smokers, aged between 35-50 years) and buffalo (3-4 years old) were collected from suspected areas Pharmaceutical, Textile, Leather tanneries linked areas, near high-ways, waste combustion areas etc. (Fig. 1). Soil (surface soil) and water samples were placed in polyethylene bags

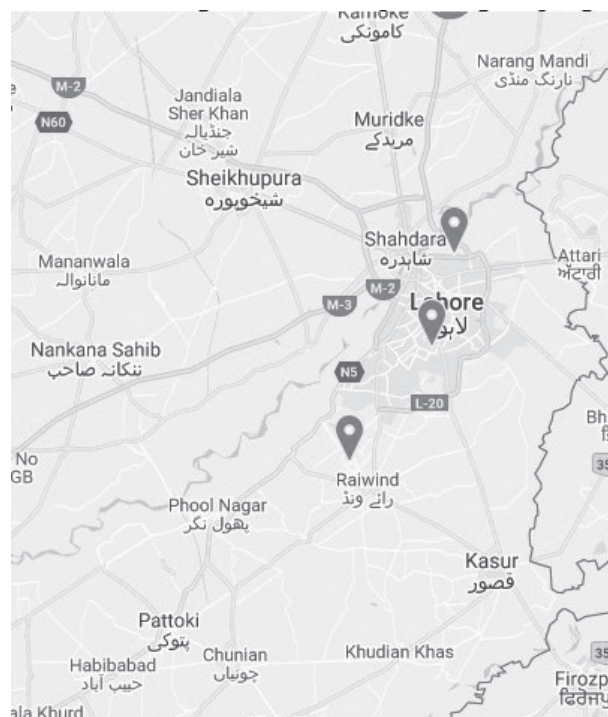


Fig. 1. Map showing sampling areas around Lahore.

as well as in sterile plastic/glass bottles at appropriate conditions until analysis. Human and animal blood samples from the respective areas were collected in heparinized blood sampling tubes. After centrifugation at 5000 rpm plasma was separated and stored at -20°C .

Chromatographic Conditions

Reversed phase HPLC analysis was performed on Shimadzu 20A system using C_{18} column having the specification (250 x 4.6 mm) with a pore size of 5 μm . Stock as well as working solutions of Phenanthrene, Biphenyl, Biphenylene, Naphthalene, Anthracene and P-Ansidine were prepared (10 mg/10 mL) by dissolving them in acetonitrile. The detection of PAHs was carried out at 247 nm. The mobile phase consists of solution A = 50 % acetonitrile in water, B = 100 % acetonitrile, both solutions were run in 75:25 ratio at a flow rate of 1.0 mL/min.

Extraction of PAHs from Soil and Water Samples

Soil sample (10 gm) and water sample (100 mL) was taken in a 250 mL separating funnel and added 50 mL n-hexane-acetone (1:1) as an extraction solvent. The mixture was shaken vigorously and allowed to settle for 2 min. This process was repeated twice and the solvent was collected in a beaker followed by drying on water bath or under nitrogen stream. The residue was reconstituted in 1 mL mobile phase, filtered through 0.22 μ polyamide filter and 75 μL was injected to the HPLC system.

Extraction of PAHs from Plasma Samples

Blood plasma (1 mL) was deproteinated with 1 mL acetonitrile followed by centrifugation at 15000 revolutions per minute for 15 min. The supernatant was separated, added 1 mL of hexane-acetone (1:1) and vortexed for 3 min. Upper layer (hexane) was separated and evaporated under nitrogen stream. Residue was reconstituted with 1 mL of mobile phase, filtered through 0.22 μ polyamide filter and 75 μL was injected to the system.

Validation of HPLC Method

Analytical method for estimation of PAHs in soil, water and plasma was validated following the validation guidelines of International Conference of Harmonization, Federal Drug Authority [18, 19]. Different parameters like LOD, LOQ, and linearity, precision, accuracy, recovery and stability were checked.

Results and Discussion

Selectivity/Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analytes in the presence of other compounds in the sample. Specificity in liquid chromatography is obtained by setting chromatographic conditions, such as mobile phase composition, column (type, temperature), and flow rate and detector wavelength. Besides chromatographic separation, the sample preparation step can also be optimized. Optimized specific HPLC conditions were followed for selectivity by analyses of blank samples (pre-extracted water and sediment samples) in triplicates. Each blank sample tested for interference, and selectivity was lower than limit of detection (LOD). The specificity of the method was determined by analyzing the sample solution containing all the PAH compounds. For this purpose, 75 μL of one of the sample solutions was injected into the HPLC system and the specificity of the method was measured in terms of the resolution between the two peaks (retention times) without overlapping of the peaks.

Limit of Detection and Limit of Quantification (LOD and LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were obtained by processing the eight aliquots of a spiked sample with smallest quantity of the standard materials to produce a valid quantifiable peak at signal to noise ratio $>3:1$ ($s/n > 3$) for a 75 μL of injection. The LOD was calculated as per the USEPA method. The limit of quantification (LOQ) was calculated for a valid quantifiable peak at signal to noise ratio $>10:1$.

Calibration Range and Linearity

In this method, six-point calibration curves were prepared with different concentration levels for different PAH compounds in soil, water and plasma having range (50-4000, 10-1600 and 10-320) ng/mL individually (Table 1). The observed linearity (R^2) ranged between 0.991-0.996 for all the above mentioned PAH compounds. Linearity and matrix effects were tested by a plot of linear regression equation and standard addition method for individual PAHs (Table 1).

Precision, Accuracy and Recovery

Intra-day and inter-day precision (repeatability) was checked by spiking the quality control (QC) concentrations of PAH analytes (Anthracene, Biphenanthrene, Biphenyl, Naphthalene, P-Ansidine and Phenanthrene) in soil, water and plasma individually ($n = 6$) and calculated its coefficient of variation (CV %).

Table 1. The linearity and regression analysis of PAHs in different nature of samples.

Sr. No.	Sample nature	PAHs	Conc. Range (ng/mL)	Linearity (R ²)	LOD (ng/mL)	LOQ (ng/mL)
1	Soil	Biphenanthrene	50-4000	0.9873	15	50
2		P-Ansidine	50-4000	0.9971	15	50
3		Naphthalene	50-4000	0.998	15	50
4		Biphenyl	50-4000	0.998	15	50
5		Phenanthrene	50-4000	0.9979	15	50
6		Anthracene	50-4000	0.9969	15	50
7	Water	Biphenanthrene	10-1600	0.9993	3	10
8		P-Ansidine	10-1600	0.9993	3	10
9		Naphthalene	10-1600	0.998	3	10
10		Biphenyl	10-1600	0.998	3	10
11		Phenanthrene	10-1600	0.9979	3	10
12		Anthracene	10-1600	0.9969	3	10
13	Plasma	Biphenanthrene	10-320	0.9979	3	10
14		P-Ansidine	10-320	0.9994	3	10
15		Naphthalene	10-320	0.9975	3	10
16		Biphenyl	10-320	0.9987	3	10
17		Phenanthrene	10-320	0.9987	3	10
18		Anthracene	10-320	0.9988	3	10

Table 2. Validation parameters (precision, accuracy and recovery) performed after spiking of different PAHs in soil, water and plasma.

Sample nature	PAHs	Quality controls conc. (ng/mL)	CV (%)	Accuracy (%)	Recovery (%)
Soil	Biphenanthrene	150	0.87	99.15	92.29±2.03
		1500	1.51	97.73	103.97±1.64
		3000	0.12	98.52	103.90±1.24
	P-Ansidine	150	2.64	102.97	88.05±1.03
		1500	3.48	98.14	100.35±2.99
		3000	1.82	102.01	105.50±1.82
	Naphthalene	150	0.79	99.23	98.42±3.49
		1500	0.57	98.14	94.12±3.18
		3000	1.54	100.25	107.76±3.24
	Biphenyl	150	0.25	97.88	82.86±2.48
		1500	0.24	101.25	98.52±0.70
		3000	0.20	100.21	103.21±0.66
	Phenanthrene	150	0.57	98.20	98.23±1.13
		1500	0.61	98.99	99.08±1.24
		3000	0.64	100.17	102.00±1.26
	Anthracene	150	0.59	97.90	98.81±0.96
		1500	0.50	100.64	98.31±0.90
		3000	0.64	99.87	101.35±0.50

Table 2. Continued.

Water	Biphenanthrene	30	1.10	97.75	96.39±1.34
		300	2.30	101.32	95.46±0.59
		1200	0.61	102.58	98.61±1.05
	P-Ansidine	30	1.91	98.55	79.12±2.13
		300	2.23	99.63	97.44±1.92
		1200	1.21	100.57	99.97±3.33
	Naphthalene	30	7.90	97.97	93.87±8.09
		300	2.46	98.60	96.91±1.60
		1200	0.97	98.66	87.78±3.46
	Biphenyl	30	0.67	99.01	84.88±2.33
		300	0.63	100.78	100.92±0.37
		1200	0.38	100.25	95.29±0.74
	Phenanthrene	30	0.39	98.36	86.83±1.46
		300	0.74	101.22	99.56±0.97
		1200	0.35	99.37	96.83±0.48
	Anthracene	30	0.70	97.84	87.86±0.53
		300	1.48	99.69	100.16±0.47
		1200	0.53	100.69	97.28±0.16
Plasma	Biphenanthrene	30	5.17	99.21	92.61 ±2.63
		100	3.71	100.01	100.27±4.67
		250	3.28	101.33	93.33±2.30
	P-Ansidine	30	3.10	101.30	94.01±6.30
		100	2.68	98.55	88.09±2.74
		250	1.64	101.01	83.22±2.30
	Naphthalene	30	5.60	98.33	73.06±1.85
		100	2.52	99.34	88.41±1.30
		250	1.99	100.65	91.31±0.69
	Biphenyl	30	1.44	97.77	93.28±1.62
		100	1.82	101.50	87.59±0.84
		250	1.69	99.89	91.15±0.78
	Phenanthrene	30	1.84	101.11	89.09±2.06
		100	2.61	98.63	94.17±3.25
		250	1.87	101.66	94.12±1.91
	Anthracene	30	0.51	98.89	78.65±0.71
		100	1.32	102.08	93.49±0.80
		250	2.20	100.32	101.86 ±2.54

It was noted that % CV not exceeded the acceptable limit as defined in the residue guidelines [20], shown in Table 2. Similarly, PAH analytes were spiked by low, medium and high QC concentrations in soil, water and plasma (n = 6) to check the accuracy and found that

all quality control samples were within the allowable limit of accuracy defined by EMEA, 2009 presented in Table 2. Recovery was also checked in soil, water and plasma using the QC samples (n = 6) and found within the admissible range, shown in Table 2.

Stability

The stability studies were conducted to check the significant degradation of PAHs in soil, water and plasma. To evaluate the freezer and room temperature stability plasma samples were kept frozen at -20°C and 25°C temperature (ambient temperature) for 24hrs. and 48hrs. The QC standard of PAH was monitored to examine stability of standard and sample solutions. PAH was found stable when freeze as well as during analysis at room temperature (Table 3).

Occurrence of PAHs

PAHs were estimated quantitatively in soil, water and blood (human and animal) samples ($n = 50$ each) collected from industrial areas, highways and incinerators from Lahore division, Pakistan. Average concentration of Biphenanthrene and P-Ansidine found in soil and water in industrial areas were $108.05 \pm 20.01 \mu\text{g/kg}$ and $258.24 \pm 81.09 \mu\text{g/kg}$ respectively. The mean concentration of Naphthalene, Biphenyl, Phenanthrene and Anthracene calculated in soil and water from industrial area samples were $509.22 \pm 111.43 \mu\text{g/kg}$, $53.44 \pm 14.84 \mu\text{g/kg}$, $266.18 \pm 69.23 \mu\text{g/kg}$ and $181.16 \pm 42.32 \mu\text{g/kg}$, respectively, shown in Table 4. Similarly, the average concentration of different PAHs including Biphenanthrene, P-Ansidine, Naphthalene, Biphenyl, Phenanthrene and Anthracene determined in the samples of highways were 7.48 ± 2.58 , 52.79 ± 5.23 , 218.60 ± 53.37 , 15.78 ± 6.70 , 128.83 ± 35.76 and $70.83 \pm 15.65 \mu\text{g/kg}$, respectively. While, the mean values of PAHs (Biphenanthrene, P-Ansidine, Naphthalene, Biphenyl, Phenanthrene and Anthracene) found in the samples collected from incinerators were 42.68 ± 8.52 , 112.49 ± 26.28 , 256.45 ± 53.75 , 26.49 ± 7.38 , 135.36 ± 39.83 and $78.50 \pm 18.26 \mu\text{g/kg}$, respectively, presented in Table 4.

Thirteen PAHs determined [21] were collected from the vicinity of the Jordan petroleum refinery and Al-Hussein thermal power stations in Zarqa region. They found that the total concentration for PAHs ranged from $0.94 \mu\text{g/kg}$ to $191 \mu\text{g/kg}$. According to [22], the PAHs compounds were found in almost all samples and the total concentrations ranged from 1.3 to 528mg/kg . It was found that all the analyzed samples were containing concentrations of more than 1mg/kg (Set by European Union) criteria that was issued by European commission regarding the concentration of PAHs in consumer articles.

The concentration of low molecular weight PAHs [23] (LMW PAHs, with 2-3 rings) ranged from 7.71 to 185.16ng.g^{-1} with an average value of 37.7% . High molecular weights PAHs (HMW PAHs, with 4-6 rings) had a relatively high concentration (12.97 - 435.85ng.g) with an average value of 62% . The concentration of low (30.95 to 501ng.g) as well as high molecular weight (7.48 to 391.75ng.g) PAHs found in the present

study is higher than the previous study as the samples were collected from PAHs prone areas in this study. Similarly, the PAHs concentrations determined [24] were found to range from 6.48 to 154ng/g in high polluted areas and the concentrations of the PAHs in the urban sites were higher than those in the suburban sites for ambient air. Vehicle and industrial emissions as well as coal/biomass combustion were the leading sources (approximately 47%) of the PAHs in the ambient air.

Meanwhile the average concentration of Biphenanthrene and P-Ansidine found in human and animal plasma samples in industrial areas were $18.46 \pm 2.96 \text{ng/mL}$ and $24.34 \pm 8.86 \text{ng/mL}$ respectively. While the mean concentration of Naphthalene, Biphenyl, Phenanthrene and Anthracene from industrial area samples were $12.48 \pm 4.56 \text{ng/mL}$, $3.61 \pm 1.89 \text{ng/mL}$, $72.72 \pm 14.60 \text{ng/mL}$ and $94.32 \pm 14.27 \text{ng/mL}$, respectively, shown in Table 5. Similarly, the average concentration of different PAHs including Biphenanthrene, P-Ansidine, Naphthalene, Biphenyl, Phenanthrene and Anthracene determined in the samples of highways were 10.31 ± 3.46 , 51.71 ± 41.88 , 34.16 ± 16.42 , 6.07 ± 1.72 , 53.88 ± 6.80 and $64.67 \pm 7.49 \text{ng/mL}$, respectively. Further, the mean values of PAHs (Biphenanthrene, P-Ansidine, Naphthalene, Biphenyl, Phenanthrene and Anthracene) found in the samples collected from incinerators were 26.90 ± 18.64 , 4.96 ± 2.15 , 5.43 ± 1.52 , 3.55 ± 1.03 , 35.92 ± 2.71 and $50.77 \pm 5.18 \text{ng/mL}$, respectively, presented in Table 5.

Naufal et al. [25] determined different PAHs in plasma in a highly exposed Chinese population and found the mean value of 13ng/mL .

Another study was reported by Singh et al. [26] in the Indian children in which they found the mean value of 358ppb of PAHs in whole blood. Singh et al. [27] also calculated the PAHs from a cohort of 56 children in which they found the higher values of PAHs (about 235 times higher) in the Indian measurements than for the control subjects.

The PAHs concentration determined in the blood samples [28] ranging between 0.156 to 3.61ng/mL were relatively higher than the levels reported in other several studies [29]. In the present study, the amount of PAHs collected from industrial, highways and incinerators linked areas determined in the human blood and animal blood ranged from 0.02 to 107.82ng/mL and 1.12 to 87.65ng/mL , respectively which is also higher and comparable to the mentioned studies. The estimation of higher concentrations of PAHs in blood may probably be due to the highly exposed areas of PAHs. Analysis of water samples for quantification of PAHs in this study showed the contamination in almost every sample which is comparable to a study conducted by [30] which also found the contamination of PAHs in all samples.

The highest amount of Naphthalene ($501.87 \mu\text{g/kg}$) was detected in soil sample collected around industrial area of Lahore while maximum concentration of Naphthalene ($516.57 \mu\text{g/kg}$) was found in the water sample, shown in Fig. 2. However, maximum

Table 3. The stability of analytical method performed after spiking of different PAHs in the quality control samples of soil, water and plasma.

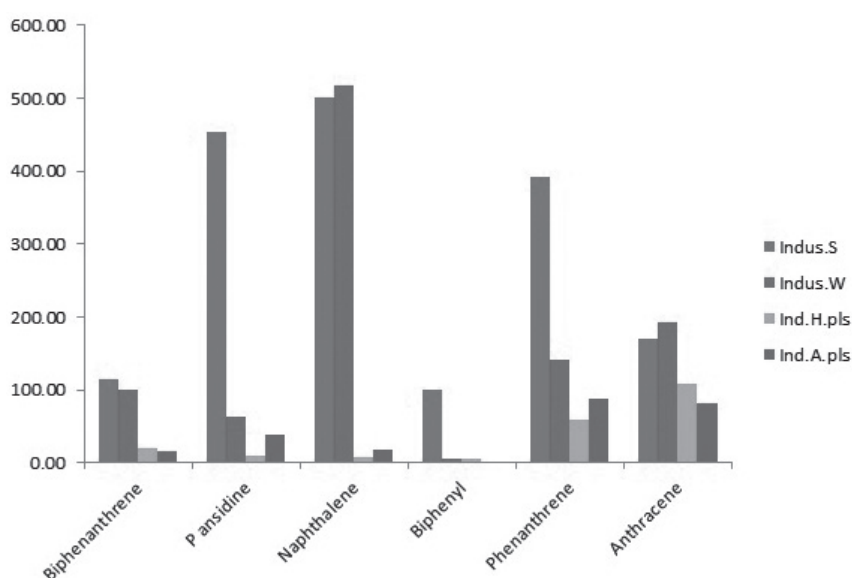
Sample nature	PAHs	Quality controls conc. added (ng/mL)	Normal	1st Thaw (24 hrs.)	2nd Thaw (48 hrs.)	
			Conc. found (mean±SD)	Conc. found (mean±SD)	Conc. found (mean±SD)	
Soil	Biphenanthrene	150	160.55±3.29	151.66±2.78	165.16±1.88	
		3000	2806.07±41.46	2998.90±29.69	2865.49±62.93	
	P-Ansidine	150	148.00±2.87	156.50±7.24	154.39±6.00	
		3000	3158.38±57.61	3218.14±33.18	2981.60±129.92	
	Naphthalene	150	171.47 ±0.88	151.87 ±3.02	168.69 ±0.49	
		3000	2995.09±42.72	3030.69±10.94	2963.33±14.44	
	Biphenyl	150	160.02±0.21	157.57±0.41	164.29±0.41	
		3000	2860.07±48.15	2931.17±4.32	2881.37±8.22	
	Phenanthrene	150	157.31±0.12	157.26±0.17	167.63±0.50	
		3000	2807.18±30.75	2893.10±6.58	2861.41±11.08	
	Anthracene	150	168.94±0.63	141.17±0.33	148.85±0.90	
		3000	2700.67±40.49	2937.97±7.50	2885.87±8.67	
	Water	Biphenanthrene	30	27.27±0.02	30.25±0.34	27.46±0.08
			1200	1209.35±1.76	1250.24±4.61	1252.14±4.26
P-Ansidine		30	29.15±0.27	29.67±0.52	32.45±0.51	
		1200	1283.77±45.44	1295.53±30.92	1198.36±13.42	
Naphthalene		30	29.97±2.00	30.09±0.70	30.65±1.16	
		1200	1189.36±1.31	1162.82±18.70	1090.83±6.04	
Biphenyl		30	29.12±0.09	34.27±1.25	26.46±0.22	
		1200	1248.90±1.50	1098.75±3.15	1189.19±6.90	
Phenanthrene		30	29.95±0.77	30.29±0.06	31.33±0.02	
		1200	1286.03±6.09	1121.94±14.78	1182.79±4.48	
Anthracene		30	26.46±0.03	32.06±0.22	29.07±0.01	
		1200	1256.03±4.43	1129.17±9.49	1231.73±0.90	
Plasma		Biphenanthrene	30	30.91±0.07	30.84±0.13	30.71±0.21
			250	229.29±7.87	242.99±2.03	233.84±7.10
	P-Ansidine	30	30.72±0.67	29.72±0.97	31.79±0.87	
		250	251.53±4.22	253.73±4.19	254.80±1.71	
	Naphthalene	30	30.80±1.17	31.11±1.46	31.30±0.61	
		250	262.42±5.05	249.99±2.59	250.48±3.72	
	Biphenyl	30	29.95±0.28	29.17±0.28	29.44±0.19	
		250	263.67±5.27	255.44±0.63	259.64±4.85	
	Phenanthrene	30	28.74±0.26	32.25±0.60	29.72±0.16	
		250	269.74±7.68	262.32±0.38	263.14±9.61	
	Anthracene	30	28.57±0.61	29.34±0.09	29.26±0.02	
		250	269.36±7.09	258.54±1.18	265.33±6.79	

Table 4. Amount of PAHs ($\mu\text{g}/\text{kg}$) detected in different samples of soil and water collected from Lahore, Pakistan.

	Nature of Sample	Polycyclic Aromatic Hydrocarbons (PAHs)					
		Biphenanthrene	P- Ansidine	Naphthalene	Biphenyl	Phenanthrene	Anthracene
Industrial area (pharmaceutical, textile and steel mill)	Soil	115.29 \pm 15.72	453.67 \pm 143.3	501.87 \pm 88.66	100.57 \pm 27.19	391.75 \pm 102.44	169.51 \pm 36.58
	Water	100.81 \pm 24.30	62.81 \pm 18.87	516.57 \pm 134.19	6.31 \pm 2.49	140.60 \pm 36.01	192.81 \pm 48.06
	\pm Mean	108.05 \pm 20.01	258.24 \pm 81.09	509.22 \pm 111.43	53.44 \pm 14.84	266.18 \pm 69.23	181.16 \pm 42.32
Highways	Soil	7.48 \pm 2.58	99.81 \pm 8.41	287.53 \pm 51.03	30.95 \pm 12.90	211.21 \pm 54.81	105.39 \pm 20.76
	Water	7.91 \pm 2.63	5.77 \pm 2.05	149.67 \pm 55.71	0.6 \pm 0.49	46.44 \pm 16.70	36.26 \pm 10.54
	\pm Mean	7.48 \pm 2.58	52.79 \pm 5.23	218.60 \pm 53.37	15.78 \pm 6.70	128.83 \pm 35.76	70.83 \pm 15.65
Incinerator	Soil	74.22 \pm 12.57	220.48 \pm 49.92	383.38 \pm 69.00	52.98 \pm 14.76	252.39 \pm 72.11	130.01 \pm 28.67
	Water	11.14 \pm 4.46	4.49 \pm 2.64	129.51 \pm 38.49	0.0 \pm 0.00	18.33 \pm 7.55	26.99 \pm 7.84
	\pm Mean	42.68 \pm 8.52	112.49 \pm 26.28	256.45 \pm 53.75	26.49 \pm 7.38	135.36 \pm 39.83	78.50 \pm 18.26

Table 5. Amount of PAHs (ng/mL) detected in different samples of blood (Human & animal) collected from Lahore, Pakistan.

	Nature of Sample	Polycyclic Aromatic Hydrocarbons (PAHs)					
		Biphenanthrene	P- Ansidine	Naphthalene	Biphenyl	Phenanthrene	Anthracene
Industrial area (pharmaceutical, textile and steel mill)	Human Blood	20.43 \pm 3.56	10.05 \pm 5.04	6.99 \pm 3.54	6.10 \pm 2.95	58.55 \pm 9.74	107.82 \pm 18.16
	Animal Blood	16.49 \pm 2.36	38.62 \pm 12.68	17.96 \pm 5.57	1.12 \pm 0.83	86.88 \pm 19.45	80.82 \pm 10.37
	\pm Mean	18.46 \pm 2.96	24.34 \pm 8.86	12.48 \pm 4.56	3.61 \pm 1.89	72.72 \pm 14.60	94.32 \pm 14.27
Highways	Human Blood	19.58 \pm 1.34	15.76 \pm 3.69	30.7 \pm 14.09	8.02 \pm 2.05	74.82 \pm 5.08	101.78 \pm 9.46
	Animal Blood	6.40 \pm 1.06	87.65 \pm 80.07	37.62 \pm 18.75	4.12 \pm 1.38	32.94 \pm 8.51	27.56 \pm 5.52
	\pm Mean	10.31 \pm 3.46	51.71 \pm 41.88	34.16 \pm 16.42	6.07 \pm 1.72	53.88 \pm 6.80	64.67 \pm 7.49
Incinerator	Human Blood	6.12 \pm 0.77	3.2 \pm 1.39	1.18 \pm 0.56	0.02 \pm 0.02	15.55 \pm 1.89	22.09 \pm 3.39
	Animal Blood	15.46 \pm 1.37	6.71 \pm 2.91	9.68 \pm 2.48	7.07 \pm 2.03	56.29 \pm 3.52	79.44 \pm 6.97
	\pm Mean	26.90 \pm 18.64	4.96 \pm 2.15	5.43 \pm 1.52	3.55 \pm 1.03	35.92 \pm 2.71	50.77 \pm 5.18

Fig. 2. PAH concentration ($\mu\text{g}/\text{kg}$) of soil, water, human plasma and animal plasma samples collected from industrial area of Lahore (n = 50).

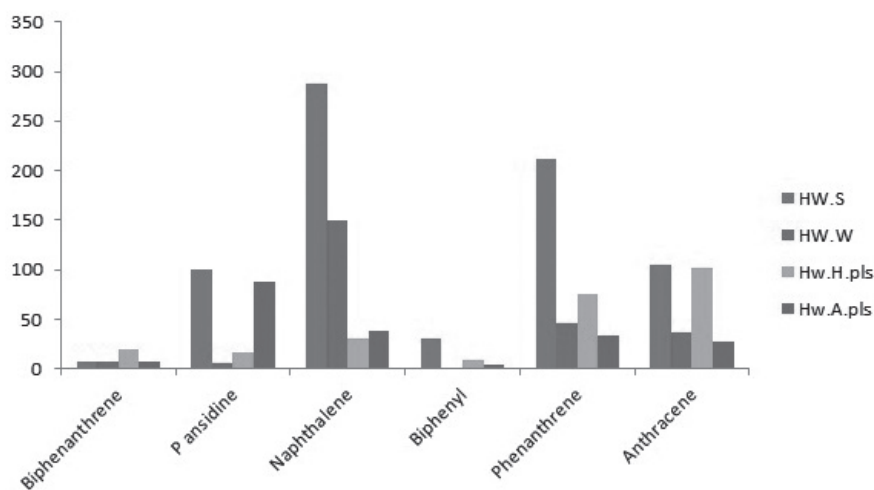


Fig. 3. PAH concentration (µg/kg) of soil, water, plasma (human and animal) samples collected near highways of Lahore (n = 50).

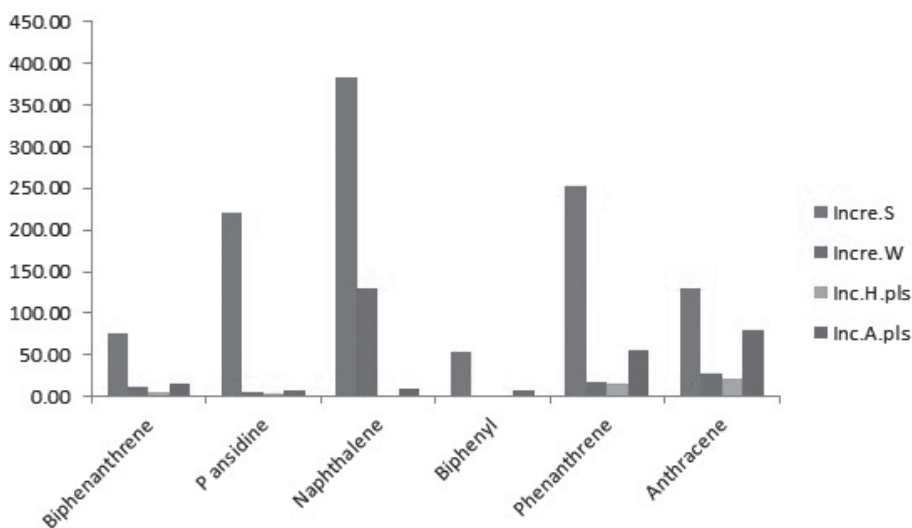


Fig. 4. PAH concentration (µg/kg) of soil, water, human plasma and animal plasma samples collected near incinerators of Lahore (n = 50).

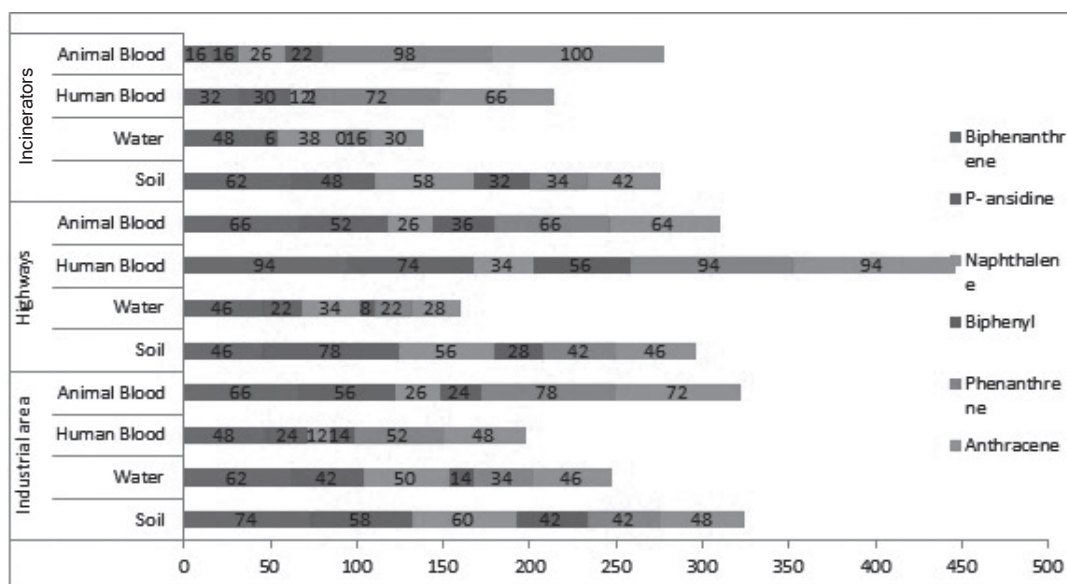


Fig. 5. The percentage of PAHs detected in different samples collected from Lahore, Pakistan.

Table 6. Netherlands “Maximum Permissible Concentrations” (MPCs) for PAHs.

PAH	MPC WATER (mg/L)	MPC SOIL (mg/kg)
Naphthalene	1.2	0.14
Anthracene	0.07	0.12
Phenanthrene	0.3	0.51
Biphenyl	1.0	33
P-Ansidine	0.5	0.5
Biphenthrene	0.2	0.51

Table 7. Sample of soil, water and plasma (human and animals) having PAHs concentration greater than the permissible limit.

Sr. No.	Sample nature	PAHs	% age of positive samples	Samples > permissible level (%)
1	Soil	Biphenanthrene	61	33
2		P-Ansidine	61	61
3		Naphthalene	58	43
4		Biphenyl	34	24
5		Phenanthrene	39	20
6		Anthracene	45	13
7	Water	Biphenanthrene	52	32
8		P-Ansidine	23	16
9		Naphthalene	41	39
10		Biphenyl	7	5
11		Phenanthrene	24	21
12		Anthracene	35	30
13	Human Plasma	Biphenanthrene	58	19
14		P-Ansidine	43	5
15		Naphthalene	19	28
16		Biphenyl	24	9
17		Phenanthrene	73	39
18		Anthracene	69	44
19	Animal Plasma	Biphenanthrene	48	6
20		P-Ansidine	41	17
21		Naphthalene	26	11
22		Biphenyl	27	3
23		Phenanthrene	81	58
24		Anthracene	79	51

concentration of Naphthalene (287.53 $\mu\text{g}/\text{kg}$) was also detected in soil sample collected near highways, shown in Fig. 3. Similarly, highest amount of Naphthalene (383.38 $\mu\text{g}/\text{kg}$) was also detected in soil sample collected from incinerators, overall for all the six analytes shown in Fig. 4. The results indicated that the industrial areas have more concentration of PAHs as compared to other

two zones like incinerators and highways. Naphthalene and Phenanthrene were also reported [31], mostly in air; the presence of these compounds was from incomplete combustion of fuels by vehicles.

PAHs concentrations were also detected in surface sediment samples from the rivers in Shanghai, China ranging from 25.1-9910 $\mu\text{g}/\text{kg}$ [32], Taihu Lake ranging

from 150-2300 $\mu\text{g}/\text{kg}$ [33], the River Nile ranging from 723-1078 $\mu\text{g}/\text{kg}$ [34]. The results of PAHs were also comparable to those found in the Baiyangdian Lake ranging from 163-861 $\mu\text{g}/\text{kg}$ [35], the Hooghly River estuary ranging from 3.3-630 $\mu\text{g}/\text{kg}$ [36], Hainan Island ranging from 20.7-990 $\mu\text{g}/\text{kg}$ [37], the Serbian rivers and canals, Serbia ranging from 29.7-1047 $\mu\text{g}/\text{kg}$ [38], and the Yangtze River Estuary, China ranging from 27.2-621 $\mu\text{g}/\text{kg}$ [39]. The comparison indicated that PAH concentrations in sedimentary were comparable in the global range.

The percentage of PAHs contaminated samples greater than its permissible level is presented in Fig. 5. Overall, it was found that Biphenanthrene and Anthracene were detected greater than the allowable limits in most of the samples. It was found that most of the soil samples quantified for PAHs were having values greater than the permissible limit, shown in Table 6. Percentage of positive samples of PAHs as well as the samples with greater values than the permissible levels in soil, plasma (human and animal) and water are presented in Table 7.

Conclusion

A simple isocratic HPLC method was developed in this study for extraction and determination of six PAHs in soil, water and blood samples. Method validated in this study has shown the analytical precision, accuracy, linearity, sensitivity and selectivity. The developed method was successfully applied for quantitative estimation of six selected PAHs in different types of samples (soil, water and blood samples). Results indicated that highly exposed areas of noncombusted carbons are at high risks of PAHs toxicity either carcinogenic or non-carcinogenic associated risks in human as well as in animals. Having considered the outcomes and the observations made in the current research, it is suggested that a continuous monitoring and assessment of PAHs in these areas should be established to avoid PAHs exposures and toxicity.

Conflict of Interest

The authors declare no conflict of interest.

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